QlAgility User Manual

For use with the QIAgility and the QIAgility Setup Manager v1.4



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REF

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Introduction

1 Introduction

Thank you for choosing the QIAgility®. We are confident it will become an integral part of your laboratory.

Before using the QlAgility, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

1.1 About this user manual

This user manual describes the operation of the QIAgility instrument with the QIAgility Setup Manager Software. It provides information about the QIAgility in the following sections:

- 1. Introduction
- 2. Safety information
- 3. General description
- 4. <u>Installation procedures</u>
- 5. Getting started
- 6. Operating procedures
- 7. QlAgility Setup Manager Software
- 8. Maintenance procedures
- 9. <u>Troubleshooting</u>
- 10. Glossary
- 11. Appendices

The user manual can be accessed by simply pressing the context-sensitive help functionality (**F1** key) provided by the QlAgility Setup Manager Software. Alternatively, the user manual can be accessed, read and printed as *.pdf file.

The functionality of the QIAgility Setup Manager Software and its available plug-ins are described in detail in the QIAgility Setup Manager Software section (section 7).

The user manual includes the following appendices:

- Appendix A:
 - o Technical data
 - Environmental conditions
 - o Waste Electrical and Electronic Equipment
 - o FCC declaration
 - Declaration of conformity

- Appendix B: Liability clause
- Appendix C: French (FR) translation of Safety Information
- Appendix D: German (DE) translation of Safety Information
- Appendix E:
 - o License terms
 - o Copyright information

Check www.giagen.com/goto/QIAgility for a newer version of the user manual.

1.2 General information

1.2.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAgility or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please visit our Technical Support Center at www.support.giagen.com.

1.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.3 Intended use of the QIAgility

The QlAgility is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The QIAgility, if used in combination with QIAGEN kits indicated for use with the QIAgility instrument, is intended for the applications described in the respective QIAGEN kit handbooks.

If the QIAgility instrument is used with kits other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The QlAgility instrument is intended for use by professional users trained in molecular biological techniques and the operation of the QlAgility instrument.

1.4 Requirements for QIAgility users

This table covers the general level of competence and training necessary for transportation, installation, use, maintenance and servicing of the QlAgility.

Task	Personnel	Training and experience
Delivery	No special requirements	No special requirements
Installation	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Routine use (running experiments)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing and annual preventive maintenance	QIAGEN Field Service Specialists only	Regularly trained, certified and authorized by QIAGEN

Safety information

2 Safety information

Before using the QlAgility, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

Note: Translations of the Safety Information in French and German are available in <u>Appendix C</u> and <u>Appendix D</u>.

The following types of safety information appear throughout this manual.

WARNING



The term **WARNING** is used to inform you about situations that could result in personal injury to you or other persons.

Details about these circumstances are given in a box like this one.

CAUTION



The term **CAUTION** is used to inform you about situations that could result in damage to the instrument or other equipment.

Details about these circumstances are given in a box like this one.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

2.1 Proper use

WARNING/ CAUTION



Risk of personal injury and material damage [W1]

Improper use of the QIAgility may cause personal injuries or damage to the instrument.

The QIAgility must only be operated by qualified personnel who have been appropriately trained.

Servicing of the QIAgility must only be performed by QIAGEN Field Service Specialists.

WARNING/ CAUTION



Risk of overheating [W2]

To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QIAgility.

Slits and openings that ensure the ventilation of the QIAgility must not be covered.

WARNING/ CAUTION



Risk of personal injury and material damage [W3]

The QIAgility is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

WARNING/ CAUTION



Risk of personal injury and material damage [W4]

Do not attempt to move the QIAgility during operation.

WARNING/ CAUTION



Explosive atmosphere [W5]

The QIAgility is not designed for use in an explosive atmosphere.

WARNING/ CAUTION



Risk of explosion [W6]

The QIAgility is intended for use with reagents and substances supplied with QIAGEN kits. Use of other reagents and substances may lead to fire or explosion.

CAUTION



Damage to the instrument [C1]

Direct sunlight may bleach parts of the instrument and cause damage to plastic parts.

The QIAgility must be located out of direct sunlight.

CAUTION



Damage to the instrument [C2]

Avoid spilling water or chemicals onto the QIAgility. Damage caused by water or chemical spillage will void your warranty.

WARNING



Risk of personal injury [W7]

The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

In case of emergency, switch off the QlAgility using the power switch at the rear of the instrument and unplug the power cord from the power outlet.

2.2 Electrical safety

Disconnect the line power cord from the power outlet before servicing.

WARNING



Electrical hazard [W8]

Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.

Intentional interruption is prohibited.

Lethal voltages inside the instrument

When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.

WARNING



UV irradiation hazard [W9]

An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp. Under no circumstances should this interlock be disabled.

To ensure satisfactory and safe operation of the QIAgility:

- Connect the line power cord to a line power outlet that has a protective conductor (earth/ground).
- Do not adjust or replace internal parts of the instrument.

- Do not operate the instrument with any covers or parts removed.
- If liquid has spilled inside the instrument, switch off the instrument, disconnect it from the power outlet and contact QIAGEN Technical Services.

If the instrument becomes electrically unsafe, prevent other personnel from operating it, and contact QIAGEN Technical Services. The instrument may be electrically unsafe when:

- The line power cord appears to be damaged.
- It has been stored for a prolonged period of time in conditions which are outside of the "Storage Conditions" outlined in <u>Appendix A</u>.
- It has been subjected to severe transport stresses.

2.3 Biological safety

Specimens and reagents containing materials from humans should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as Biosafety in Microbiological and Biomedical Laboratories, HHS (www.cdc.gov/od/ohs/biosfty/biosfty/biosfty.htm).

2.3.1 Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store and dispose of such samples according to the required safety regulations.

WARNING



Samples containing infectious agents [W10]

Some chemicals used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, 2 pairs of gloves and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of infectious agents as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH† or COSHH‡ documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

^{*} OSHA: Occupational Safety and Health Administration (United States of America).

[†] ACGIH: American Conference of Government Industrial Hygienists (United States of America).

[‡] COSHH: Control of Substances Hazardous to Health (United Kingdom).

2.4 Chemicals

WARNING



Hazardous chemicals [W11]

Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.

Always wear safety glasses, gloves and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH† or COSHH‡ documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

2.4.1 Toxic fumes

If working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

2.5 Waste disposal

Used plasticware and containers may contain certain hazardous chemicals, or contagious/biohazardous materials. Such wastes must be collected and disposed of properly according to local safety regulations.

For information about how to dispose of the QIAgility, see Appendix A.

2.6 Mechanical hazards

WARNING



Moving parts [W12]

Avoid contact with moving parts during operation of the QlAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.

^{*} OSHA: Occupational Safety and Health Administration (United States of America).

[†] ACGIH: American Conference of Government Industrial Hygienists (United States of America).

[‡] COSHH: Control of Substances Hazardous to Health (United Kingdom).

WARNING



Moving parts [W13]

To avoid contact with moving parts during operation of the QIAgility, the instrument must be operated with the lid closed.

If the in-built lid sensor is not functioning correctly, contact QIAGEN Technical Services.

CAUTION



Damage to the instrument [C3]

Do not apply any manual force to the Y-arm (i.e. the pipetting head).

We strongly recommend restarting the system if the Y-arm was accidentally manually moved.

To ensure satisfactory and safe operation of the QIAgility, follow the guidelines below:

- Only use recommended tips and tip racks.
- Keep the instrument lid closed whenever possible.
- When calibrating with the lid open, always stand clear of the instrument when making adjustments. The
 lid interlock is disabled during calibration. Therefore, do not click with the mouse or press any of the
 keys on the keyboard when looking closely at calibrations. Wait until the robotic arm has completed its
 movements before moving any plates or tubes.
- Do not place any items onto the robotic arm.

During run, click on the **Pause** button to pause the instrument before removing plates or tubes from the worktable. Wait until the robotic arm has completed the current movement. This may take up to 10 seconds.

2.7 Maintenance safety

Perform the maintenance as described in the <u>Maintenance procedures</u>. QIAGEN charges for repairs that are required due to incorrect maintenance.

WARNING/ CAUTION



Risk of personal injury and material damage [W14]

Only perform maintenance that is specifically described in this user manual.

WARNING/ CAUTION



Risk of electric shock [W15]

Do not open any panels on the QlAgility.

Risk of personal injury and material damage

Only perform maintenance that is specifically described in this user manual.

CAUTION



Damage to the instrument [C4]

Do not use solvents or reagents containing acids, alkalis, or abrasives to clean the QIAgility.

CAUTION



Damage to the QIAgility lid [C5]

Do not use alcohol, solvents, reagents containing acids, alkalis, or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use distilled water only.

CAUTION



Damage to the QIAgility side panels [C6]

Never clean the instrument side panels with alcohol or alcohol-based solutions.

CAUTION



Relocating the QlAgility [C7]

The most important component of the packaging is the wooden support that immobilizes the Y-arm. This support must be used when the QlAgility is moved.

2.8 Symbols on the QIAgility

Symbol	Location On the back of the instrument, and on the front edge of the worktable	Description Warning symbol
	Near the tip ejector outlet, and on the front edge of the worktable	Biohazard symbol
	On the front edge of the worktable	Squeezing symbol
CE	Type plate on the back of the instrument	CE mark for European Conformity
C Us	Type plate on the back of the instrument	CSA listing mark for Canada and the USA
	Type plate on the back of the instrument	Legal manufacturer
X	Type plate on the back of the instrument	Waste Electrical and Electronic Equipment (WEEE)
F©	Type plate on the back of the instrument	FCC mark of the United States Federal Communications Commission
	Type plate on the back of the instrument	RCM (former C-Tick) for Australia (supplier identification N17965)
25)	Type plate on the back of the instrument	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)
[]i	Type plate on the back of the instrument	Consult instructions for use

General description

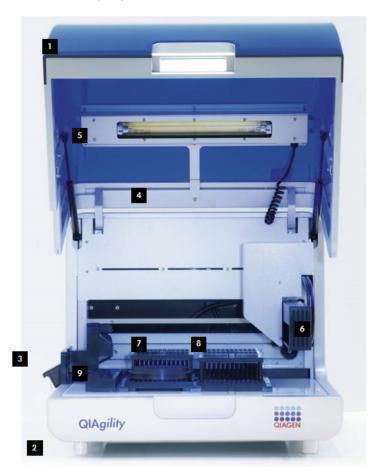
3 General description

The QlAgility is a compact benchtop instrument that enables rapid, high-precision PCR setup. For added flexibility and ease of use, the QlAgility has been designed to support almost all tube and plate formats, as well as the Rotor-Disc[®] for the Rotor-Gene[®] Q instruments. The QlAgility is operated via a computer with an user-friendly software. Further information on the QlAgility Setup Manager Software are available here.

The QlAgility instrument provides:

- Automated PCR setup in almost all formats
- Convenient, easy-to-use software
- Standardized results and increased productivity
- Elimination of manual pipetting steps
- Seamless integration with QIAGEN sample technologies

3.1 QlAgility instrument



- 1 Lid
- 2 Feet
- 3 Tip ejector
- 4 HEPA filter
- 5 UV lamp

- 6 Robotic arm with pipettor head
- 7 Tip rack holder and tips
- 8 Tip rack holder and tips
- 9 Master mix block and reagent block

3.1.1 Lid

CAUTION

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Damage to the QIAgility lid [C5]

Do not use alcohol, solvents, reagents containing acids, alkalis, or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use distilled water only.

The lid of the instrument provides both an isolated enclosure and a safety mechanism. The instrument lid incorporates a magnetic sensor to determine whether or not the lid is closed. Under no circumstances should this sensor be bypassed.

The instrument lid must be closed for a run to proceed. Opening the instrument lid while the instrument is performing a run causes the run to pause.

The instrument lid may remain open during calibration. Do not interfere with robotic arm movement during these calibrations and keep all body parts out of reach of the robotic arm while it is moving.

Important: Care should be taken when opening the lid during a run, because it takes up to 10 seconds for the instrument to complete the current movement and for the pause to take effect. Under no circumstances should the sensor be bypassed.

3.1.2 Feet

The feet of the instrument have a non-slip base to ensure that the QIAgility remains securely positioned on a laboratory bench. Do not attempt to slide the instrument on a bench. Always lift the instrument to avoid damage to the base of the feet.

3.1.3 Tip ejector

Tips used on the worktable are discarded through the tip ejector located on the left side of the QIAgility. The tip ejector is held in place by a locating block, allowing easy placement and removal for cleaning.

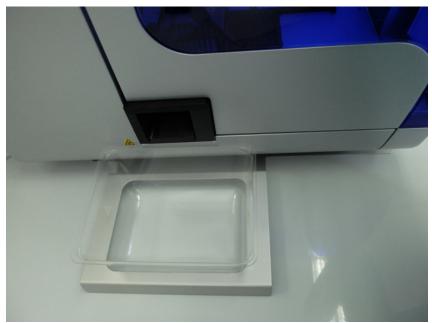
Note: Details on removing and replacing the tip ejector are described in section Cleaning the tip ejector.

Tip ejector

3.1.4 Tip disposal box

Discarded tips are collected in a tip disposal box, located at the side of the QIAgility. To ensure correct positioning under the tip ejector, the tip disposal box is held in place by the tip disposal box holder. The tip disposal box has a capacity of approximately 350 tips.

Lock the tip disposal box holder with the instrument's feet on the left side of the instrument and then place the tip disposal box into the tip disposal box holder.



Alignment of tip disposal box holder to the instrument and the tip disposal box.

3.1.5 HEPA filter

The HEPA filter system provides positive clean air pressure on the worktable beneath the lid. The HEPA filter system consists of two HEPA filter cartridges.



HEPA filter

3.1.6 UV lamp

A UV lamp in the instrument lid provides efficient worktable decontamination and helps to prevent cross-contamination.

Note: Before using the UV lamp, the tip ejector must be placed into the instrument.

For increased safety, an electronic interlock ensures that the UV lamp operates only when the instrument lid is closed. Under no circumstances should the interlock be disabled.

During UV irradiation, the robotic arm moves. All items must be removed from the instrument to ensure the worktable is fully exposed.

The UV lamp does not irradiate certain areas, including the pipettor head, due to construction constraints.

To operate the UV lamp, the UV lamp bulb needs to be connected to the UV plug. For more details, see section <u>Using UV lamp</u>.

3.1.7 Pipettor head and robotic arm

Liquid-handling tasks are performed by the pipettor head, which provides a motor-driven, backlash-compensated pipetting mechanism. Tips attach to the pipettor piston of the pipettor head.

The robotic arm controls the movement of the pipettor head in the X-, Y- and Z-directions.

The Y-axis arm of the robotic arm provides support for the pipettor head. Under no circumstances should any force be applied to the Y-arm. This may misalign the arm and cause pipetting errors.

Note: To prevent accidental damage, the wooden Y-arm support should always be fixed or locked before the QlAgility is moved.

3.1.8 Worktable

The worktable is the instrument surface upon which items, such as adapters, labware and tips are placed. The QIAgility Setup Manager Software automatically calculates the worktable layout before each run.

3.1.9 Accessory panel

All instrument electronics are housed in the rear of the instrument. The accessory panel, located at the rear of the QlAgility, provides ports for connection to the instrument.



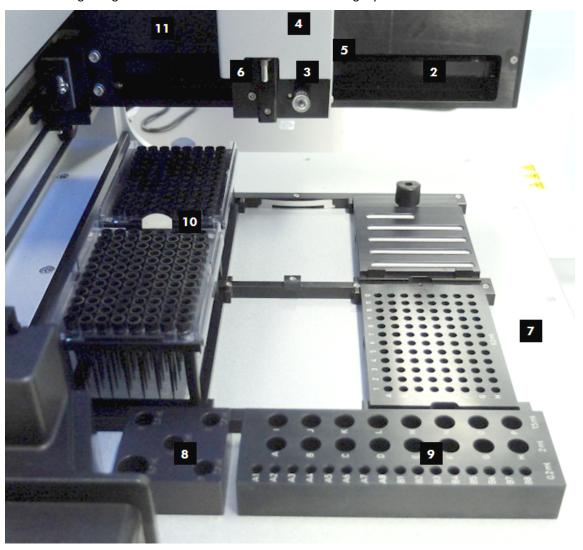
QIAgility accessory panel

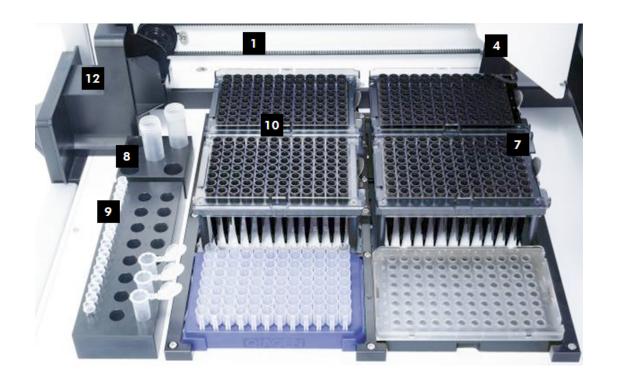
- 1 Accessory 1
- 2 Accessory 2
- 3 Accessory 3
- 4 Serial RS-232 socket

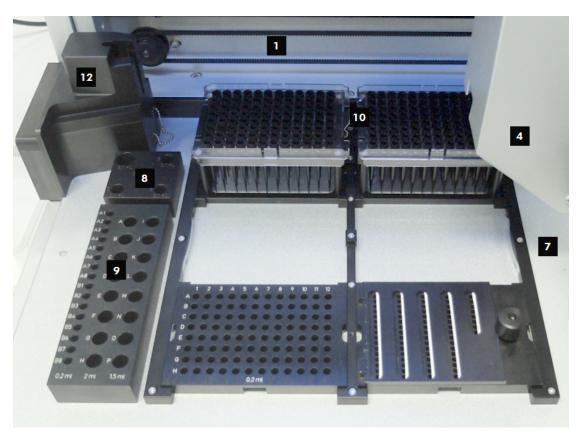
- 5 USB socket
- 6 Accessory power outlet
- 7 Main power input socket
- 8 Power switch

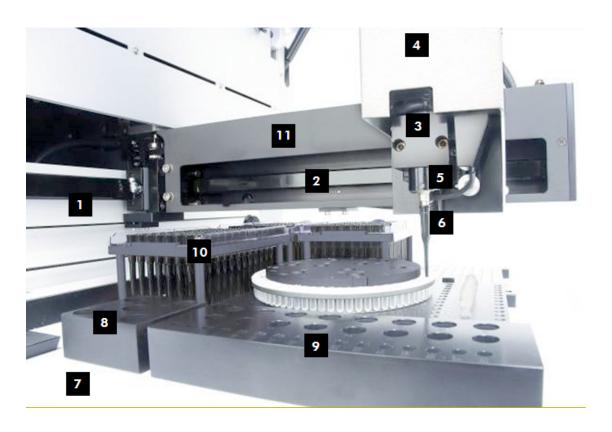
3.1.10 Internal features of the QIAgility

The following images show the internal features of the QIAgility.









1	X-axis	7	Worktable
1	X-dxi3	,	WORKINDIC
2	Y-axis	8	Master Mix Block
3	Z-axis	9	Reagent Block
4	Pipettor head cover	10	Tip Rack Holders and tips
5	Pipettor head	11	Y-arm
6	Pipetting hub	12	Tip ejector

Part	Description
X-axis	The X-axis directs the left/right movement of the pipettor head.
Y-axis	The Y-axis directs the forwards/backwards movement of the pipettor head.
Z-axis	The Z-axis directs the upwards/downwards movement of the pipettor head.
Tip ejector	The tip ejector enables tip removal from the pipettor head.
Pipettor head cover and pipettor head	The pipettor head is a single channel, able to pipette 1–200 μ l and is located under the protective pipettor head cover. The pipettor head consists of a motor-driven backlash-compensated pipetting mechanism.
Y-arm	The Y-arm supports the pipettor head. Under no circumstances should the user apply force to the Y-arm. This may misalign the Y-arm and cause pipetting errors.
Worktable	The worktable has 6 SBS positions (127.76 mm x 85.48 mm) in which adapters and labware can be placed. In addition, the worktable supports both Master Mix Blocks and Reagent Blocks.

3.2 Liquid-level tracking

The QlAgility can track the liquid level using liquid-level sensing or liquid-level estimate. The QlAgility Setup Manager Software ensures optimal use of the feature during runs.

Liquid-level sensing

For maximum accuracy and precision, the QIAgility is equipped with a liquid-level sensor. This feature enables the instrument to automatically detect when the pipet tip makes contact with the liquid as the tip descends. When aspirating, the tip is placed below the liquid surface, at the minimum insertion depth required to collect the aspirate volume. When dispensing, the tip is placed at the liquid surface and is retracted as liquid is ejected. This minimizes contact between the tip and the liquid, reducing liquid carryover.

The liquid-level sensor relies on conductive tips to detect the change in capacitance when the tip contacts the liquid inside a tube – the smaller the volume of liquid inside the tube, the lower the change in capacitance. For correct functioning of the liquid-level sensor, it is essential that the labware (plates, tubes, adapters) located on the instrument worktable has been correctly selected in the software. See section Managing labware for details about how to do this.

If a liquid is nonconductive (e.g., pure water, oil), the level sensor will not detect any change in capacitance.

Liquid-level estimate

This feature enables the instrument to estimate the liquid level, using information from the run configuration. The software tracks the movement of liquid between wells and estimates the liquid level during all liquid transfers. This information is used to determine the optimal tip placement. Levels of accuracy and precision comparable to those obtained with liquid-level sensing are obtained. The pipet tip moves to a calculated position so that, after aspiration, the pipet tip remains below the liquid level.

3.3 Computer

The QlAgility is operated via a computer.

A laptop with the correct specifications for operation of the QlAgility and QlAgility Setup Manager Software is supplied as part of the QlAgility system. However, if a different computer is used to operate the QlAgility or to run the QlAgility Setup Manager Software, then a computer hosting system with the following specifications is recommended.

Computer specifications:

- Windows[®] 7 Professional (32- or 64-bit) with Service Pack 1 or higher; Windows 10 (64-bit) with minimum required version 1607
- At least 1 GHz or faster CPU
- At least 1 GB RAM for 32-bit OS, or 2 GB RAM for 64-bit OS
- At least 16 GB free hard drive capacity for 32-bit OS, or 20 GB free hard drive capacity for 64-bit OS
- At least 1360 x 768 screen resolution
- Pointer device (mouse or similar)

To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe[®] Reader[®] can be downloaded at **www.adobe.com**.

Additional configuration steps:

- Turn off all automatic processes or services such as indexing or similar, which could produce a high workload.
- Ensure that energy saving options, hibernation and the screen saver are deactivated during the operating times of the QIAgility.
- For Windows 10 only if **UEFI Boot** is used, the **Secure Boot** option must be disabled. Otherwise, the Windows Device Driver Installation of the QIAgility device driver will fail during installation of the QIAgility Setup Manager Software.
- Ensure that the Federal Information Processing Standards (FIPS) policy is disabled.

Note: In case of any concern regarding the Windows configuration, please contact your local IT support.

Note: If you close the laptop lid during a run, the run will be interrupted and stopped.

If connecting the computer to a network, be sure to consider the following:

- Ensure that update processes are scheduled to download and install only outside the operating times of the QIAgility.
- Turn off auto-restart after an installation of Windows security patches to prevent automatic computer restart during the operating times of the QIAgility.

Note: In order to complete a security update installation, perform a manual restart later.

If virus protection software is required, ensure that the following tasks are scheduled to run outside the operating times of the QIAgility:

- Scans (also ensure that files that are created by the QIAgility Setup Manager Software are not scanned during creation or on access)
- All kind of updates

Note: Some virus protection software might interfere with QlAgility Setup Manager Software and cause the software to run very slowly.

Note: The QIAgility Setup Manager Software is tested by QIAGEN using the original image supplied with your laptop without connecting the laptop to the internet. Updates on your operating system might lead to unexpected behavior of the QIAgility Setup Manager Software. In such cases, please contact your local IT support.

Note: The minimal screen resolution can be used in combination with small fonts. However, medium size fonts can be used in combination with an improved resolution (e.g., the medium font size (125%) setting in Windows 7 requires a minimum screen resolution of 1680 x 1050). Larger font sizes are not supported.

Installation procedures

4 Installation procedures

This section provides instructions on unpacking and installing the QIAgility.

Note: For your own safety and to prevent damage to the instrument, observe the safety instructions.

4.1 System delivery

The following items are delivered:

- QlAgility instrument
- Laptop
- QlAgility Setup Manager Software and instructions for use
- Unpacking Instructions (printed version)
- Packing List (printed version)
- Volume Calibration Report (printed version)
- QlAgility Calibration Kit
- Accessories

Note: We recommend checking the packing list before installation. If anything is missing, contact QIAGEN Technical Services.

Adapters

The QIAgility is supplied with the following adapters:

- 2 x Adapter, 96 x 0.2 ml PCR Tubes
- 1 x Adapter, Flip-Cap 32 x 1.5 ml Tapered Base
- 1 x Adapter, 72 x 0.1 ml Strip Tubes
- 1 x Master Mix Block, 5 x 5 ml
- 1 x Master Mix Block 1 x 5 ml, 4 x 2 ml
- 1 x Master Mix Block, 1 x 5 ml, 4 x 1.5 ml
- 1 x Reagent Block, 16 x 0.2 ml / 2 ml Flat / 1.5 ml Tapered Tubes
- 4 x Tip Rack Holder for 96-Tip Rack

The adapters are intended to hold tubes, plates and tips.

Tubes and plates

Several types of tubes and plates can be used with the QIAgility. A set of labware is already preinstalled in the software and can be found in the **Configuration** environment. The following tubes are supplied with the instrument:

- 5 ml free-standing tubes
- 200 μl PCR tubes

Note: Supplied tubes are not guaranteed to be sterile.

Important: Tubes supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). We recommend that once a particular tube type is chosen, to always purchase that consumable from the same manufacturer to avoid frequent recalibration.

Conductive Filtered Tips

200 μ l or 50 μ l Conductive Filtered Tips can be used on QIAgility. Supplied tips are carbon impregnated. See section Insert tips to tip rack holder for details about how to insert tips into a Tip Rack Holder.

Important: Do not use tips from other suppliers.

4.2 Requirements

QlAgility is a high-precision liquid-handling device and has specific requirements regarding the installation site. These requirements are explained in the following chapter.

4.2.1 Installation site

Choose the position for the QIAgility carefully. A change in position necessitates a recalibration. Place the QIAgility in its final position.

The requirements for the QIAgility location and position are given below:

- The QIAgility must be located out of direct sunlight, away from heat sources and away from sources of vibration and electrical interference. Refer to <u>Appendix A</u> for the operating conditions (temperature and humidity).
- The site of installation should be free of excessive drafts, moisture and dust, and should not be subject to large temperature fluctuations.
- Use a level workbench that is dry, clean, vibration-proof and large enough to accommodate the QlAgility, computer and tip disposal box. Refer to <u>Appendix A</u> for the weight and dimensions of the QlAgility.
- The QIAgility must be placed within approximately 1.5 m of a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.
- Ensure that the switch and the main power input socket at the rear of the instrument are easily reachable. Maintain sufficient clearance at the rear to unplug the power line from the main power input socket.
- Ensure that the instrument lid can fully open upwards without obstruction.

- The QlAgility must not be installed near generators of static electricity (e.g., ionizers, large-screen televisions).
- The QIAgility should be installed in a laboratory environment that is free of excessive airborne dust.

Note: We recommend that the QIAgility does not share a wall socket with other laboratory equipment, and that the QIAgility is plugged directly into its own wall socket.

Important: The wall socket shall be easily accessible.

Important: Position the QIAgility in a way that allows easy access to the power supply cable at the rear. Meeting these location and position requirements will allow you to immediately disconnect the instrument from the power supply should a need or emergency arise.

WARNING/ CAUTION



Risk of overheating [W2]

To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QIAgility.

Slits and openings that ensure the ventilation of the QIAgility must not be covered.

CAUTION



Damage to the instrument [C1]

Direct sunlight may bleach parts of the instrument and cause damage to plastic parts.

The QIAgility must be located out of direct sunlight.

4.2.2 Power requirements

The QlAgility operates at these power conditions: 100–240 V, 250 VA, 50–60 Hz, 5 A fuse.

Make sure the voltage rating of the QIAgility and laptop are compatible with the AC voltage available at the installation site. Main supply voltage fluctuations are not to exceed \pm 10% of nominal supply voltages.

4.2.3 Grounding requirements

To protect operating personnel, and for correct functioning of the liquid-level sensor, ensure that the QIAgility is correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

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4.3 Unpacking and installation

4.3.1 Unpacking the QIAgility

Important: For unpacking of the QIAgility, refer the unpacking instructions delivered with the instrument. The unpacking instructions can also be downloaded at www.qiagen.com/goto/QIAgility.

Before unpacking the QlAgility, move the package to the site of installation and check whether the package is damaged. In case of damage, contact the transporter of the package.

WARNING/ CAUTION

Risk of personal injury and material damage [W3]



The QlAgility is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

- 1. Remove the lid from the QIAgility shipping container.
- 2. Check that the QIAgility is not damaged and that there are no loose parts. If anything is damaged, contact QIAGEN Technical Services.
- 3. Read the packing list to check that you have received all items (see <u>System delivery</u>). If anything is missing, contact QIAGEN Technical Services.
- 4. Keep the package in case you need to transport or ship the QIAgility in the future.
- 5. Carefully place the QIAgility onto the workbench where it will be used. For site requirements, refer to section <u>Installation site</u>. When lifting the QIAgility, slide your fingers under both sides of the instrument and keep your back straight.
- 6. Remove transport lock from pipetting arm.

4.3.1.1 Releasing transport locks

CAUTION

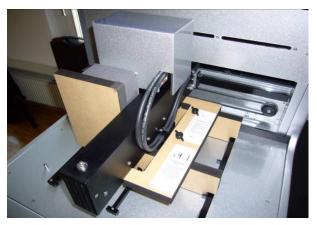
ON Relocating the QIAgility [C7]



The most important component of the packaging is the wooden support that immobilizes the Y-arm. This support must be used when the QIAgility is moved.

To release the transport locks:

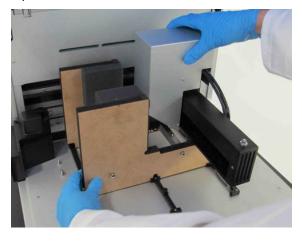
1. Unscrew the two screws and remove the horizontal bar.





2. Move the pipettor head to the right and remove the transport lock from the worktable.





3. Unscrew the Z-axis locking screw. When this screw is removed, the pipettor head will drop.



4. Store the transport lock and the Z-axis screw with the rest of the packaging.

4.3.2 Installation of AC power cord

Connect the QIAgility to the power outlet as follows:

- 1. Ensure that the power switch of the QIAgility is set to the "Off" position.
- 2. Plug the power cord into the main power input socket.
- 3. Plug the power cord into a grounded power outlet.

Important: The wall socket should be easily accessible.

Important: The QIAgility Setup Manager Software must be installed on the computer before the QIAgility is switched on (see section <u>Software installation</u>).

4.3.3 Connecting the computer to the QIAgility

Before attempting to connect the QIAgility to the laptop, ensure the following conditions have been met:

- The QIAgility has been properly set up and is in its final location (see section <u>Unpacking the QIAgility</u>).
- The laptop is in its final location near the instrument.

Note: Before connecting the QIAgility to the computer for the first time, ensure that the QIAgility Setup Manager Software has been installed (see section <u>Software installation</u>).

Note: Connect the computer to the QIAgility via the USB interface.

To connect via the USB interface:

Connect the USB cable between the USB port on the instrument and the USB port on the computer.



USB cable attached to the accessory panel.

Note: The QlAgility Setup Manager Software recognizes connection to the QlAgility as a USB device even when the instrument is switched off.

4.3.4 UV lamp

The UV plug is integrated within the lid. To connect the UV lamp, proceed as follows:

- 1. Open the lid.
- 2. Connect the delivered UV lamp bulb into the UV plug and turn it by 90°.

Note: Hold the UV lamp bulb at both ends when fixing it into the UV plug. Always wear safety gloves.

WARNING

\wedge

UV irradiation hazard [W9]

An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp. Under no circumstances should this interlock be disabled.

4.4 Software installation

The QlAgility Setup Manager Software must be installed before the QlAgility is connected to the laptop for the first time. The software will generally be installed on the laptop by a QlAGEN Field Service Specialist on the day of instrument installation, if the QlAgility was purchased with an installation and startup training. A user with Windows administrator privileges is required to install the QlAgility Setup Manager Software, but such privileges are required for software installation only. If the QlAgility was purchased without an installation and startup training, follow the steps below to install the software:

For computer system requirements, see section Computer.

1. Set up the computer as described in the computer installation instructions provided with the computer.

- 2. Before installing the QlAgility Setup Manager Software, shut down all other software and make sure that the QlAgility is not connected to the computer.
- 3. If a previous version of the software is already installed on the computer, refer to <u>Updating QlAgility Setup Manager Software</u>. Otherwise, follow the instructions in <u>Installing QlAgility Setup Manager Software</u>.

Note: We recommend having no other software installed on the laptop that controls the QIAgility other than the QIAgility Setup Manager Software. In particular, the legacy QIAgility Software must not be installed.

Note: Default settings are recommended where appropriate for all installation steps.

Note: The QlAgility Setup Manager Software version 1.2 is the first version supporting Windows 10 (64-bit).

4.4.1 Installing QIAgility Setup Manager Software

Things to do before starting:

- 1. Ensure that the computer meets the minimum requirements (see <u>Computer</u>).
- 2. Use a Windows account with administrator privileges.

Note: Windows administrator privileges are required for software installation only.

3. Close any programs running on the computer and make sure that the QIAgility is not connected to the computer (i.e., unplug the USB cable from the computer).

To install the QIAqility Setup Manager Software from a Removable Media:

4. Insert the QIAgility Setup Manager Software Removable Media into the computer. The setup wizard automatically opens the QIAgility Setup Manager Software setup window. Proceed to step 7.

Note: Automatic setup is disabled on laptops provided by QIAGEN.

Note: If the setup does not start automatically, double-click the **setup.exe** file in the Removable Media drive folder. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking if you want to allow the program to install. Allow the program to install.

To install the software from a web download:

QlAgility Setup Manager Software is available for download at <u>www.qiagen.com/goto/QlAgility</u>. Before installing, read the information provided on the web page.

4. Download the installation package .zip file.

Note: If the computer running the QIAgility Setup Manager Software is not connected to a network, download the installation package from another computer to a USB stick. Copy the installation package to the QIAgility Setup Manager Software computer.

- 5. Extract the installation package .zip file.
- 6. Double-click on the **setup.exe** file. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking you if you want to allow the program to install. Allow the program to install.

- 7. The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft .NET Framework. This may take some time. If you click **No**, the QlAgility Setup Manager Software will not be able to operate.
- 8. Click **Next** to start the installation.
- 9. Follow the instructions that appear in the setup wizard.

Note: Default settings are recommended where appropriate for all installation steps.

- 10. Before the installation is completed, the appropriate version of the device driver will be installed if it is not already present. In this case, the Device Driver Installation Wizard will open. Click **Next** to proceed. Depending on the Windows configuration, a Windows security confirmation message may be displayed to indicate that QIAGEN GmbH is attempting to install drivers. Click **Install** to install the QIAgility driver. After driver installation, click **Finish** in the Device Driver Installation Wizard. **Note:** If you do not install the driver software, the QIAgility Setup Manager Software will not be able to operate the QIAgility.
- 11. After installation, click **Exit Setup** in the Removable Media menu, or **Finish** in the wizard.
- 12. Connect the QlAgility to the computer.
- 13. The QlAgility Setup Manager Software can now be started.

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your local IT support.

Note: In case other messages are displayed, refer to the troubleshooting section System setup.

4.4.2 Install plug-ins

The QlAgility Setup Manager Software setup automatically installs all currently available plug-ins. To view the list of available plug-ins, click **Help** in the menu bar of the QlAgility Setup Manager Software, and then **About**. In the dialog that is shown click the tab **Loaded plug-ins**. Click **Close** to close the dialog. For further details about plug-ins, refer to <u>Plug-in concept</u>.

New plug-in versions will be made available over time. Please check the QIAgility website at www.giagen.com/goto/QIAgility for additional information.

Update the QIAgility Setup Manager Software to upgrade plug-ins to a newer version. Follow the instructions in <u>Updating QIAgility Setup Manager Software</u>.

After the QlAgility Setup Manager Software has been upgraded, the new versions of plug-in wizards will be available in the **Home** environment, having replaced the previous versions.

4.4.3 Uninstalling QIAgility Setup Manager Software

Important: During uninstallation of the QIAgility Setup Manager Software, all experiments, templates, reports, saved output files, etc. that have been saved in any subfolder of **C:** \Users\Public\QIAGEN\QIAgility will be removed.

We recommend moving all user files to another location outside C:\Users\Public\QIAGEN\QIAgility before uninstalling the software.

Note: It is not possible to uninstall an individual plug-in.

To automatically uninstall the QIAgility Setup Manager Software together with all its plug-ins, follow these steps:

- In the Windows Start menu, select: All Programs / QIAGEN / QIAgility / Uninstall QIAgility Setup Manager Software
- 2. Follow the instructions of the installer to uninstall the software.

To manually uninstall the QIAgility Setup Manager Software or any plug-ins or wizards, follow these steps:

- 1. Open the Control Panel by clicking on the Windows Start menu and selecting Control Panel.
- 2. Depending on your layout settings, either click **Programs and Features** or **Programs** / **Uninstall Programs**.
- Select QlAgility Setup Manager Software to be uninstalled and click Uninstall/Change.
 Note: Depending on the Windows configuration, there may be a pop-up message asking if you want to allow the program to install. Allow the program to install.
- 4. Follow the instructions of the installer to uninstall.

4.4.4 Updating QIAgility Setup Manager Software

If a previous version of the QIAgility Setup Manager Software is already installed, it can be updated using the software package that is available at www.qiagen.com/goto/QIAgility. Before updating, read the information provided on the web page. Experiments and user settings will be preserved. Customized labware will be deleted and must be installed again after the software is updated.

Things to do before starting:

- 1. Ensure that the computer meets the minimum requirements (see Computer).
- 2. Use a Windows account with administrator privileges.

Note: Windows administrator privileges are required for software installation only.

3. Close any programs running on the computer and make sure that the QIAgility is not connected to the computer (i.e., unplug the USB cable from the computer).

To install the software from a web download:

QIAgility Setup Manager Software is available for download at <u>www.qiagen.com/goto/QIAgility</u>. Before installing, read the information provided on the web page.

4. Download the installation package .zip file.

Note: If the computer running the QIAgility Setup Manager Software is not connected to a network, download the installation package from another computer to a USB stick. Copy the installation package to the QIAgility Setup Manager Software computer.

- 5. Extract the installation package .zip file.
- 6. Double-click on the **setup.exe** file. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking you if you want to allow the program to install. Allow the program to install.

- 7. The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft .NET Framework. This may take some time. If you click **No**, the QIAgility Setup Manager Software will not be able to operate.
- 8. Click **Next** to start the installation.
- 9. If you are updating from QIAgility Setup Manager Software version 1.0 or 1.1, a message will appear to inform you that a wrong SiLabs USB driver version has been detected that will be replaced during installation by the new driver version. This new driver version is required to operate the QIAgility Setup Manager Software. Click OK to proceed with the installation.
- 10. Follow the instructions that appear in the setup wizard.

All new software components will be installed. Existing plug-ins will be updated and newly available plug-ins will be added automatically. User profiles, labware settings and calibrations created before the update will be available after the update. Experiments, reports and sample files will also be available.

Note: Default settings are recommended where appropriate for all installation steps.

Note: A message will appear to inform you that some files that need to be updated by the setup are still in use by the QlAgility Core Engine Service. The QlAgility Core Engine Service is a service of the QlAgility Setup Manager Software that always runs on the computer. Select the option **Close the applications and attempt to restart them.** The setup will stop and restart the QlAgility Core Engine Service during installation. If you select the option **Do not close applications. A reboot will be required**, the installation will continue, but it will only be completed after the computer is rebooted.

Note: If you are updating from version 1.0, a message may appear to inform you that during uninstallation of the QIAgility Setup Manager Software, all experiments, templates, reports, saved output files, etc. that have been saved in any subfolders of **C:\Users\Public\QIAGEN\QIAgility** will be removed. Click **OK** to acknowledge the message. Despite the message, these items will still be available after the setup.

11. If the message appeared at the beginning of installation to inform you that the SiLabs USB driver will be replaced by a new driver version, the Device Driver Installation Wizard will open. Click **Next** to proceed. A security confirmation message is shown that QIAGEN GmbH drivers attempt to be installed. Click Install to install the driver. After driver installation click Finish in the Device driver Installation Wizard.

Note: If you do not install the new driver version, the QIAgility Setup Manager Software might not be able to operate the QIAgility properly.

12. After installation, click **Finish** in the wizard.

Note: If you selected the option **Do not close applications. A reboot will be required.** during installation, you will need to restart the computer before the QlAgility Setup Manager Software is ready to operate.

- 13. Connect the QlAgility to the computer.
- 14. The QlAgility Setup Manager Software can now be started.
- 15. Use one of the previously created user IDs for login.

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your local IT support.

Note: In case of other messages displayed, refer to the troubleshooting section System setup.

Note: Experiments from a previous plug-in version can be opened with a newer plug-in version.

4.4.5 Upgrading from legacy QIAgility Software

Before upgrading from old versions of the QIAgility Software (version 4.X) to the QIAgility Setup Manager Software, you must first uninstall the legacy QIAgility Software. It is not possible to have both the legacy version of the QIAgility Software and the QIAgility Setup Manager Software installed on the same computer. Data migration of protocols from old to new software is not possible and not provided.

After the successful uninstallation, install the QIAgility Setup Manager Software as described in section Installing QIAgility Setup Manager Software.

Getting started

5 Getting started

This chapter provides information about basic configuration that needs to be completed before the QIAgility can be used for runs.

Some of the tasks need to be repeated occasionally, such as instrument calibration after servicing the QIAgility, or labware managament and labware calibration when changing labware or supplier.

5.1 Start the software

The QIAgility Setup Manager Software can always be started without having the instrument connected or switched on, for example, to prepare experiments for the next day. However, in this case, certain areas of the software that require the instrument to be switched on will not be available, such as worktable and run step for experiments, calibration or system checks.

After starting the computer on which the QIAgility Setup Manager Software will run, wait two minutes before opening the application.

You can switch on the QIAgility later after the software has already started. The QIAgility automatically connects to the QIAgility Setup Manager Software. This may take around 5 to 10 seconds.

To start the QIAgility:

 Double-click on the SetupM desktop icon, or open the Windows Start menu and select: All Programs / QIAGEN / QIAgility / QIAgility Setup Manager Software.



2. The software starts and displays the login dialog.

Note: Authentication is always required.

- If you are starting the software for the first time after installation, log in by entering the user name
 admin into the User ID field and entering the password admin into the Password field. Click OK.
 Otherwise, log in with your user ID and password.
- 4. The software opens in the **Home** environment.
- 5. If you are starting the software for the first time, continue with <u>Customizing settings</u>.

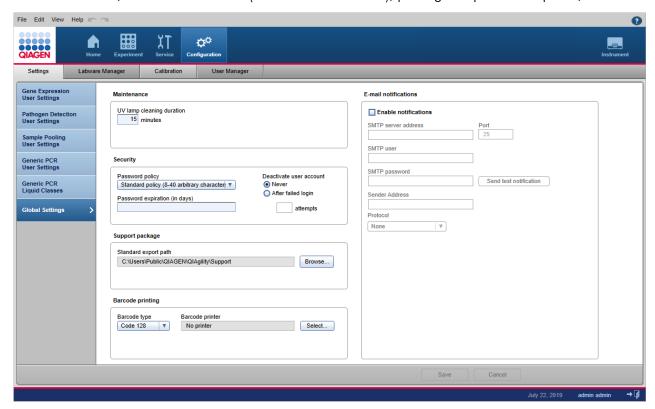
Note: The QlAgility will not initialize when the software is started; however, it will initialize when someone attempts to use the instrument.

Note: If you go to the **Customer** settings for the first time, a "**VER**" will show on the icon because the instrument is not fully calibrated. Before you can start a run, <u>instrument calibration</u> needs to be performed or completed.

The QIAgility User Manual (this publication) can be accessed at any time from within the software by pressing the **F1** key, or by clicking the help button located above the main toolbar.

5.2 Customizing settings

The **Global Settings** tab of the **Configuration** screen enables users to define general settings such as default directories, e-mail server details (for e-mail notifications), printing and password options, and more.



Maintenance options

Enter the time duration for the UV lamp cleaning procedure in the **Maintenance** section of the window. The UV lamp cleaning procedure can be initiated from the **Cleaning Procedure** tab of the **Service** environment. QIAGEN recommends a duration of 15 minutes.

Maintenance



Security options

Specify the security and authentication settings in the **Security** section of the window.

Security



Option

Description

Password policy

There are two different options for the password policy:

- Standard policy (8–40 arbitrary characters): select this option if authentication is required.
- No policy (0–40 characters): select this option if no authentication is required. This policy allows you to use short passwords or even to use an empty password.

Deactivate user account

Specify whether the user account should be deactivated after failed login attempts:

- Never: select this option if an account should never be deactivated.
- After failed login: select this option if a user account should be deactivated after a certain number of failed login attempts. Enter the number in the field below.

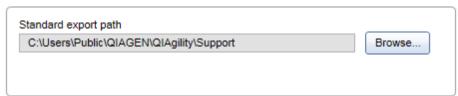
Password expiration (in days)

Specify the number of days after which user passwords expire. Users will be forced to change their passwords after the set amount of time.

Support package

Set the standard export path for the support package by clicking the **Browse** button. This path will be used as the initial path when a support package is created from the **Support package** tab of the **Service** environment.

Support package



Barcode printing

Use this option if you intend to print a barcode for output labware. Select the barcode type that fits your needs. Select the barcode printer by clicking **Select**. A dialog offers a list of printers that are available from the computer, i.e., all printers which have been configured in Windows. Select **No printer** if you do not want to use this option. Barcode printing is then not available.

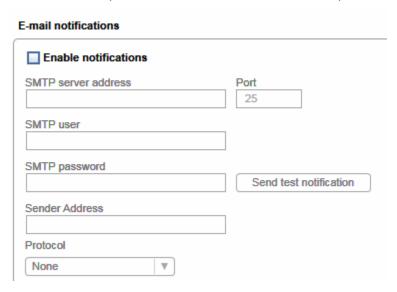
Barcode printing



E-mail notifications

If you would like to be informed via e-mail about certain instrument events, such as errors or finish of a run, activate the **Enable notifications** option. The actual notification options can be found in the **User Settings** of the individual plug-ins.

The general network settings for the mail server must to be entered in order for the e-mail notification to function. In case you do not know these details, contact your network administrator.



Option	Description
SMTP server address	Specify the address of the SMTP server.
SMTP user	Specify the user for authentication on the SMTP server.
SMTP password	Enter the users password for authentication on the SMTP server.
Sender address	Specify the address of the sender of notifications.
	Note: Some SMTP servers require that the sender address is related to the SMTP user and password.
Protocol	Specify whether the SMTP server requires encryption (SSL or TLS) or not.

Use the **Send test notification** button to test if an e-mail is sent to the e-mail address that is configured for the currently logged in QlAgility Setup Manager Software user.

5.2.1 User settings for Gene Expression experiments

The **Configuration** environment provides settings for the Gene Expression Plug-in. These settings are specific for a user.

Report / Export

Description
Check this option if the report shall open automatically after it has been created.
Note: The report will always be saved first and then opened.
Note: To view reports, a PDF reader must be installed on the computer.
The Gene Expression Plug-in provides a Basic report and an Advanced report. For details, refer to section Report.
Select the paper format: A4 or US Letter.
Select Save to origin experiment directory if you want to save the report side by side with the experiment file.
Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.
Note: This selection serves as a starting point for the file dialog that opens when you create a report.
Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.
Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.
Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiment	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C:\ .
	Initially, the path is set to $C:\Users\Public\QIAGEN\QIAgility\Experiments.$
Template	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
	Initially, the path is set to $C:\Users\Public\QIAGEN\QIAgility\Templates$.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
	Initially, the path is set to $C:\Users\Public\QIAGEN\QIAgility\Reports.$
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file after the run.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification



A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that use also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QlAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.2 User settings for Pathogen Detection experiments

The **Configuration** environment provides settings for the Pathogen Detection Plug-in. These settings are specific for a user.

Report / Export

Option	Description
Display report after saving	Check this option if the report shall open automatically after it has been created.
	Note: The report will always be saved first and then opened.
	Note: To view reports, a PDF reader must be installed on the computer.
Report type	The Pathogen Detection Plug-in provides a Basic report and an Advanced report. For details, refer to section <u>Report</u> .
Report format	Select the paper format: A4 or US Letter.
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C:\.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Experiments.
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Templates.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Reports.
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file after the run.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification



A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.3 User settings for Sample Pooling experiments

The **Configuration** environment provides settings for the Sample Pooling Plug-in. These settings are specific for a user.

Report / Export

Option	Description
Display report after	Check this option if the report shall open automatically after it has been created.
saving	Note: The report will always be saved first and then opened.
	Note: To view reports, a PDF reader must be installed on the computer.
Report format	Select the paper format: A4 or US Letter.
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.
Select xslt file	If you need the output file in another format other than the QDEF format, an <code>.xslt</code> file can be selected that defines a transformation from the QDEF format to another format. If an <code>.xslt</code> file has been selected, every time you save an output file from the <code>Run</code> screen (see <code>Run</code> completion), the transformation will be started automatically based on the created QDEF file using the selected <code>.xslt</code> file. As a result, two output files will be created: one in QDEF format, and one in the format defined by the <code>.xslt</code> file. The file created by the <code>.xslt</code> will have the same file name as the QDEF file, but with an extension <code>.csv</code> . It will be saved side by side with the QDEF file.
	As an example, the Sample Pooling Plug-in provides one .xslt file that transforms the output file into a .csv file:
	$\label{lem:c:program} $$ Files\QIAGEN\QIAgility\QIAgility\Data\KitConfiguration\PSP\PSP_SampleInformation\\File.xslt$
	Note: If any other file format needs to be generated, please contact an Application Service Specialist.

Default pipetting order

To define the default pipetting order used for sample input batches (first line) and pool batches (second line).

Default pipetting order



For regular rectangular labware, e.g., 96-well plates, you can select between **Row by Row** and **Column by Column** order. For all other labware, select between **Ascending** and **Descending** pipetting order. For sample input batches, the selected pipetting order will be used for all sample input batches. For pool batches, you can select the pipetting order that is most often used. During an experiment setup, define the pipetting order for the pool batch individually in the <u>Sample-pool mapping dialog</u>.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C: \.
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
Sample batch input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file in the Labware Selection step.
	Also, when you scan the input labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
Pool batch output files	The selected directory serves as a starting point for the file dialog that opens when you save a batch file after the run.
	For experiments created with this plug-in.
Unused samples	The selected directory serves as a starting point for the file dialog that opens when you save an unused samples file after the run.
	For experiments created with this plug-in.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification



A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.4 User settings for Generic PCR experiments

The **Configuration** environment provides settings for the Generic PCR Plug-in. These settings are specific for a user.

Report / Export

Option	Description	
Display report after saving	Check this option if the report should open automatically after creation.	
	Note: The report will always be saved first and then opened.	
	Note: To view reports, a PDF reader must be installed on the computer.	
Report type	The Generic PCR Plug-in provides a Basic report and an Advanced report. For details, refer to section Report.	
Report format	Select the paper format: A4 or US Letter.	
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.	
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.	
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.	
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.	
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.	
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.	
Select output file converter directory	The selected directory contains the files that define conversion of the sample output file from the QDEF format to another format that is required, e.g. by a PCR cycler software.	
	$Initially, the path is set to C: \Users \Public \QIAGEN \QIAgility \Output Converters.$	

Normalization

Option	Description
Normalization target	Select one of Sample mass or Sample concentration . Depending on the selection, normalization calculation will be based on mass or concentration. When normalizing to a specific concentration the sample will be diluted to the chosen target value and afterwards transferred to the output labware.
	Changing the selection will take effect for new experiments, only.
	Note: The default normalization target is Sample mass.

Sample validation

Option Description

Enable sample loading validation

Select this option if you want to validate loading of sample tubes using a handheld scanner during worktable loading. If checked, the **Validate sample loading** button will be available in the Worktable step.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

=	
Subject	Description
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start, the starting point is C:\ .
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Experiments.
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Templates.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Reports.
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file or a dilution file after the run.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification



A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run, as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.5 Generic PCR Liquid Classes

Liquid classes define the pipetting and tip handling for respective tip transfers. Liquid classes are defined by QIAGEN.

You can view information about the liquid classes in the **Configuration** environment, in the **Generic PCR Liquid Classes** tab, or you can refer to the table below.

Name	Version	Description
Aqueous liquid to reaction (master) mic	1.0.0	Example: MgCl ₂ solution - use for all reagents with low viscosity (main solvent water) added to a final reaction (master) mix
Control or Std. to output or for dilution	1.0.0	Example: Positive/Negative control or Standards - use for all assay controls or standards (incl. stock) transferred to final assay well or for creation of a standard dilution series.
		Note: In case NTC is used from the water provided in the kit, without using a separate input tube for NTC and the water, the liquid class "Water to reaction (master) mix" is recommended
Diluent for dilution	1.0.0	Example: Diluent - use for all water-based diluents for preparation of standard series or for normalization of

Diluent to dilution	1.0.0
Enzyme to reaction (master) mix	1.0.0
Mix of reaction (master) mix (final)	1.0.0
Primer-Probe or IC to reaction (master) mix	1.0.0
Reaction (master) mix (final) to output	1.0.0

samples/templates, or any kind of dilution

Example: Diluent - use for all water-based diluents which are pipetted to the dilution plate during normalization. This liquid class is specifically predefined within the advanced pipetting paramater of the **Normalization** sceen, when performing normalization requiring dilution labware

Example: DNA polymerase - use for all pure enzyme reagents (main solvent glycerol (or similar)) added to the reaction (master) mix

Example: Reaction mix created by QlAgility - use to optimize mixing of the final reaction (master) mix.

This liquid class is automatically selected within the advanced pipetting parameter of the **Mixes** screen.

In case the data reveal a non-homogenous final reaction (master) mix, the mixing repetitions can be adjusted (e.g. might be required for reaction (master) mixes requiring preparation in a 5 ml tube).

Example: Primer/Probe or internal control - use for all primer/probes or internal controls with low viscosity (main solvent water) added to the final reaction (master) mix.

Example: Final reaction (master) mix - use for distribution of final reaction (master) mix to final assay wells.

This liquid class is automatically selected in the **Mixes** screen and has been developed for reaction

Sample for dilution	1.0.0
Sample to output	1.0.0
Sample to output include premix	1.0.0

(master) mixes within a standard viscosity range.

Example: Sample/Template - use for transfer of samples/templates to dilution plate during normalization.

This liquid class is automatically selected within the advance pipetting parameter of the **Normalization** screen, if normalization requires dilution labware.

In case data reveal a nonhomogenous mixing of the sample/templates and dilution buffer, the mixing repetitions and/or maximum volume for the dilution well can be adjusted.

Example: Sample/Template - use for transfer of samples/templates to the final assay well.

This liquid class is specifically predefined in the **Sample/Labware** screen and has been developed for water-based samples/templates.

In case data reveal a nonhomogenous mixing of sample/templates and final reaction (master) mix, the mixing repetitions can be adjusted within the custom pipetting parameters.

Example: Sample/Template – use for transfer of samples/templates to final assay well.

This liquid class is specifically predefined in **Sample/Labware** screen and has been developed for water-based samples/templates.

In case data reveal a nonhomogenous mixing of

Viscous liquid to reaction (master) 1.0.0

Water to reaction (master) mix 1.0.0

sample/templates and final reaction (master) mix, the mixing repetitions (pre and post) can be adjusted within the custom pipetting parameters.

Example: 10x buffer - use for all viscous reagents containing glycerol or similar components added to the final reaction (master) mix.

Example: Nuclease-free water - use to add water to the final reaction (master) mix.

Note: This liquid class is recommended to be used for NTC, if NTC shall be served from the same water tube as used for the final reaction (master) mix preparation.

In case of any dilutions (normalization or standard series), it is recommended to define a separate dilution liquid and assign the respective "Diluent to dilution" liquid class.

5.3 Managing users

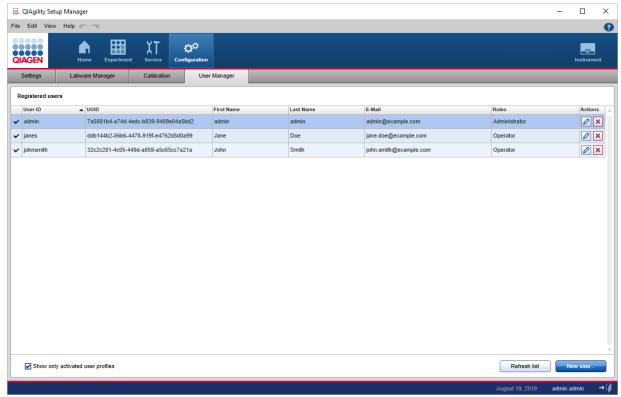
When starting the QIAgility Setup Manager Software for the first time, we recommend that you <u>change the password</u> from the default **admin**, and create user profiles to suit your needs.

Users assigned the administrator role are able to add new user profiles or activate, deactivate and modify existing user profiles. User profiles can be deleted, if necessary.

Manage users in the **User Manager** tab of the **Configuration** environment.

- 1. Click **Configuration** in the main toolbar.
- 2. Click the **User Manager** tab.

3. The **User Manager** screen is displayed.



Registered users table in the User Manager

modi

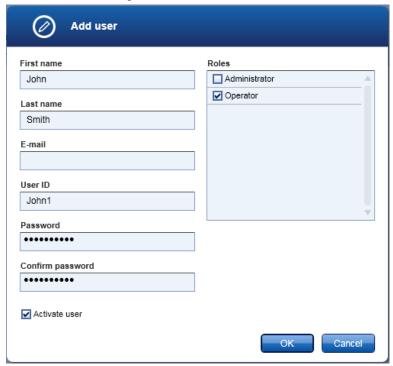
Creating a user

Note: To configure the QlAgility, a user profile with administrator role is required. To operate the QlAgility, the user role operator is sufficient. The initially provided user **admin** has the user role administrator and is sufficient to configure and operate the QlAgility. For details refer to the <u>user roles concept</u>.

Note: If an audit trail is required, create individual user profiles with user role operator for each person working with the QIAgility. Additionally, ensure that the standard password policy has been configured in the **Global Settings**. We recommend to create two user profiles with role administrator.

1. Click the **New user** button on the bottom right.

2. The **Add user** dialog is shown:



3. Enter the first name, last name, e-mail and an user ID in the corresponding fields.

Note: E-mail is mandatory for administrators, but optional for operators.

Note: The following restrictions apply to the User ID and name fields:

Field	Restriction
User ID	Must be unique for each user
	May not be empty
	May not contain more than 40 characters
	Numerical and non-numerical characters are allowed
	Must contain at least one non-numerical character
	Spaces are not allowed (in general, white spaces are not allowed)
	Once the user profile has been created, the field can no longer be changed
First/Last name	May not contain more than 50 characters
	Numerical and non-numerical characters are allowed
	May not be empty

4. Enter a password in the **Password** field, and enter it again in the **Confirm password** field.

Note: As long as the entered password does not meet the configured <u>password policy</u>, the field will remain yellow and a hint will be shown in a tooltip. The **Confirm password field** will remain yellow if it differs from the **Password** field.

5. The **Activate user** check box (Activate user) is checked by default. This means that the created user profile may be used for the next login.

Note: To prepare a user profile for later use, create a deactivated user profile by removing the check from this check box. To activate it later, follow the steps below for activating/deactivating a user.

6. Activate the check box in the **Roles** table that should be assigned to the user.

Note: At least one role must be assigned.

- 7. Click OK.
- 8. The Add user dialog closes and the new user is added to the Registered users table.

Modifying a user

The data, password and roles of a user can be modified using the **Edit user** icon in the **Actions** row of the **Registered users** table.



- Open the User Management screen by clicking Configuration in the main toolbar and then clicking the User Manager tab.
- 2. Click the **Edit user** icon (a) in the **Actions** row for the user you want to change.

3. The **Edit User** dialog is opened:



4. Modify the user data as needed.

Note: The **User ID** can no longer be modified.

Note: The password fields show 8 bullets and not the actual password. If you click in the password field, both fields will become empty and you will need to enter and confirm a new password that meets the configured password policy.

In case you clicked in the password field and do not want to change the password, click Cancel.

- 5. Click OK.
- 6. The changes to the user's data are saved.

Note: If you change your own user account, you will need to log out and log back in again for the changes to take effect.

Deleting a user

This will permanently delete a user. As an alternative to the permanent deletion, you can temporarily deactivate a user.

1. Open the **User Management** screen by clicking **Configuration** in the main toolbar and then clicking the **User Manager** tab.

2. Click the **Delete user** icon in the **Actions** row for the user you want to delete.



3. The following confirmation dialog is opened:



4. Click **OK** to permanently delete the user.

Activating/deactivating a user

A user profile can temporarily be deactivated and be activated later.

- 1. Open the **User Management** screen by clicking **Configuration** in the main toolbar and then clicking the **User Manager** tab.
- 2. Click the **Edit user** icon (a) in the **Actions** row for the user you want to change.
- 3. The **Edit User** dialog is opened.
- 4. Use the **Activate user** check box (✓ Activate user) to activate or deactivate a user:

To activate a user: Add a check to the check box

To deactivate a user: Remove the check from the check box

□

- 5. Click **OK**.
- 6. The user will be activated or deactivated according to your changes.

There is an option on the **User Manager** screen to show only activated user profiles. Add or remove the check from the check box to activate or deactivate the option, respectively:

Show only activated user profiles

Note: If you deactivate a user profile while this option is activated, the user profile will not be listed in the **Registered users** table. To show the deactivated user, you must first remove the check from this box to deactivate this option.

5.3.1 Changing password

User passwords may be changed by following the steps below. Each user may change his or her own password.

- 1. From the **File** menu select **Change password**.
- 2. The **Change password** dialogs opens.



Change password dialog

- 3. Enter the current password into the **Old password** field.
- 4. Enter a new password into the **New password** field, and enter it again in the **Confirm new password** field.

Note: As long as the entered password does not meet the configured <u>password policy</u>, the **New password** field will remain yellow and a hint will be shown in a tooltip. The **Confirm new password** field will remain yellow if the contents differ from the **New Password** field.

Note: The **No policy** option allows users to set and save an empty password. To set an empty password, leave the **New password** and **Confirm new password** fields empty. However, if the **New password** field remains yellow, this indicates that the **Standard policy** is configured and an empty password is not allowed.

5. Click **OK** to save the new password.

Use the new password for the next login.

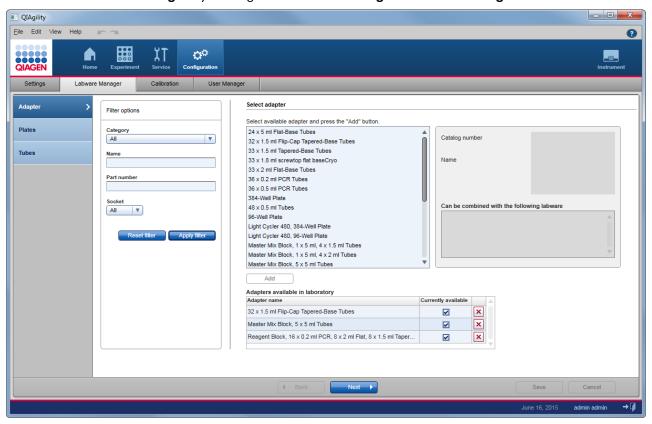
If an empty password has been set, leave the **Password** field empty during login.

5.4 Managing labware

The QIAgility Setup Manager Software contains a labware library that includes all labware supported by the QIAgility.

During run setup, the software automatically plans the worktable for the run. As a basis for this plan, you must first indicate which labware (e.g., adapters, plates or tubes) is available in your laboratory. This information is managed in the **Labware Manager**.

Access the Labware Manager by clicking the Labware Manager tab in the Configuration environment.



This screen contains a step marker with three steps (**Adapter**, **Plates**, **Tubes**) on the left. The software distinguishes between the different labware types, because they must be treated differently during worktable planning:

• Adapter: An adapter is placed on the worktable in order to hold other labware such as tubes or plates.

- Plates: For example, 96-well plates, 48-well plates or Rotor-Discs for the Rotor-Gene Q. Some plates
 can be placed directly on a worktable position, while others require an adapter. Plates usually occupy an
 entire worktable position, while a Rotor-Disc occupies 2 positions.
- Tubes: Single tubes or tube strips. Tubes always need an adapter to be placed on the worktable.

The Filter options area in the middle is used to search for a specific labware.

The **Select adapter**, **Select plates**, or **Select tubes** area on the right shows the search results together with a picture and further details.

Use the **Add** button to confirm and add the selected labware to the **Available in laboratory** list. The **Next** and **Back** buttons in the button bar allow you to advance or move backwards one screen.

Defining a specific labware as available

- 1. Click **Configuration** to change to the **Configuration** screen.
- 2. Click the Labware Manager tab.
- 3. The Labware Manager screen will be displayed, and the Adapter step will be active.
- 4. Use the options in the **Filter options** area to filter for a specific adapter.

The available filter options are:

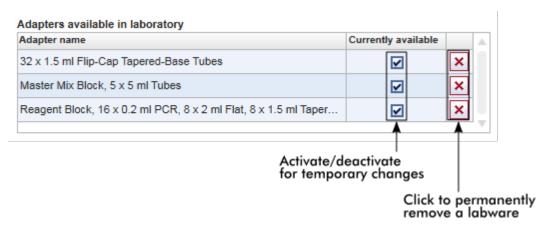
Option	Description
Category	Labware category, such as standard 96-well, Rotor-Gene Q, etc.
Name	Labware name
Part number	Catalog number of the labware
Socket (available in Adapter step only)	Labware suitable for a specific position on the worktable
Manufacturer (not available in Adapter step)	List of labware manufacturers
Tube/Well size (not available in Adapter step)	Tube/well size range from < 0.5 ml to > 2 ml

- 5. Click **Apply filter** to commit your filter query.
- 6. All adapters matching your query are listed in the **Select adapter** area.
- 7. Click on the desired adapter and then click **Add** to add it to your available in laboratory list.
- 8. The selected adapter is added to the **Adapters available in laboratory** list at the bottom.
- 9. Click **Save** to save the list of available adapters.
- Repeat steps 4–9 for the **Plates** and the **Tubes** steps.
 Either click on the desired step in the step marker or click **Next** in the button bar.

Important: Tubes or plates supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). Therefore, it is important to select the correct plates and tubes. We recommend that once a particular tube type is chosen, to always purchase that consumable from the same manufacturer to avoid frequent recalibration.

Removing labware permanently or temporarily

Use the interactive buttons in the **Adapters available in laboratory** table to temporarily or permanently remove a labware. Use the temporary deactivation if a labware is out of stock but will be available later. Remove it permanently if you will not be using this labware in the future. If you permanently remove a labware and then change your mind, you can add the labware again.



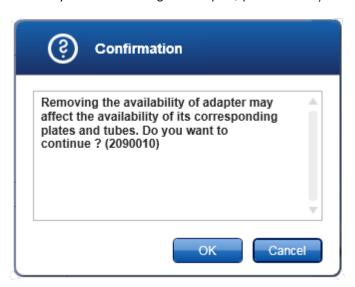
To **temporarily** remove a labware

- 1. Deactivate the check box in the **Currently available** column.
- 2. The icon changes from \square to \square .
- 3. The selected labware will be deactivated and will no longer be available in the **Experiment** environment.

To reactivate the labware, simply activate the check box again.

To **permanently** remove a labware

- 1. Click the **Remove labware** icon (X) in the column of the labware you want to remove.
- 2. A confirmation dialog will be shown (the text may vary depending whether you are removing an adapter, plate or tube):



3. Click **OK** to permanently remove the selected labware.

Before the labware can be used, instrument calibration is required.

5.5 Instrument calibration

QlAgility is a high-tech precision instrument, which is able to perform movements in the micrometer range. An instrument calibration must be performed before the QlAgility can be used, to ensure absolute precision positioning and pipetting. No runs can be started if the required calibrations have not been performed.

Calibration includes:

• Volume calibration

The actual volume calibration is performed at the manufacturer's site and only requires that the user enters the measured calibration data.

Volume calibration defines the number of units that the P-axis motor must move to accurately deliver a given volume of liquid.

X-Y and Z-axis calibration

The X-Y and Z-axis calibration must be performed at the user's site and is required for the tip ejector, the tip racks and all labware used on the QIAgility.

X- and Y- axis calibration defines the distinct position of a labware on the worktable to ensure proper operation of the instrument. Z-axis calibration defines the worktable height of each labware to maximize precision of liquid transfers and to ensure optimal conditions for liquid-level detection. Before using any items on the worktable, X-Y and Z-axis calibration must be performed for each labware to be used on each worktable position.

The lid interlock is disabled during calibration. The robotic arm will move during calibration when the lid is raised.

WARNING

Risk of personal injury [W7]



The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

WARNING

Moving parts [W12]



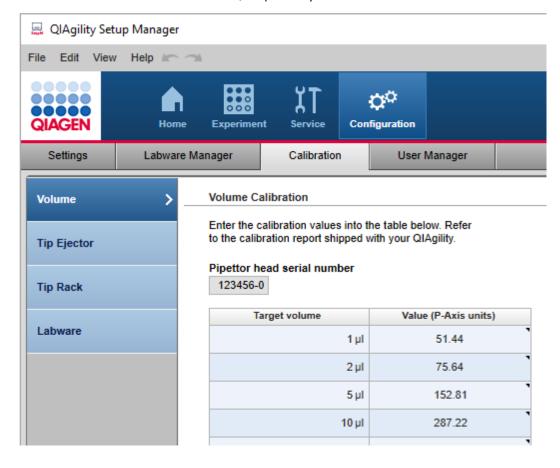
Avoid contact with moving parts during operation of the QlAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.

Important: Clicking on **Perform calibration** or a positioning button will immediately move the robotic arm in the selected direction for a specific movement step.

To ensure your personal safety:

- Always stand clear of the instrument when starting calibration or making adjustments.
- Do not click any of the software buttons with the mouse or press any of the keys on the keyboard while
 parts of your body are within the instrument worktable or when looking closely at calibration positions.
- Wait until the robotic arm has completed its movements before moving any plates or tubes.

The calibration tasks are bundled in the **Calibration** tab of the **Configuration** environment. All calibration steps are built in a wizard-style format, that allows you to move forward or backward one screen using the **Next** or **Back** button in the button bar, respectively.



The calibration comprises the following steps:

Calibration step

Volume

Tip Ejector

Tip Rack

Labware

Description

Enter the volume calibration data that was shipped with the instrument in the calibration report. No further interaction is required. See Calibrate volumes.

Perform, and if necessary, correct and test the calibration of the tip ejector. Manual adjustment of XY-axis calibration is possible if necessary. Z-Axis calibration is performed automatically without user interaction. See <u>Setup tip ejector</u>.

Perform and manually correct the XY-axis calibration of the tip rack. See <u>Calibrate tip racks</u>.

Perform X-Y and Z-axis calibration for all labware that will be used on the QlAgility. See Calibrate labware.

Select the labware, the corresponding adapter(s) and/or cooling block and the worktable position for which the labware should be calibrated, and add to a calibration work list.

Based on the work list, the labware calibration wizard will guide you through all XY-axis calibrations, followed by the Z-axis calibrations for each selected labware.

Manually correct the XY-axis calibration for each XY-axis calibration step.

It is strongly recommended to perform the Z-axis calibration automatically whenever possible. However, the software also provides the possibility to perform Z-axis calibration manually.

Note: QIAGEN recommends that you follow the steps from top to bottom starting with the **Volume** calibration and finishing with the **Labware** calibration step. For the first calibration, this order is required, and the next step will only be available after the previous step has been completed.

Note: If the QIAgility is moved, we recommend recalibration, starting from step **Tip Ejector**. Click on the **Tip Ejector** step to start there.

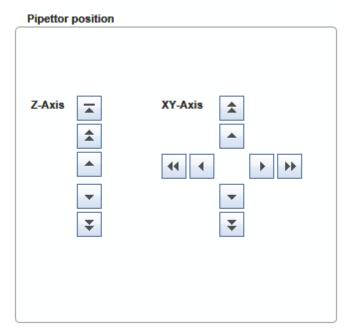
Note: After servicing the pipettor head, recalibration is required, starting from step **Volume**.

Note: Calibration data can be saved and restored for backup purposes using the **Export calibration data** and **Import** buttons at the bottom of the screen. For details, see <u>Saving and restoring calibration data</u>.

5.5.1 Calibrate positioning buttons

For manual corrections to calibration of positions, the software provides several positioning buttons.

The positioning buttons are arranged in two groups, one for the Z-axis and one for the XY-axis.



The following table shows the movement steps for the positioning buttons:

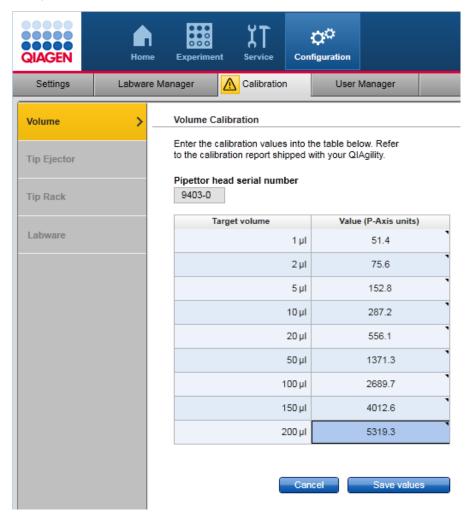
Z-axis XY-axis

Button	Movement	Button	Movement
_	Moves up to the highest possible position (Z-home)	4 →	Slow left / slow right
	Slow up	44 >>	Fast left / fast right
	Slow down		Slow forward
*	Fast up	-	Slow backward
*	Fast down		Fast forward
		*	Fast backward

5.5.2 Calibrate volumes

Volume calibration defines the number of units that the P-axis motor must move to accurately deliver a given volume of liquid.

The QIAgility is shipped with a calibration report. The data in this calibration report have been measured and adjusted by the manufacturer individually for each individual pipettor head to ensure accuracy of volumes. The data in the calibration report must be entered in the software before the QIAgility can be used.



Follow these steps to enter the volume calibration data:

1. Click on Configuration to change to the Configuration screen.

2. Click on the **Calibration** tab.

Note: If the instrument is not connected, there will be a warning sign on the **Calibration** tab. When you click it, a message will be displayed that the instrument needs to be connected first. The volume step will be displayed without the pipettor head serial number and with the column **Value (P-axis units)** empty. No calibration functionality will be available, because all calibration information is related to an individual pipettor head. In this case, connect the instrument and relaunch the software.

- 3. The **Volume** step is displayed by default.
- 4. Enter the pipettor head serial number from the calibration report into the **Pipettor head serial number** field. Ensure that the calibration report shows the same serial number.
- 5. For the first target volume entry in the table (1 μ l) enter the corresponding P-axis value from the calibration report to the **Value** column.

Note: If no volume calibration data has been saved yet for this pipettor head, the cells of the **Value** column are empty. They are displayed in yellow to indicate that a value is mandatory.

- 6. Repeat the previous step for all volumes from $2 \mu l$ to $200 \mu l$.
- 7. Click **Save values** to save the data or click **Cancel** to cancel the volume data input and revert any values to their previous values.

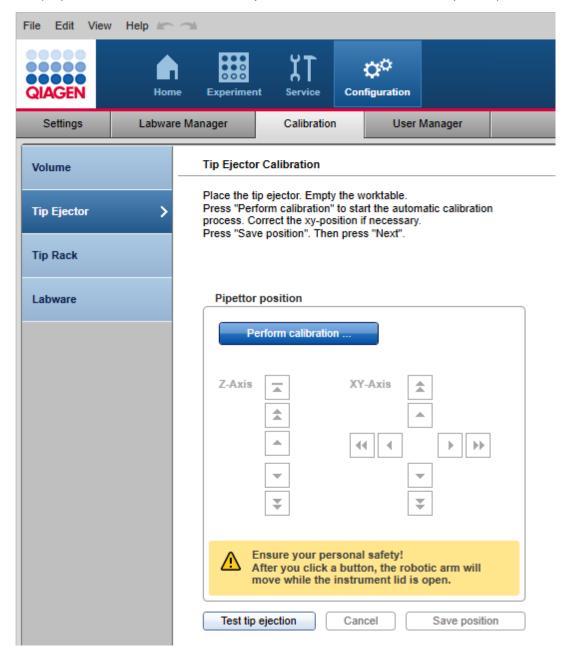
Click the **Next** button to continue with **Tip Ejector** calibration.

Note: The **Next** button is available only if volume calibration data for the current pipettor head have been saved.

5.5.3 Setup tip ejector

The **Tip Ejector** step allows the instrument to determine the location of the tip ejector. The location of the tip ejector must be calibrated to ensure proper operation of the instrument. This must be done carefully to avoid damaging the instrument.





The lid interlock is disabled during calibration. The robotic arm will move during calibration when the lid is raised.

WARNING



Risk of personal injury [W7]

The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

WARNING



Moving parts [W12]

Avoid contact with moving parts during operation of the QIAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.

Important: Clicking on **Perform calibration** or a positioning button will immediately move the robotic arm in the selected direction for a specific movement step.

To ensure your personal safety:

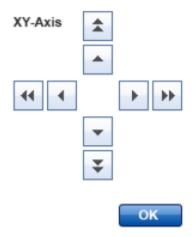
- Always stand clear of the instrument when starting calibration or making adjustments.
- Do not click any of the software buttons with the mouse or press any of the keys on the keyboard while parts of your body are within the instrument worktable or when looking closely at calibration positions.
- Wait until the robotic arm has completed its movements before moving any plates or tubes.

Follow these steps to calibrate the tip ejector:

- Install the tip ejector. For details, refer to <u>Tip ejector</u>.
 Ensure that the tip ejector is securely mounted to the worktable. During the process, the body of the pipettor head is touched against the tip ejector.
- 2. Install the tip disposal box. For details, refer to Tip disposal box.
- 3. Ensure that the pipettor head is not holding any tips.
- 4. Empty the worktable.
- 5. Keep the lid open during the calibration procedure.
- 6. Click **Perform calibration** to start the automatic calibration process. The head starts to move and automatically locates the tip location.
- 7. The message dialog opens asking whether the pipettor hub is above the ejector slot. Carefully visually check the positioning on the instrument (from both the front and the side).

8. Click **Yes** if the positioning is correct. In this case, the XY-axis calibration of the tip ejector will be automatically saved, and the Z-axis calibration will start immediately.

Or click **No** to manually adjust the XY positioning. In this case, the manual XY-axis positioning buttons will be enabled. Use the buttons to position the pipettor head directly centered above the tip ejector. To confirm and save the XY-axis calibration, click the **OK** button on the bottom right of the positioning button. The Z-axis calibration will start immediately.



For details about the buttons, refer to <u>Calibration positioning buttons</u>.

- 9. The Z-axis calibration starts automatically. The pipettor head moves up and down and stops when the calibration is finished. No user interaction is needed for the Z-axis calibration. The **Save Position** button will be enabled after the Z-axis calibration has finished.
- 10. Click Save Position to save the tip ejector calibration. The calibration information is saved.
- 11. We recommend testing whether the calibration was successful by clicking **Test tip ejection** (see procedure below). Afterwards, continue with the next calibration step (Tip rack) either by clicking **Next** in the button bar or **Tip Rack** in the step marker pane.

Testing the tip ejector calibration

The following procedure starts a test to determine whether the calibration of the tip ejector was successful. During the test, the pipettor head will move.

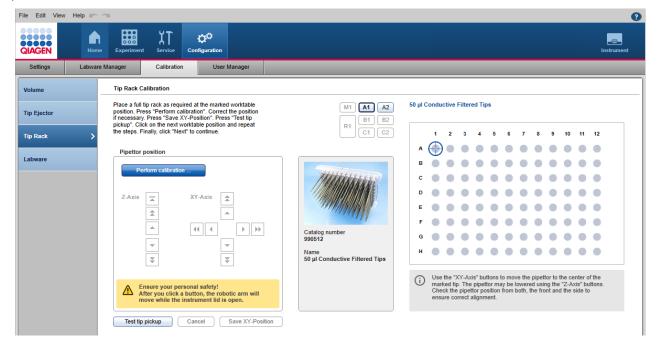
- 1. Click the **Test tip ejection** button to start the test.
- 2. A confirmation dialog opens. When you click **OK**, the pipettor head starts to move immediately to the right to its home position.
- 3. Another dialog opens requesting you to place a tip onto the pipettor head. Manually place a tip onto the pipettor head and click **OK**. The pipettor head starts to move immediately.
- 4. The pipettor head moves to the tip ejector and ejects the tip.

 If the tip is not correctly ejected, there might be an error with the calibration. Repeat the tip ejector calibration as described above.

5.5.4 Calibrate tip racks

The tip rack calibration is essential to ensure a reliable pickup of tips.

Tip rack calibration is available only if the volume and tip ejector calibrations have already been performed.



Proceed as follows:

- 1. Carefully place a full tip rack of 50 μ l tips in worktable position A1. It is important to follow the instructions in <u>Insert tips to tip rack holder</u> for details about how to place tips.
- 2. Carefully place a full tip rack of 200 μ l tips in worktable position A2.
- 3. If not activated, click A1 from the worktable selector:

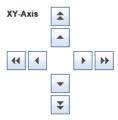


- 4. Keep the lid open during the calibration procedure.
- 5. Click **Perform calibration** to start the calibration. The pipettor head starts to move and positions itself above position A1 of the rack in worktable position A1.

6. Carefully inspect whether the pipettor head is positioned over the center of the tip in position A1. Look from the front and from the side. In case you have difficulties seeing the position, use the buttons of the Z-axis position control to lift and lower the pipettor head.

Note: If you still have difficulties seeing the position, you can click **Cancel** just below the pipettor position control. Remove the tip from A1 and any tips around it. Then click **Perform calibration** and calibrate into the A1 position of the tip rack, instead. Replace the tips after step 9.

7. Adjust the XY-positioning manually. Use the buttons of the XY-axis control to position the pipettor head directly centered just above the calibration position. For details about the buttons, refer to <u>Calibration positioning buttons</u>.



- 8. Click Save XY-Position to save the calibration data for tips at worktable position A1.
- 9. A message pops up indicating that the pipettor will move to the home position. Confirm and stand clear of the instrument while the pipettor is moving.
- 10. To test if the calibration was successful, click **Test tip pickup** (see below).
- 11. Click A2 from the worktable selector and repeat steps 4 to 9 for the tip rack at worktable position A2.

Note: Tip rack calibration on A1 and A2 is the minimum requirement. We recommend that you also calibrate $50 \,\mu$ l tip racks on positions B1 and B2 in the later <u>Labware calibration</u> step. This is because the software will plan additional tip racks on these positions, if possible, to support completely using previously started tip racks and minimizing tip reloading during the run.

Testing the tip rack calibration

The following procedure starts a test to determine whether the calibration of the tip rack was successful. During the test, the pipettor head moves to the tip rack, picks up a tip and drops it at the tip ejector.

- 1. If not activated, click the worktable position you want to test: A1 or A2.
- Click the **Test tip pickup** button to start the test.
 Note: The button is active only if a calibration has been already performed on that worktable position.
- 3. The pipettor head moves to the tip ejector, ejects the attached tip and picks up the next available tip from the tip rack at the selected worktable position.
- 4. Confirm the message with **OK**. If the tip pickup failed, repeat the tip rack calibration for the selected worktable position. If the instrument picked up the tip successfully, continue with the next calibration.

5.5.5 Calibrate labware

All labware and adapters must be calibrated before use, to ensure that the pipettor knows their exact positions on the worktable. In addition, every labware, adapter and worktable position combination must be calibrated, because the same labware with different adapters at different positions has different coordinates. For example, the QIAGEN Elution Microtubes RS labware used in both positions B1 and B2 must be calibrated twice: once in position B1 and a second time in position B2. Also, different adapters of the same type may show calibration variances. Therefore, be sure to always calibrate the adapter that will be used.

Labware calibration is performed in the software using a convenient wizard, which guides users through all necessary steps for the set of labware to be calibrated.

The first step of labware calibration is to select the set of labware (or labware/adapter combinations), and the worktable positions on which the labware will be calibrated. This information is collected in the work list. See <u>Select labware to calibrate</u>. The calibration wizard will then guide the user through the following calibration steps.

The second step is the preparation of the worktable, i.e., placing required tips. See <u>Prepare worktable for labware calibration</u>.

In several steps, all labware and labware/adapter combinations will be XY-axis calibrated on the worktable positions as defined in the work list, one step for each labware or labware/adapter combination in a specific worktable position. The user will correct the XY-positioning manually and save the calibration data for each step, before continuing with the next step. See <u>Calibrate X- and Y-axis</u>.

Afterwards, in several steps, all labware and labware/adapter combinations will be Z-axis calibrated, one step for each labware and labware/adapter combination in the work list. For most labware, the software provides an automatic Z-axis calibration function, which should be used whenever possible. However, it is also possible to decide on each step individually to calibrate the Z-axis manually. Calibration data needs to be saved for each step, before continuing with the next step. See <u>Calibrate Z-axis</u>.

Note: Make sure to calibrate each individual adapter that is used with the QlAgility, even if you have already calibrated an adapter of the same type. When performing experiments, only use the exact adapter that was calibrated.

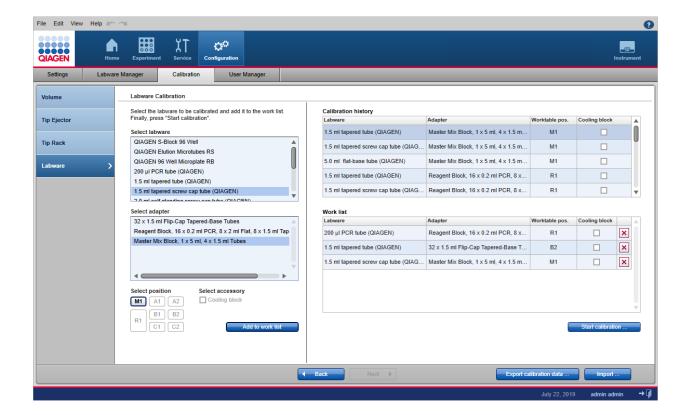
5.5.5.1 Select labware to calibrate

The first step of labware calibration is to select a set of labware along with the adapters and worktable positions on which the labware will be calibrated in the work list. Initially, the work list is empty.

The labware calibration step will only be available if all previous calibration steps have been performed.

All labware that has already been calibrated for the currently connected instrument will be listed in the **Calibration history** table. Initially, the table is empty.

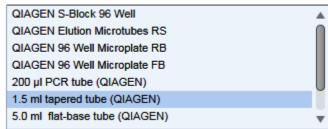
Note: The calibration history will also be empty after the pipettor head has been changed, because calibration data are individual for an instrument and a pipettor head.



Follow these steps to define the list of labware that should be calibrated:

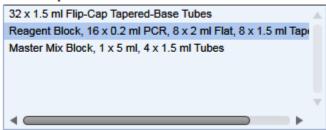
1. Select the desired labware from the **Select labware** list. Only one item can be selected.

Select labware



2. If the selected labware can be used with adapters, select the desired adapter from the **Select adapter** list. Only one item can be selected.

Select adapter



3. Select the worktable position in which the selected labware will be used. Only one position can be selected.

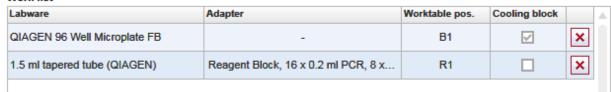


4. **Optional step:** if you intend to use a cooling block for the labware on the selected worktable position, select the **Cooling block** option.

Note: The cooling block check box will only be available if the currently selected labware or labware/adapter combination can be used with a cooling block.

5. Click **Add to work list** to save the selected combination of labware, adapter (optional), position and cooling block (optional) to the work list. The combination is added to the the **Work list** table:

Work list



The work list above shows for example that the first labware "QIAGEN 96 Well Microplate FB" has no alternative adapters to be selected and is calibrated with a cooling block. The last entry is calibrated with a specific adapter on R1 without cooling block.

- 6. Repeat steps 1–5 for all labware, adapter and worktable position combinations to be calibrated together in a session.
- 7. Finally, click **Start calibration** to start the calibration wizard. Based on the work list, the wizard creates the necessary XY-axis and Z-axis calibrations.

After all calibration steps have been performed, all items from the work list will appear in the calibration history table and the work list will be empty.

Note: If the calibration wizard is canceled, the work list will still contain the items that have not been completely calibrated.

Note: A labware item is correctly calibrated only if both XY- and Z-calibration have been performed. When creating the work list, take into account the time required for a complete calibration. Sometimes it is better to create a smaller work list and to calibrate the labware in subsets.

It is not necessary to calibrate every labware on every worktable position. As a starting point, use the following recommendations as a guide (for details, refer to the kit handbooks):

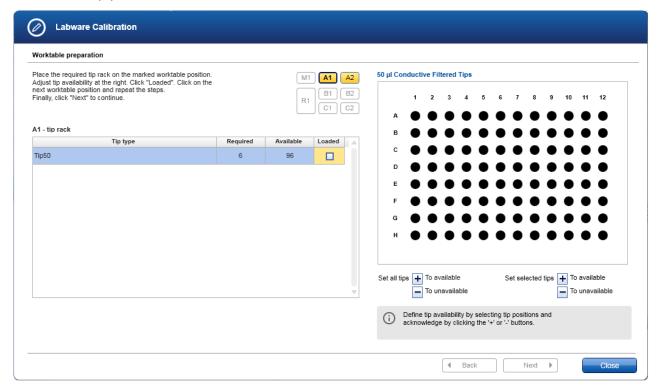
- Calibrate labware that will contain the samples (input) on worktable position C1. In case you intend to use Rotor-Gene Q adapters for output, calibrate the input labware on worktable position C2 instead.
- Calibrate labware that is intended for output on worktable position C2. However, Rotor-Gene Q adapters must be calibrated on B1/C1.
- In addition to the tip rack calibration, calibrate 50 μl tips and 200 μl tips on B1, B2, C1 and C2. The software will plan additional tip racks on these positions, if possible, to support completely using previously started tip racks and minimizing tip reloading during the run. If you plan to do sample pooling experiments, you will need to additionally calibrate 50 μl tips on A2 and 200 μl tips on A1.

Note: It is not possible to combine tip racks and other labware in one work list.

• Calibrate the labware provided with the QIAgility and the tubes provided with the QIAgility Calibration Kit on worktable positions M1 and R1, because this labware is used for QIAGEN Kits.

5.5.5.2 Prepare worktable for labware calibration

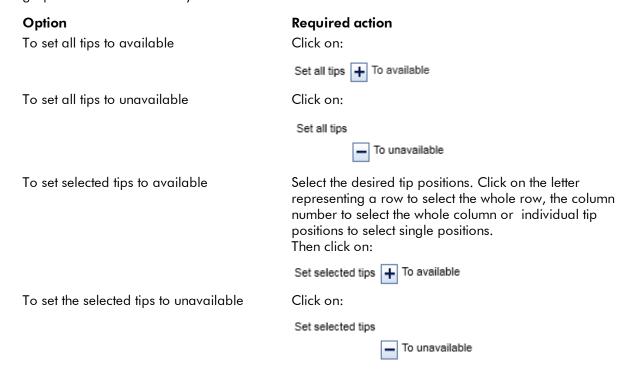
The worktable must be prepared before the labware calibration can be started. The calibration requires 50 μ l and 200 μ l tips. The required number of each type of tips will be displayed in the **Required** column of the **Worktable preparation** dialog. Use the tip availability buttons at the bottom right of the dialog to define the available tip positions.



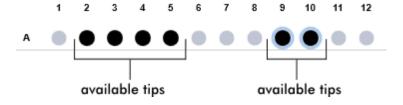
Preparing the worktable

1. Place the required tip rack containing a sufficient number of tips in worktable position A1. The dialog displays the required number of $50 \,\mu$ l and $200 \,\mu$ l tips in the **Required** column of the table. Initially, the buttons A1 and A2 are displayed in yellow to indicate that the number of available tips, initially set to zero, is insufficient and that the loaded state has not yet been confirmed.

2. A visualization of the tip rack is displayed on the right. Use the tip availability buttons below the rack graphic to set the availability of the rack.

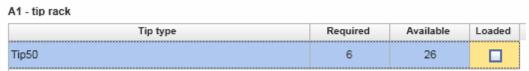


Available tip positions are displayed in dark color (A2–5, A9–10) and unavailable positions are displayed in gray (A1, A6–8, A11–12). Selected tips are surrounded by a light blue circle (A9–10):



Note: Ensure that the visualization on the screen is identical to the actual tip loading of the tip rack on the worktable.

Click the Loaded check box in the last column of the tip rack table on the left to confirm that you have loaded the tip rack onto the worktable. The color changes from yellow to blue:



4. Click **A2** in the worktable position:

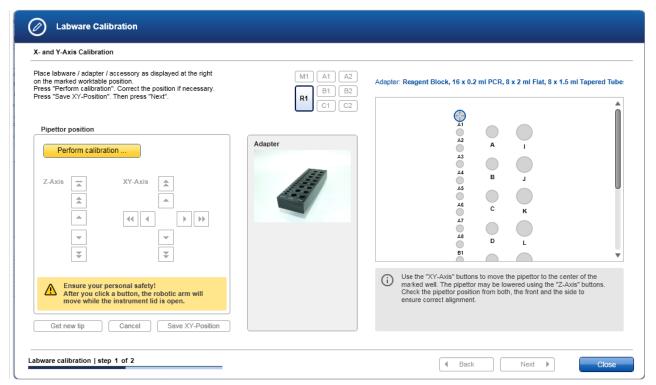


- 5. Place the required tip rack into worktable position A2.
- 6. Repeat steps 2–3 for the tip rack at position A2.
- 7. Once you have placed a sufficient number of available tips into both positions A1 and A2, and have confirmed the **Loaded** state for both tip racks, the **Next** button will be activated.
- 8. Click **Next** to continue to the next step, the XY-axis calibration of the labware.

5.5.5.3 Calibrate X- and Y-axis

Each individual XY-axis calibration step is provided in a separate screen.

The calibration <u>positioning buttons</u> are displayed on the left side of the window, along with advice and notifications regarding the calibration. An image of the labware or labware/adapter combination to be calibrated in this step is displayed in the center. The name of the labware or names of the labware/adapter combination is displayed on the right side. The graphic on the right shows a schematic of the labware with the position used for the calibration marked with crosshairs. A progress bar is displayed at the bottom left indicating the number of calibration steps remaining for the actual work list.



XY-Axis calibration of the reagent block adapter. Position A1 will be used for calibration.

To perform XY-axis calibration for one labware item, follow the steps below:

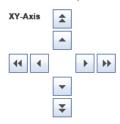
1. Place the labware, adapter or accessory listed at the top right onto the worktable position indicated in the worktable indicator:



- 2. Click **Perform calibration** to start the XY-axis calibration.
- 3. The pipettor head starts moving and positions itself above the dedicated calibration position (marked with crosshairs in the schematic).

Note: If a tip rack is calibrated, any attached tip will be ejected from the pipettor head before it moves to the calibration position.

- 4. **Optional step:** If no tip is attached to the pipettor head, click **Get new tip**. The pipettor head picks up the next 200 μ l tip and returns to the dedicated calibration position.
- 5. Carefully inspect whether the pipettor head is positioned in the center of the marked position. Look from the front and from the side. In case you have difficulties seeing the positioning, use the buttons of the Z-axis position control to lift and lower the pipettor head. Adjust the XY-positioning manually. Use the buttons of the XY-axis control to position the pipettor head directly centered above the calibration position. For details about the buttons, refer to Calibration positioning buttons.



- 6. Click **Save XY-position** to save the XY-calibration position. A message will be displayed, and the pipettor head will move to the home position, while keeping an attached tip. Alternatively, click **Cancel** and repeat from step 2 on.
- 7. Click **Next** to move forward to the next calibration step.

Note: The Next button will only be activated after the Save XY-position button has been clicked.

Note: If the work list contains remaining XY-calibrations for any labware, the next step will be the XY-calibration for the next labware item. The first Z-calibration step will only be displayed once all XY-calibration steps for the work list have been performed.

You can return to the previous calibration step by clicking the **Back** button. From the first calibration step, this will lead back to the initial screen of the labware calibration (work list).

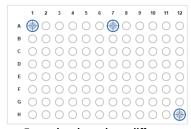
You can stop the labware calibration by clicking the **Close** button on the bottom right. In this case, none of the labware items from the work list are completely calibrated, because all Z-calibrations must still be

performed. The labware calibration wizard will be closed and the initial labware calibration screen will be shown. All labware items will remain in the work list, allowing you to start calibration again at a later time.

5.5.5.4 Calibrate Z-axis

Each individual Z-axis calibration step is provided in a separate screen. The first Z-calibration step will only be displayed after all of the XY-calibration steps for the work list have been performed.

The screen is very similar to the XY-calibration screen. The graphic on the right shows a schematic of the labware with the positions used for the calibration marked with crosshairs.



Example where three different positions are used for z-axis calibration.

To perform Z-axis calibration for one labware item, follow the steps below:

 Place the labware, adapter or accessory listed at the top right onto the worktable position indicated in the worktable indicator.

For tubes, ensure that tubes are placed in all positions marked with crosshairs in the schematic. Use the scroll bars of the schematic to scroll down, in case some labware positions are not visible.



 You can either perform an automatic calibration by leaving the check box next to **Automatic** activated or make a manual calibration by deactivating the option. We recommend using the automatic calibration option whenever possible.



Automatic variant selected.

- 3. Click the **Perform calibration** ... button. The pipettor head ejects the tip, picks up a new 50 μ l tip and moves to the calibration position.
- 4. The pipettor head automatically moves several times down to the bottom of the labware using several tips. The pipettor head repeats this procedure for all calibration positions marked with crosshairs.
- 5. Click **Save Z-position** to save the Z-axis calibration data.

 Click **Next** to move forward to the next Z-calibration step, or click **Close** if this was the last calibration step. To determine if this was the last calibration step, look at the progress bar in the lower left of the window.

Note: The **Next** button will only be activated after clicking the **Save Z-position** button, if there is a following Z-calibration step. The **Next** button is not activated after the last step.

You can return to the previous calibration step by clicking the **Back** button. From the first calibration step, this will lead back to the initial screen of the labware calibration (work list).

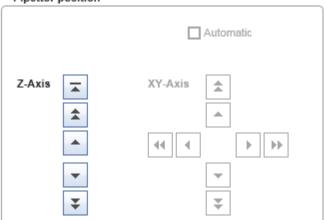
You can stop the labware calibration by clicking the **Close** button at the bottom right. Only the labware items from the work list for which both the XY- and Z-calibrations have been performed are completely calibrated. The labware calibration wizard will be closed and the initial labware calibration screen will be shown. All labware items which have not been completely calibrated will remain in the work list, allowing you to start calibration again at a later time.

Manual calibration

Note: We recommend using the automatic calibration option whenever possible.

- 1. Click the **Perform calibration** ... button. The pipettor head ejects the tip and picks up a new 50 μ l tip.
- 2. The pipettor head moves to the calibration position. There is only one calibration position for the manual calibration procedure. The Z-axis positioning buttons become activated.
- 3. Click **Get new tip**. The pipettor head picks up the next 50 μ l tip and returns to the calibration position.
- 4. Use the Z-axis positioning buttons to carefully lower the tip so that it is close to the base of the tube or well (approximately 0.5 mm above tube or well base). The distance may be checked by carefully lifting the well from its support. In case the tip is damaged, click **Get new tip** and repeat to ensure a good calibration result.

Pipettor position



5. Click **Save Z-Position** to save the Z-axis calibration data. Alternatively, click **Cancel** and repeat from step 3 on.

6. Click **Next** to move forward to the next Z-calibration step, or click **Close** if this was the last calibration step. To determine if this was the last calibration step, look at the progress bar in the lower left of the window.

Note: The **Next** button will only be activated after clicking the **Save Z-position** button, if there is a following Z-calibration step. The **Next** button is not activated after the last step.

5.5.6 Saving and importing calibration data

The QIAgility Setup Manager Software includes a function to save and restore the calibration data. Use this function if for any reason another computer will be used to control the QIAgility, or if the QIAgility Setup Manager Software must be reinstalled.

Calibration data files have the file extension *.xcd.

Saving calibration data

- 1. Click the **Export calibration data** button in the button bar.
- 2. The file dialog is shown.
- 3. Select the target directory and enter a file name.
- 4. Optional step: Select the desired information to be saved by activating the corresponding check boxes:



Option	Description
Volume calibration	Include volume calibration data.
Instrument calibration	Include general instrument calibration data (e.g., for tip ejector, tip rack)
	Note: Instrument calibration data can only be saved together with volume calibration data.
Labware calibration	Include labware calibration data.
	Note: Labware calibration data can only be saved together with volume and general instrument calibration data.

Note: If you intend to change the laptop that controls the QIAgility, or if the QIAgility Setup Manager Software needs to be reinstalled, check all options.

Click **OK** to confirm and close the file dialog.

6. The calibration data will be saved under the provided file name with the file extension .xcd.

Importing calibration data

To import calibration data that was previously saved on another laptop for the currently connected QIAgility, proceed as follows.

Note: The import function will only be available if volume calibration has already been performed. This ensures that only calibration data that match the current instrument and pipettor head are imported.

- 1. Click on **Import** in the button bar.
- 2. The file dialog is shown.
- 3. Navigate to the directory containing the calibration data file and select it.
- 4. From the options area of the file dialog, select the calibration data you want to import by activating the corresponding check boxes.

Options ✓ Volume calibration ✓ Instrument calibration (Tip Ejector, Tip Rack) ✓ Labware calibration

Option	Description
Volume calibration	Include volume calibration data
Instrument calibration	Include general instrument calibration data (e.g., for tip ejector, tip rack)
	Note : Instrument calibration data can only be imported together with volume calibration data.
Labware calibration	Include labware calibration data.
	Note : Labware calibration data can only be imported together with volume and general instrument calibration data.
	Important : If you have already calibrated labware using this laptop before importing, use this option cautiously. Importing the labware calibration data may result in a situation in which data for labware items that are not part of the file will be kept, and calibration data for labware items that are part of the file will be replaced.

Note: If you changed the laptop that controls the QlAgility, or if the QlAgility Setup Manager Software has been reinstalled, check all options.

5. Click **Open** to import the selected calibration data set from the selected calibration file.

Note: If the selected calibration data file to be imported does not correspond to the current instrument and pipettor head, the import will be declined.

Note: During import, the selected calibration data will be read from the file, and will replace the current data as appropriate.

6. The selected calibration data will be imported and will be used for subsequent runs.

Operating procedures

6 Operating procedures

This section describes how to operate the QIAgility.

Before proceeding, we recommend that you familiarize yourself with the features of the QIAgility by referring to the section General description.

The QlAgility lid must remain closed during operation of the instrument. Only open the lid when the instrument is not operating or when instructed to do so by the software.

Note: Opening the lid during operation of the QlAgility will cause the system to pause. The instrument will complete the current movement, which may take up to 10 seconds.

WARNING/ CAUTION



Risk of personal injury and material damage [W4]

Do not attempt to move the QlAgility during operation.

WARNING



Moving parts [W12]

Avoid contact with moving parts during operation of the QIAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.

WARNING



Moving parts [W13]

To avoid contact with moving parts during operation of the QlAgility, the instrument must be operated with the lid closed.

If the in-built lid sensor is not functioning correctly, contact QIAGEN Technical Services.

6.1 Starting a run

The QlAgility Setup Manager Software provides two plug-ins that have been developed to directly facilitate specific PCR applications. Each plug-in supports several QlAGEN Kits. A third plug-in has been solely designed to meet your sample pooling requirements for convenient setup of your PCR experiments. A fourth plug-in is available for a kit-independent, easy and convenient, qPCR setup, which includes a sample normalization function. The fifth plug-in allows you to set up PCR experiments that incorporate serial dilution. For details on different experimental setup possibilities, refer to Working with experiments.

Before starting a run, read the QIAgility User Manual and the kit handbook for the kit that will be used.

To start a new run, proceed as follows:

- Start the QIAgility Setup Manager Software (see <u>Start the software</u>) if it is not yet operating.
 Note: For the first steps, the instrument does not need to be switched on.
- 2. If the **Home** screen is not visible, click on the **Home** icon from the main tool bar.
- 3. Create a new experiment using one of the following alternatives:
 - a) Create a new empty experiment by clicking an entry in the **Create Experiment** list. For details, refer to <u>Create an empty experiment</u>.
 - b) Use a template to create an experiment by clicking on it from the **Favorite Templates** list. If the desired template is not listed here, click on the **Create from other template** button at the bottom to open the file dialog. For details, refer to <u>Create an experiment using a template</u>.
 - c) Use a saved experiment as the starting point for the new experiment. In the Recent Experiments list, position the mouse pointer over the name of the desired experiment and select the Use as template for new experiment option from the context menu icon that appears. For details, refer to Create an experiment from an existing experiment.

The new experiment will be created and displayed in the **Experiment** environment.

4. Step by step, enter all necessary parameters and sample data. Follow the instructions given by the software. Click **Next** to go to the next step. Before entering the **Worktable** step, proceed with step 5.

Note: For details about the steps and parameters, press the **Help** button to access the help page for the current step.

Note: The number and kind of steps and parameters may vary, depending on the plug-in to be used. For details, refer to Working with experiments.

Note: Some steps may only become available after the previous one has been completed and after all required data have been entered. Required data are indicated with a yellow field. For details, refer to <u>General software usage</u>.

- 5. Empty the tip disposal box.
- 6. Before entering the **Worktable** step, ensure that the instrument is connected to the software. If the instrument is not yet connected, switch it on now.

Note: The instrument needs to be switched on and connected to the software before entering the **Worktable** step.

- 7. Close the lid.
- 8. Click **Next** to enter the **Worktable** step. The software automatically plans the worktable and checks the instrument calibration status. If the instrument has not been used before, it will initialize.
- 9. Prepare the reagents and samples.
- 10. Open the instrument lid.
- 11. Load the worktable. Follow the instructions of the software as it guides you through all worktable positions. For each item to be loaded, add a check to the **Loaded** check box to confirm.

Note: In order to confirm all **Loaded** check boxes in one step, e.g., for an input plate, click the **Confirm all** button below the table.

Follow the instructions in <u>Loading tips</u> and <u>Insert tips to tip rack holder</u> for details about how to place tips.

Follow the instructions in <u>Loading adapters and labware</u> for details about how to place adapters and labware

Follow the instructions in Loading reagents and samples to ensure run accuracy.

Note: For details, press the **F1** key or the **Help** button to open the help page.

Note: If you want to load the worktable in a different order from that proposed by the software, click the worktable position from the worktable selector. The software jumps to the corresponding step. Load the position and click **Next** or another yellow worktable position to load. Blue worktable position buttons will indicate when all worktable positions have been loaded.

Note: A yellow Calibrate labware button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started. Before clicking the button, remove all filled labware from the worktable position. Then click the Calibrate labware button. The software automatically prepares a labware calibration worklist. Ensure that you have the empty labware at hand for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility. The first XY-calibration screen is displayed. Follow the loading instructions on the screen, and perform all XY- and Z-calibration steps. When the calibration steps have been completed, the worktable screen will be displayed again. Load (or re-load) all calibrated positions that are displayed in the table.

- 12. Close the instrument lid.
- 13. If the experiment has not yet been saved, save it now by clicking the **Save** button in the button bar. The file dialog opens. Select a folder and enter a name.

Note: If an experiment has been saved before, the software will always automatically save the latest changes to the experiment when the run is started.

- 14. Click Start run to start the run.
- 15. The run will start and the progress will be displayed in the **Run** step.

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

6.1.1 Loading tips

The software tracks the tip usage during operation. The interactive graphic on the right side of the screen shows the current tip availability according to that tracking. However, the software cannot detect if tip racks have been reloaded or removed in the mean time.

An example below demonstrates the confirmed loading of a tip rack with 50 μ l tips on worktable position B2. The tip rack has been filled with 40 tips. A total of 136 tips are available for the run because the loading of a full tip rack with 50 μ l tips has already been confirmed for another worktable position.

QIAgility User Manual 02/2022

B2 - Tip rack

Tip type	Required	Available	Loaded	Α
50 μl Conductive tip (QIAGEN)	105	136	V	

Tip loading instruction.

Required tip type

The required tip type to be loaded to the worktable position is displayed in the **Tip type** column of the table. Ensure that the tip rack you load matches the tip type specified here.

Note: To ensure optimal tip pickup, it is important to follow the instructions in the section <u>Insert tips to tip</u> rack holder.

Required number of tips

The required number of tips for the run is displayed in the **Required** column.

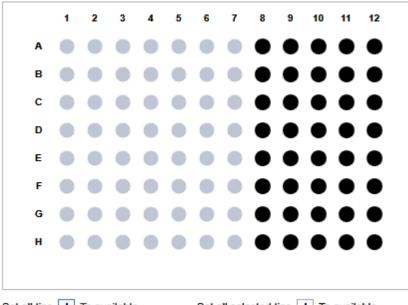
Note: The software always displays the total number of required tips of that type for the run.

Note: If according to the tip tracking, the number of currently available tips of a particular tip type is not sufficient for the run, or if more than 96 tips of the same tip type will be required, the software will plan an additional worktable position for tips of that tip type, if possible. However, it is possible not to make use of this additional tip rack for a run (see **Confirm loading** below).

Tip availability

The software tracks the tip usage during operation. The interactive graphic on the right side of the screen shows the current tip availability according to that tracking. However, the software cannot detect if tip racks have been reloaded or removed in the mean time. For the run, the software relies on the information displayed in the graphic. Therefore, it is important to set the same tips available in the graphic as the tips that are placed on the tip rack on the worktable.

50 µl Conductive Filtered Tips



Set all tips + To available Set all selected tips + To available

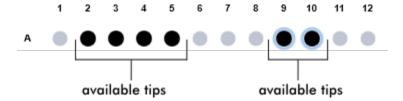
To unavailable - To unavailable

Interactive rack to define tip availability

Use the tip availability buttons below the graphic to set the availability of the rack.

Option	Required action
To set all tips to available	Click on:
	Set all tips + To available
To set all tips to unavailable	Click on:
	Set all tips
	To unavailable
To set selected tips to available	Select the desired tip positions. Click on the letter representing a row to select the whole row, the column number to select the whole column or individual tip positions to select single positions. Then click on:
	Set selected tips + To available
To set the selected tips to unavailable	Click on:
	Set selected tips
	To unavailable

Available tip positions are displayed in dark color (A2-5, A9-10) and unavailable positions are displayed in grey (A1, A6-8, A11-12). Selected tips are surrounded by a light blue circle (A9-10):



Available number of tips

According to the settings selected in the tip rack graphic on the right, the software will display the number of available tips of that tip type in the column **Available**.

Note: The software will display the total number of available tips of that type, summarizing the available tips of that tip type from all worktable positions.

Note: You can start the run, even if it is not possible to load enough tips. The software will pause the run at a certain time and ask you to reload more tips.

Confirm loading

Confirm that:

- you have loaded a tip rack
- of the correct tip type, and
- you have correctly set the tip availability in the graphic on the right

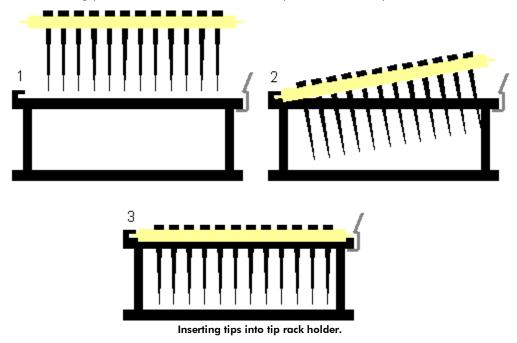
To confirm, either check the box next to **Loaded** in the table, or click on **Confirm all**.

Note: In order to indicate that a planned tip rack on a worktable position will not be loaded for the run (e.g., because there are already enough tips of that tip type on other worktable positions), set the tip availability for all tips of that worktable position to **Unavailable** and do not check the **Loaded** check box.

6.1.2 Insert tips to tip rack holder

To ensure optimal tip pickup, insert the tips as described below.

The following picture outlines how to insert a tip rack into the Tip Rack Holder.



Note: Sometimes, the tip rack holder may not fit perfectly into the worktable position. In this case, check that the tip rack holder is shifted towards the upper left corner of the worktable position and is aligned with the corner, to ensure calibration and run accuracy.

Note: Sometimes, the tip rack may not fit perfectly into the tip rack holder. In this case, check that the tip rack itself is shifted towards the upper left corner of the tip rack holder, to ensure calibration and run accuracy.

6.1.3 Loading adapters and labware

To ensure calibration and run accuracy, it is important that all adapters and labware are loaded correctly onto the worktable.

Adapters

Sometimes, an adapter may not fit perfectly into the worktable position. In this case, check that the adapter is shifted towards the upper left corner of the worktable position and is aligned with the corner.

Plates

Sometimes, plates that can be loaded directly onto the worktable may not fit perfectly into the worktable position. In this case, check that the plate is shifted towards the upper left corner of the worktable position and is aligned with the corner.

If the plate is loaded into an adapter, the plate may not fit perfectly into the adapter. In this case, check that the plate itself is shifted towards the upper left corner of the adapter.

Tubes

Check that the tubes fit correctly into the adapter. Sometimes, labels on the outside of tubes may prevent the tubes from sitting deep in the adapter. In this case, remove the label from the outside of the tube, or manually push the tube so that it sits deep in the adapter.

6.1.4 Loading reagents and samples

To ensure run accuracy, it is important that all reagents and samples loaded onto the worktable are free from air bubbles, including in the bottoms of the tubes. If necessary, briefly centrifuge the tubes to remove any bubbles.

For gene expression and pathogen experiments:

Use original QIAGEN kit reagents in their original tubes to ensure correct liquid level detection. The volumes displayed in the software are the minimum required volumes, so consider adding extra to avoid liquid level errors (i.e., "Insufficient liquid") during the run.

For viscous liquids (e.g., Master Mix), it is particularly important that you use the original kit reagent tubes, because they already contain extra liquid. If you transfer only the minimum required volume to another tube, the liquid level detection may detect insufficient liquid and display an error message.

For generic PCR. generic PCR with normalization, PCR with serial dilution and other workflow experiments:

For generic PCR runs, original QIAGEN kit reagents or self-developed reagents can be used in any available tubes.

The volumes displayed in the software are the minimum required volumes, already taking extra volume into consideration, to avoid liquid level errors (i.e., "Insufficient liquid") during the run. Keep in mind, especially for viscous liquids (e.g., Master Mix), that original tubes already contain extra liquid by production. It is also possible to define an additional volume for the input liquid.

6.2 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table.

The instrument icon in the main toolbar as well as the progress indicator on the run step show the overall run progress.

Run progress

Task	Progress
Prepare Reaction Mix Base	
Prepare Standard Series	
Prepare Target Reaction Mix	
Distribute Liquids to Output Plate	
Overall progress	
Start time	Approximate end time
17:40	17:59
Run progress.	

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

6.3 Pause/cancel a run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Pausing and resuming a run

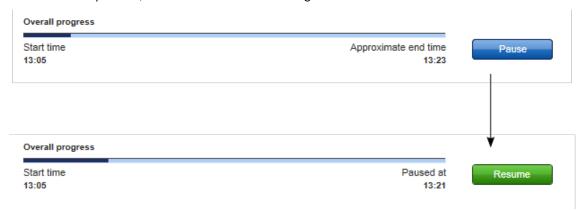
Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

1. Click the Pause button.

2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished!

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

Stopping a run

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

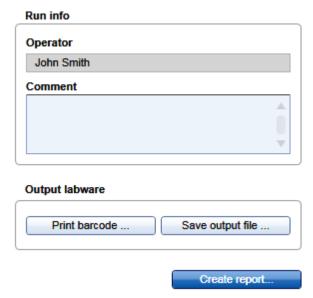
To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished!

6.4 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

Depending on the plug-in, several options may be available on the right side of the **Run** step screen. For details, refer to the specific chapters on <u>gene expression</u>, <u>pathogen detection</u>, <u>sample pooling</u>, <u>generic PCR</u>, <u>generic PCR with normalization</u>, and <u>PCR with sample dilution</u> experiments or click **Help** from the run screen.



Options of the Gene Expression plug-in

As an example, the Generic PCR Plug-in offers the following options:

Option	Description
Comment	You can enter a comment about the run in the Comment field. It will be part of the report.
Print barcode	You can print a barcode for the output labware; this requires that a printer is selected in the Global Settings tab of the Configuration environment.
Save output file	An output file can be saved for the cycler; this depends on the cycler configuration.
Create report	A report can be created for documentation purposes.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in Cleaning the QlAgility.

6.5 Finishing work and shutting down

For instructions on how to clean the QIAgility, refer to section Cleaning the QIAgility.

To shutdown the software and the instrument:

- 1. Close the lid if the instrument has been used. This allows the pipettor head to move to the home position.
- 2. Click in the top right corner of the screen, or select **Exit** from the **File** menu to shut down the software

Note: The software cannot be closed if a run is still active. In this case, the software will display an error message.

- The software shows a confirmation message if there are any unsaved changes in experiments. If you
 are unsure, click Cancel and save the changes first. Otherwise, click OK to confirm that the software
 should discard all changes and shut down.
- 4. If the QIAgility has been used, the instrument discards the tip, completes its homing routine, and then the pipettor head lowers to a known safe location.
- 5. If the QIAgility has been used, the software displays a message for a few seconds when it is safe to switch the instrument off. Then the software closes.
- 6. If the instrument is on, switch it off now.
- 7. As the instrument is switched off, a small click occurs as the pipettor head drops 1–2 mm further. All the instrument axes are disengaged and can be moved freely.

Note: We recommend that you close the software at the end of the working day.

Note: Normally, you should not switch off the instrument without closing the software. If the pipettor head holds a tip it will not be discarded. As the instrument is switched off, the pipettor head will drop approximately 70 mm. However, if it is necessary to switch off the instrument without the software, this will not damage the instrument but may result in a tip being picked up.

QIAgility Setup Manager Software

7 QIAgility Setup Manager Software

QlAgility Setup Manager Software is an easy-to-use application with a modern user interface to assist you in your workflow. It has been specially developed for use with the QlAgility instrument and uses a modern task-focused GUI-design to facilitate the interaction with the software.

QlAgility Setup Manager Software is optimized for the use of QlAGEN kits. It provides a plug-in concept whereby each plug-in has its own experiment setup, specialized to the needs of the kind of experiments and supporting a specific set of kits.

Find more information on the plug-in of your choice in the following sections:

- Working with Gene Expression experiments
- Working with Pathogen Detection experiments
- Working with Sample Pooling experiments
- Working with Generic PCR experiments
- Working with Generic PCR with normalization experiments
- Working with PCR with serial dilution experiments
- Working with Other Workflow experiments
 - Working with Normalization Only experiments
 - Preparing Reaction Mix experiments
 - Preparing and Distributing Reaction Mix experiments
 - Preparing Standard experiments

For detailed information about which kits are supported, refer to www.giagen.com/qoto/QIAgility.

7.1 Basic concepts and general software usage

The following chapters will give you an insight into the underlying concepts and the general software usage.

7.1.1 Concepts

The following concepts are essential to understand how QIAgility Setup Manager Software works and get the most out of your workflow:

- User roles
- Experiments and templates
- Plug-in concept
- Protection of files

7.1.1.1 User roles concept

User roles are an essential concept in the QlAgility Setup Manager Software. A user role defines the rights and permissions of a user. At least one user role must be assigned to every user. There are two user roles in the QlAgility Setup Manager Software:

- Operator
- Administrator

The user roles define the rights and permissions a user can have in the QIAgility Setup Manager Software. The following table describes the permissions required to enter the tabs for specific environments:

Chuj	Home	Experiment		Configuration			Instrument
Ensitonment			User Manager	Labware Manager	Calibration	Settings	
Operator	√	✓	×	√	√ (except Volume)	(except Global Settings	√
Administrator	√	✓	√	✓	✓	✓	✓

Enkin	Service					
Ensitonment	Cleaning Procedure	Maintenance	System Check	Support Package	Audit Trail	
Operator	✓	✓	✓	✓	×	
		(except Replace HEPA Filter)				
Administrator	✓	✓	✓	✓	✓	

The user role is assigned when a new user is created.

Note: Multiple user roles can be assigned to a user. A specific user can have both operator and administrator user roles. However, in this case, use the user role administrator.

Note: For the initial instrument setup, it is convenient to work with a user who has the administrator user role assigned.

7.1.1.2 Experiments and templates concept

To facilitate the process of defining experiments, the QIAgility Setup Manager Software provides the option to use templates.

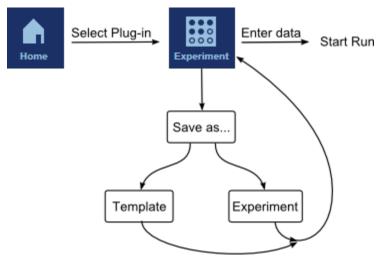
The general workflow is to select a plug-in from the **Home** environment, define the experiment in the <u>Experiment environment</u> and finally <u>start the run</u>. It is not necessary to finish the definition of an experiment in one step. A save function is available at any time, allowing you to save your progress and continue later. To facilitate the experiment definition, you can use <u>templates</u> or reuse previously run experiments.

- Save an experiment as a template for later reuse.
 - Templates are previously saved experiments that inherit the data of the experiment. The software allows you to save the experiment as template at different steps. For more details, refer to <u>Create a template</u>. <u>Experiments created using a template</u> will automatically be populated with data from the selected template. Use this function to facilitate frequently performed runs with the same experiment setup.
- Save an experiment as a quick-order template for later reuse.
 - This option is available for experiments created with the Generic PCR Plug-in, only. Use this function to facilitate runs on a regular basis with exactly the same experiment setup.

 Quick-order templates are previously saved experiments that inherit the complete data of the experiment, including assignment and layout of the output labware. For more details, refer to Create a template.

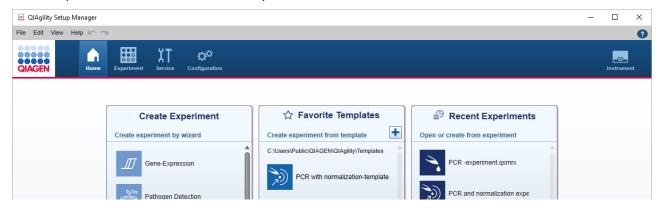
 Experiments created using a quick-order template will automatically use the complete run setup data from the selected template. The settings are not visible and cannot be modified for the current run setup. Instead, experiment setup is reduced to sample definition and loading the worktable. Always keep the original experiment for your reference. Sample names need to be defined for the same input positions as in the original experiment, however, not all of these input positions need to be used in the run. In this case, worktable calculation will create different positioning and volume of reagents.
- Use a previously saved experiment to create a new experiment. Use this function if you want to repeat the
 same experiment again. For more details, refer to <u>Create an experiment from an existing experiment</u>. If
 you often create an experiment based on the same experiment, consider creating a template and to
 adding it to the favorite templates list.

The following graphic explains these functions:



Experiments and templates have different file extensions, which are also plug-in specific. For example, the file extension for gene expression experiments is *.qsmgx, and for templates is *.qsmgt (QlAgility Setup Manager Gene Expression Experiment and Template, respectively). Generally, the file extension for an experiment ends with x, and for a template with t. The file extension for a quick order template ends with qt.

Templates and experiments can be selected from the **Home** environment, to set up a new experiment based on the experiment data in the selected template.



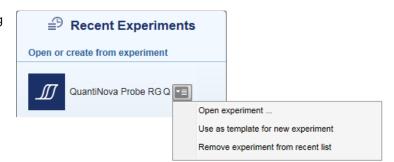
To use a template or quick-order template, click on the template entry in the **Favorite Templates** area.

For details, refer to <u>Create an experiment using a template</u>.



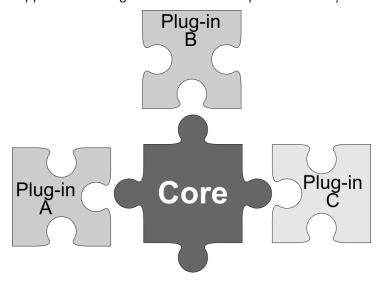
To use an existing experiment as the starting point for a new experiment, click on the desired experiment entry in the **Recent**Experiments area, click on the context menu that appears during a mouse-over and select Use as template for new experiment.

For details, refer to <u>Create an experiment</u> from an existing experiment.



7.1.1.3 Plug-in concept

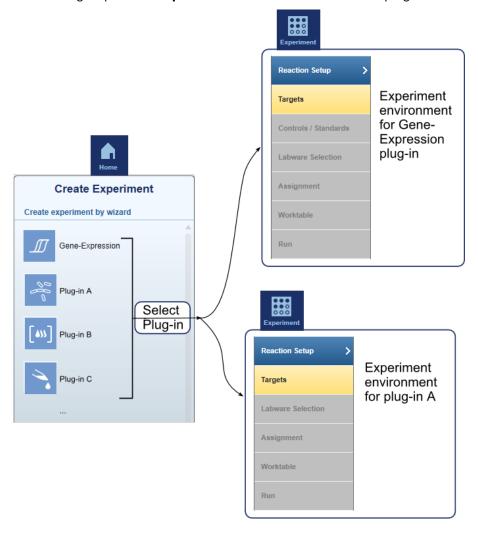
Plug-ins provide users with more specialized ways to set up experiments (e.g., more or less restrictions, support in handling controls and other special features).



A plug-in comes with its own experiment setup, report and settings (e.g., notification settings).

All available plug-ins are listed in the **Create Experiment** area of the **Home** environment. When you click on one of the plug-ins to create a new experiment, the **Experiment** environment will be opened and the steps of the step marker will be created according to the instructions of the plug-in. The steps, step names, number of steps and their order are all plug-in dependent. Refer to following sub-chapters of <u>Working with experiments</u> for detailed information about the different experiment setups.

The following graphic illustrates the relationship between selecting a plug-in in the **Home** environment and the resulting steps in the **Experiment** environment for different plug-ins:



7.1.1.4 Protection of files

QlAgility Setup Manager Software guarantees the protection of records. The system ensures that no user can modify or delete any record.

Files, configuration files and experiments are protected using checksum, which enables detection of inappropriate modifications. A checksum is a numerical value assigned to a file by the QIAgility Setup Manager Software, and is based on the content of the file. The file is invalid if the checksum does not match the file's content or is missing.

In the event that a file is modified outside the QIAgility Setup Manager Software, the file can no longer be used. If the modified file is a configuration file, it is possible that the software can no longer be used. If the modified file is an experiment, the experiment can no longer be loaded.

Note: We strongly recommend not altering the software files outside the software environment.

7.1.2 General software usage

The following section describes the GUI (graphic user interface) and the user interaction provided in the QIAgility Setup Manager Software. Fundamental topics such as entering data and working with tables are explained:

- Use of color
- Displaying errors and warnings
- Entering data
- Working with tables
- Selecting samples

7.1.2.1 Controlling the mouse

The following terms for controlling the mouse are used in this manual.

Left-click Click with the left mouse button.

Click Click with the left mouse button.

Right-click Click with the right mouse button.

7.1.2.2 Use of color

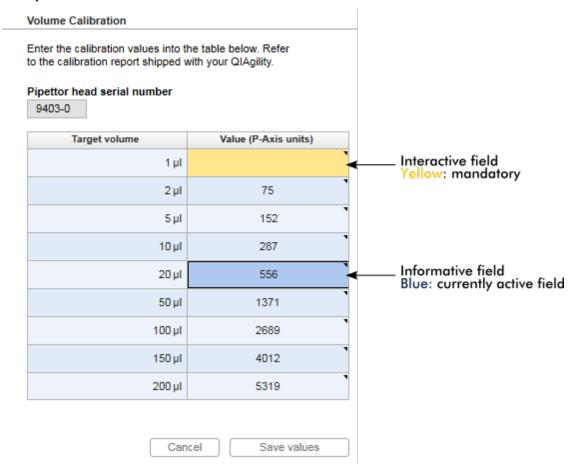
For an optimal user interaction, the QIAgility Setup Manager Software uses a dedicated color concept for presenting information. The coloring assists you in your workflow.

The following table provides an overview of the different colors used in the software and their meanings:

Color	Description
Light blue	The field is interactive and clickable.
Dark blue	The field is selected or focused.
Gray	The field is read-only and cannot be activated.
Yellow	The field requires input.

The following examples give an explanation of the color concept.

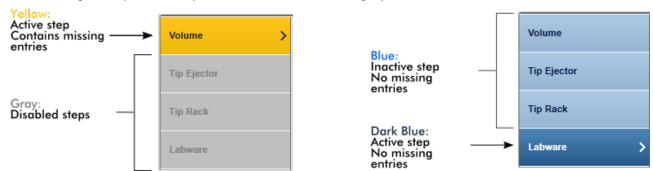
Example 1



Volume calibration screen with one mandatory value still missing.

Example 2

During experiment setup and instrument calibration, a step marker guides you through the necessary steps. The coloring concept of the step buttons is illustrated in the graphic below:



The step marker guides through the instrument calibration.

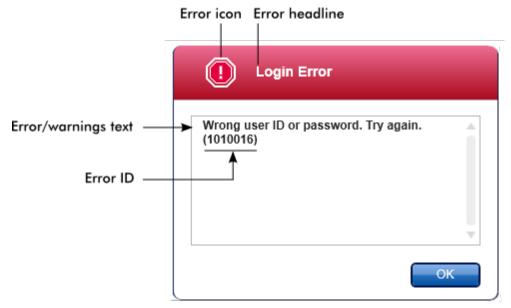
The active **Volume** step still requires missing values. Because the **Volume** calibration is required before any other calibration, the following steps are still disabled and inactive. If the **Volume** step contains all mandatory entries, it changes to blue color, and the next step **Tip Ejector** becomes available. It will remain yellow as long as the required entries or tasks are missing.

7.1.2.3 Displaying errors and warnings

Errors and warnings are essential information that indicates a problem or an erroneous situation. QIAgility Setup Manager Software differentiates between 4 different problem levels:

Priority	Name	lcon	Description of the functionality	User action
1	System error	•	A combination of unacceptable incidents	User interaction required
2	Validation error	\triangle	An error that occurs due to a missing or invalid user input	User interaction required
3	Warning	\triangle	Situation could be optimized by further input	User interaction possible, but not mandatory
4	Information	(i)	A message containing additional information about the current situation	User interaction not possible

The general structure of an error message is described below. When communicating with QIAGEN Technical Services, provide the error ID, which is unique and unambiguously characterizes the error situation. If you receive a message that contains a **Help** button, click on it to get more information.



7.1.2.4 Entering data

Shortcuts

The following hot keys are available in QIAgility Setup Manager Software:

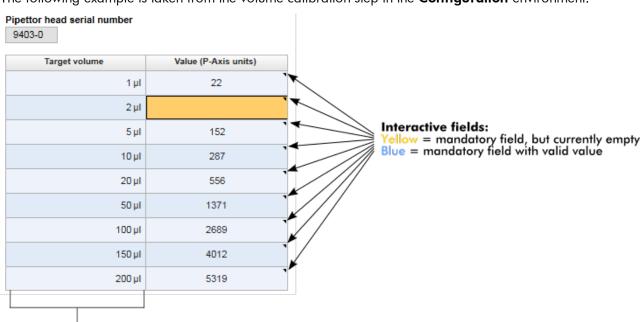
- F1 to open the help file
- Copy and paste operations (CTRL + C and CTRL + V)
- Navigation (tab key, cursor keys)

While entering data, the following keyboard shortcuts can be used:

- Escape to cancel the input
- Return to commit an input

Identifying interactive fields in tables

In <u>tables</u>, all interactive fields into which data can be entered are marked with a black triangle symbol (\neq) in their upper right corner. Left-click in the field with the mouse, and then enter data.



The following example is taken from the volume calibration step in the Configuration environment:

Editable menu: adding new data to an editable menu



Non-interactive fields:

No **▼**-icon available

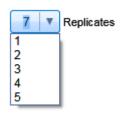
Preset values, cannot be modified

If a menu is editable, its text becomes selected if you click on it. The value list can be expanded by clicking on it.

If the value you need is missing, simply start typing the value as long as the text is selected. If the value list is empty, simply start typing into the (light-blue) menu field.



In this example, the value 7 has been entered.

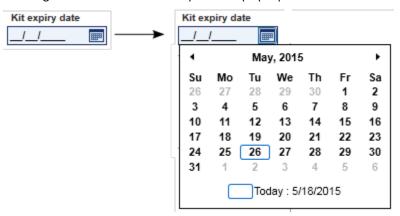


The entered data (in this example, 7) can be used as value. However, it is not added to the list of values.

Date picker: entering date in date fields

Dates can either be entered manually into date fields using the keyboard or by using an interactive date picker in the form of a pop-up calendar (.). A calendar icon is positioned to the right of every date field.

Clicking on the calendar icon opens the pop-up calendar.



Change to the previous or next month by clicking the arrow icons (and). Hovering over the year label displays additional control arrows, which can be used to quickly jump to the next (up arrow) or previous year (down arrow):



Step-by-step procedure to enter a date using the date picker

- 1. Click on the calendar icon 🗐 next to the date field. The calendar pop-up is shown.
- 2. Continue using the following scheme:

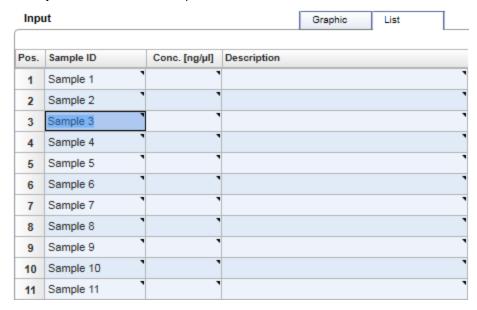
То	Do this
Change the year	Hover the mouse pointer over the year.
	Date is displayed in blue. Additional control arrows are shown.
	Click the up arrow to change to the next year.
	Click the down arrow to change to the previous year.
Change the month/day	Click the left arrow to change to the previous month.
	Click the right arrow to change to the next month.
	Click the date of the desired day.

3. The date picker disappears and the date field is populated with the selected date.

7.1.2.5 Working with tables

Tables are an essential element of the QIAgility Setup Manager Software. They are widely used to display and enter data. The interactive icon () marks cells in which you can enter data.

Example: Table to enter sample names



Entering data:

- To enter data into one cell, left-click in the cell and then enter the data.
- To move to the cell below, press the **Enter** key.
- To move to the right cell, press the **Tab** key.

This table has a context menu that is opened when you right-click on a cell:



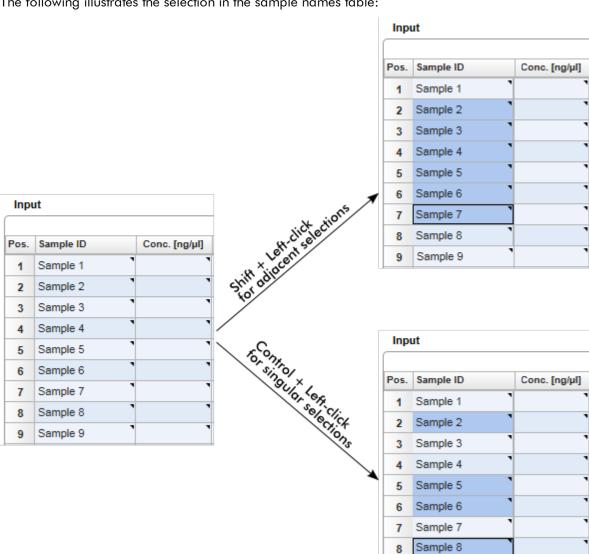
Option	Description
Сору	Copy the content of the selected cell(s) to the clipboard
Paste	Paste the clipboard content to active table cell(s). If the clipboard contains data from multiple cells, the table cells will be consecutively filled with the pasted data.
Auto-generate sample names	Sample names are generated automatically for the selected cells by taking the text in the selected cell and adding an incrementing number to the text. Example: If a cell contains "Sample," then the sample IDs Sample 1, Sample 2, etc. are generated.

7.1.2.6 Selecting samples

You can use left-clicking in combination with the **Shift** and the **Control** keys to make selections in a table or in a sample graphic:

Note: For selecting of samples, click the **Pos.** column and not the **Sample ID** column.

Shift + Left-click	To select adjacent table cells, first click on the starting cell for the selection, hold down the Shift key, move the cursor to the last cell in the selection, left-click, and release the Shift key.
Control + Left-click	To select singular cells, hold down the Control key while left-clicking on the desired cells. The selected cells will be added to the selection.
Left-click and drag	To select a rectangular area of positions.
	For rectangular sample graphics only.



The following illustrates the selection in the sample names table:

7.1.3 General elements

QIAgility Setup Manager Software uses a modern task-focused GUI design to facilitate the user's interaction with the application. The following general user interface elements are described in this section:

Sample 9

<u>Menu</u>

Main toolbar

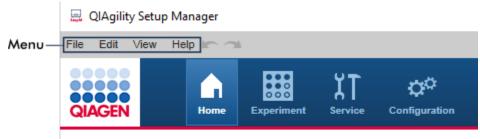
Button bar

Status bar

7.1.3.1 Menu

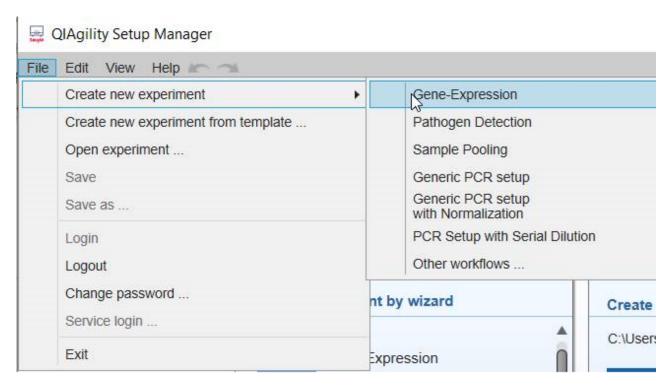
The main menu has four menu entries:

- File
- Edit
- View
- Help



Main menu

File menu



Option	Description
Create new experiment	Create a new experiment without any data. The default experiment name is set to Experiment_MM_DD_YYYY.
Create new experiment from template	Create a new experiment by selecting a previously saved template file. The data of the new experiment will be populated with all available experiment data from the template file; sample information will not be included.
Open experiment	Open an experiment.
Save	Save the current experiment. If you have not changed the default name (Experiment_MM_DD_YYYY), the file selection dialog is displayed in which you can enter the new file name along with an optional comment.
Save as	Save the current experiment under a new file name.
Login	Log in to the application. This option is only available after software start or logout.
Logout	Log out of the application. The login screen will be shown.
Change password	Change your current password. The Change password dialog is shown in which you will enter your current password and the new password.
Service login	This login is used by QIAGEN service personnel only.
Exit	Close the application.

Edit menu



Option Description

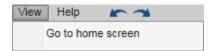
Undo the last operation.

Redo Redo the last operation.

Note: As an alternative to using the **Edit** menu, you can use the undo and redo commands by clicking on the following icons in the menu:



View menu

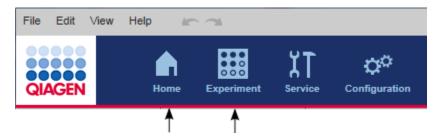


Option

Description

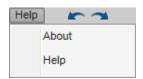
Go to home screen

Change to the home screen.



Note: As an alternative to using the **View** menu to change to the **Home screen**, you can also use the **Home** and **Experiment** buttons from the main toolbar to switch between these two environments.

Help menu



Option

About

Help

Description

Opens the **About** dialog with the copyright notice and version number (at the top right) of the software.

Opens the software help file.

7.1.3.2 Main toolbar

The main toolbar contains two areas:

- Environment icons
- Instrument icons



Environment icons

You can switch to another environment by clicking the appropriate button from the main toolbar. The icon of the currently active environment is highlighted.

Used to jump to the home screen. From here, you can create or open experiments. (Home) Used to jump to the home screen. From here, you can create or open experiments. (Home) Used to set up experiments. (Experiments) Bundles tasks for maintenance, system check and support of QIAgility. (Service) Bundles the tasks' custom settings, user and labware management, and calibration. (Configuration)

Instrument icons

Configuration

The instrument icon is placed on the right of the toolbar. Use it to inspect the connection, running and maintenance status of the instrument.

If you click the instrument icon, the software switches to:

- The run screen of the running experiment, if a run is in progress
- The maintenance schedule, if no run is in progress

Action Symbol **Description** Instrument connected. A run can be started. Instrument Instrument not connected. Prior to starting a run, switch on the instrument. **Note:** It can take some time until the software connects to the instrument. For details, refer to Start the software. **Note:** The icon cannot be clicked. Instrument running. The progress bar If you are in the process of setting up the below the icon shows the overall run next experiment, you can click the icon to switch to the run step of the running progress. experiment. Instrument To return to the setup, click the experiment name of the experiment you worked on. Run has just finished. The progress bar The same as for running instrument. shows run completion. Instrument Click the icon to switch to the run step of An error occurred during the run. the current experiment to show the error message. Instrument The instrument is not fully calibrated. Before you can start a run, instrument calibration needs to be performed or completed. A service task is due. After the reminder, If no run is in progress, click the icon to the symbol persists until the service task has switch to the maintenance schedule for

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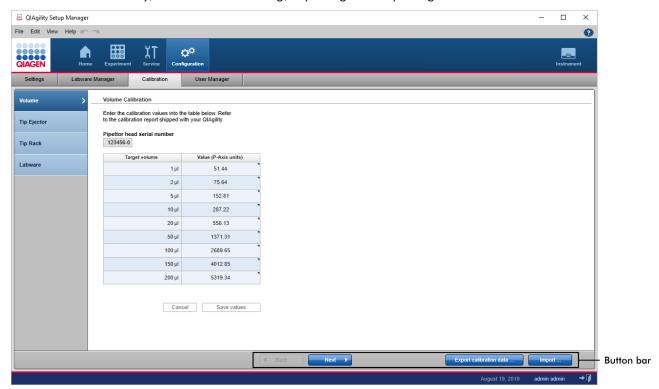
details.

been completed successfully.

Instrument

7.1.3.3 Button bar

The button bar is placed at the bottom of the screen. It contains buttons specific for the selected screen of an environment. Generally, the buttons for saving, exporting and importing can be found here.



7.1.3.4 Status bar

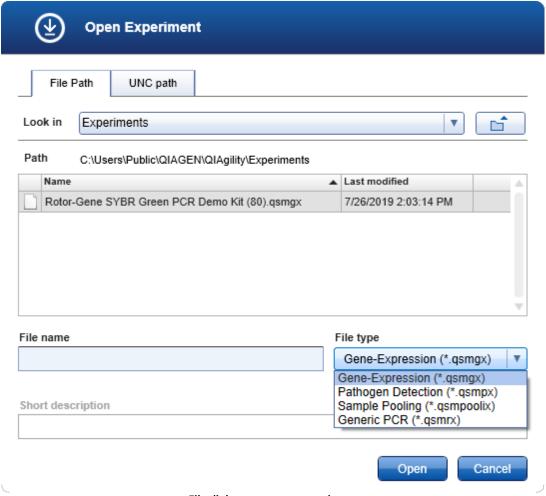
The status bar is always visible and gives an overview of the session status.



Button	Description
Date	Show the current date.
User name	Shows first and last name of the user who is currently logged in.
Log out button	Logs out the current user. If there are unsaved data, a warning will appear.

7.1.3.5 File dialog

The file dialog is used in various situations to open or save experiments or templates, to import sample information, or to save a report or an output file after a run.

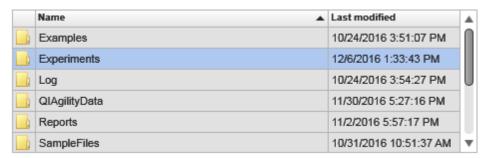


File dialog to open an experiment

Path indicates the current path. The initial path shown when the file dialog opens can be configured in the user settings for the plug-in.

Note: When the file dialog opens for the first time after clicking the **Open other experiment** or **Create** from other template button from the **Home** screen, the initial path will be **C:**.

The list in the middle of the dialog shows the sub-folders of the current path and all files with the extension selected in the **File type** menu.



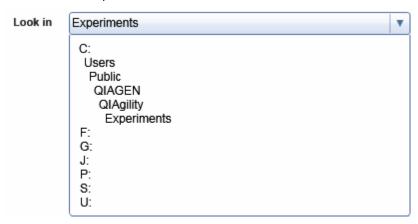
List of sub folders.

To open a file:

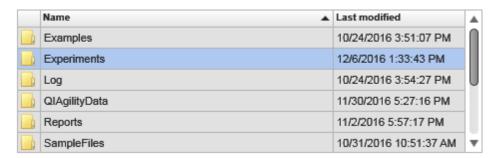
1. Navigate to the folder where the file is stored.

Use the button to navigate to the parent folder.

The **Look in** menu provides the option to select another drive by clicking the drive letter, or to select one of the parent folders by clicking it. The list shows all drive letters that are configured in the **Windows** file explorer.



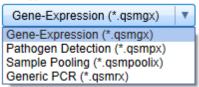
To navigate to a sub folder, either left-click the sub folder name in the list and then click **Open**, or double-click the name.



The Path changes to the sub folder and the list shows the content of the selected sub folder.

2. Ensure that the correct file type is selected. The **File type** menu lists all file extensions that are available for the current situation. The various file extensions are described further below.

File type



Experiment types

If you change the selected file type, the list of files will be updated to show all files of the newly selected file type.

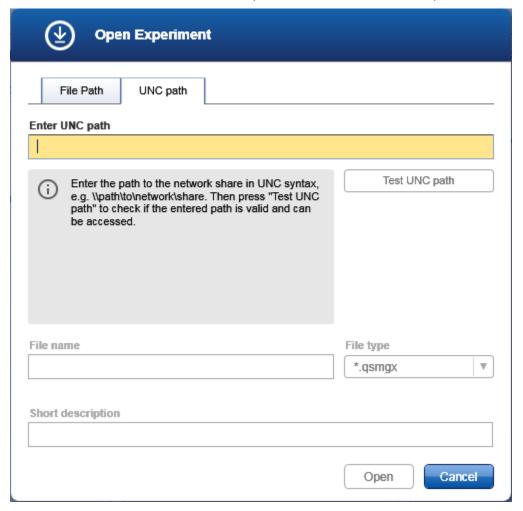
3. To open the file, either left-click the file name in the list and then click **Open**, or double-click the file name to open the file.

To save a file:

- 1. Navigate to the folder where you want to store the file. Use the navigation options described above.
- 2. Ensure that the correct file type is selected.
- 3. Enter the file name into the **File name** field.
- 4. Click **OK** to save the file.

File extension	Description
*.qsmgx	Gene expression experiment
*.qsmgt	Gene expression template
*.qsmpx	Pathogen detection experiment
*.qsmpt	Pathogen detection template
*.qsmpoolix	Sample pooling experiment
*.qsmpoolit	Sample pooling template
*.qsmrt	Generic PCR experiment
	Generic PRC with normalization experiment
	PCR with serial dilution experiment
	Other workflows
*.qsmrt	Template
*.qsmrqt	Quick order
*.xml, *.csv, *.txt	For sample input files
	$\textbf{Note:} \ \ \textbf{Find examples in the folder: C:\Users\Public\QIAGEN\QIAgility\Examples}$
	Note: For *.csv and *.txt files it is important to adhere to the syntax. In general, the file represents a comma separated table (CSV), where each line in the file represents a row in the table and a comma (,) represents the cell border to the next column. Like in a table, it is possible to leave the content of a table cell empty, but you cannot omit the commas in a line.
*.xml	For saving an output file
*.smp	For saving an output file using the XSLT converter for Rotor-Gene
*.txt	For saving an output file
*.pdf	Note: The file type of output files depends on the selected converter. For reports

The QlAgility Setup Manager Software provides the option to use **UNC** syntax to open files from and save files to network shares that are not directly known in the Windows file explorer.



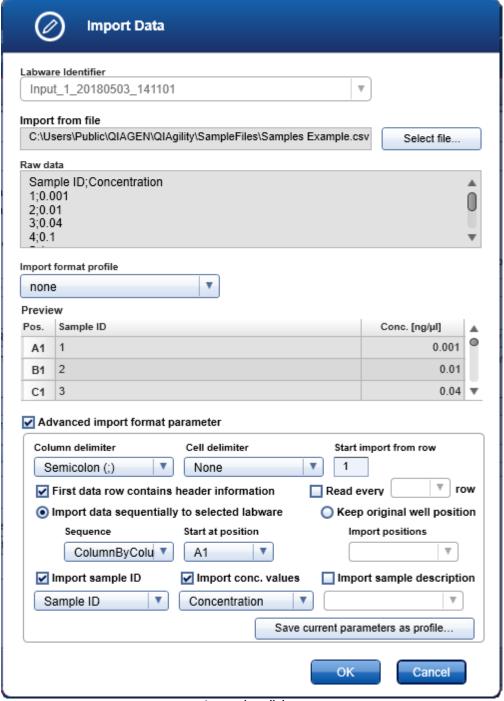
- 1. In the file dialog, click **UNC path**.
- 2. Enter the path to the network share using the UNC syntax (e.g., \\path\to\network\share).
- 3. Press the **Test UNC path** button to test whether the entered path is valid and can be accessed.
- 4. Enter the file name without the file extension into the **File name** field.
- 5. Select the file extension from the **File type** menu.
- 6. Optionally, enter a short description of the file.
- Click Open or OK to open or save the file, respectively.

If you encounter issues accessing the network share, contact your network administrator and ensure that the path has been entered correctly and is accessible.

7.1.3.6 Import data dialog

The **Import data** dialog is used in various workflows of the Generic PCR Plug-in to import sample information from a .csv file. The dialog is available from the **Samples/Labware** screen by clicking **Import data from CSV file**.

- The dialog for the input labware can be opened from the Labware identifier menu at the top.
 Note: If you are using more than one labware, you have to choose first the correct input plate from the Labware identifier menu.
- 2. Click **Select file**. The file dialog opens and shows all files with file type **.csv** that are available in the Sample Input Files folder, as specified in the <u>QG_PGP_user_settings</u>. Select the desired file and click **Open**. For details about the file dialog refer to <u>File dialog</u>.
- 3. The software loads the file into the dialog. The **Raw data** field shows the sample data as present in the file. In general, the data in the file are expected to represent a comma separated table, where each line in the file represents a row in the table, corresponding to a sample position, and a column delimiter represents the cell border to the next column. The **Preview** table below shows how the data would be imported into the samples table of the software.



Import data dialog

- 4. If the data in the **Preview** table are displayed correctly, proceed to step 9.
- 5. **Optional:** If the parameters to import from certain file types have been saved previously (see step 7), select them from the **Import format profile** menu. The **Preview** table changes according to the parameters in the selected profile. If the data are displayed correctly, proceed to step 9.

- Tick the box next to Advanced import format parameters. Additional options and parameters become visible.
- Change the parameters according to your needs. The **Preview** area changes according to the 7.

Option	Description
Column delimiter	Click the menu below to select the character that delimits the columns in a row, i.e., this character is repeated between the different properties of the sample position in the Raw data field.
	If you change the selection, a previous column selection in options Import sample ID , Import conc. values and Import sample description , below, will be cleared.
Cell delimiter	Click the menu below to select a cell delimiter. Such a character could be used to surround the characters of a (e.g., description) property. This might be necessary, if the property value includes the column delimiter character. If you are unsure, start by selecting None .
	Example: In this example, the column delimiter is, (comma), and the file contains a description text of a sample like this – Could be difficult, but give it a try. With cell delimiter None, the "," inside the description text would lead to an incomplete import of the description value Could be difficult. To solve the incomplete import of the description text, use a cell delimiter in the following way: in the file, surround the description of that sample with quotation marks, e.g., "Could be difficult, but give it a try." Then for import, select this cell quotation mark delimiter ("). The software will recognize that all characters between the quotation marks will belong to the same cell and will import the description text correctly – Could be difficult, but give it a try.
	If you change the selection, a previously made column selection in options Import sample ID , Import conc. values and Import sample description , below, will be cleared.
Start import from row	Count the lines in the Raw data field until the line where the actual sample data start (including a possible header row describing the columns). If the data starts in the very first line, enter 1.
First data row contains header information	Tick the box if the line with the number entered in the

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Start import from row field contains the column header. If ticked, the headers are available for

Read every ... row

Import data sequentially to selected labware

Sequence

Start at position

selection in options Import sample ID, Import conc. values and Import sample description, below.

The software expects exactly one row in the .csv file per sample/replicate.

If the sample file contains exactly one row per sample/replicate, do not tick the box. During import the software will read every data row from the file.

Tick the box, if the sample file contains several rows for each sample/replicate, e.g., based on target. In this case specify which rows shall be read.

Initially, the box is not ticked.

Note: If the selections made lead to the wrong rows per sample, enter the number of the first row of the file to be read into the field **Start import from row** and clear the box next to **First data row contains header information**, even if the file contains column header information.

Select the option, if you want to import the sample data from the file subsequently to the positions in the labware. As a consequence, positions would be filled up, if the sample file does not contain information for all subsequent positions.

Specify **Sequence** and **Start at position**.

If selected, the options **Sequence** and **Start at position** become active and the option **Keep original well position** becomes unselected.

Only active, if the option **Import data sequentially to selected labware** has been selected and if the selected labware supports this option.

Select one of Column by column or Row by row.

Only active, if the option **Import data sequentially to selected labware** has been selected.

Click the menu below to select the position on the labware for which the import shall start. The menu contains all positions of the currently selected labware in the order specified by option **Sequence**. The first labware position is preselected. You can select another position, if, for example, you want to leave the first row or column empty.

Example: If you selected **Column by column** and **Start at position** A2, the first column on the labware will be empty, the data of the sample described in the

first data row (after a header, if present) of the file will be imported into position A2, the data from the sample in the next row of the file into position B2, and so on.

Select this option, if you would like to keep the sample positions.

Specify the file column that contains the sample positions in the option **Import positions**.

If selected, the option **Import positions** becomes active and the option **Import data sequentially to selected labware** becomes unselected.

Only active if the option **Keep original well position** has been selected.

From the menu below, select the column that contains the sample positions.

If the option First data row contains header information has been selected, the menu contains the column headers. Select the appropriate column. Otherwise, the menu contains Column 1, Column 2. In this case, count the columns in the Raw data field starting at the left and select the appropriate column.

Tick the box, if you want to import sample IDs (sample names). In the menu below, select the file column which contains the sample IDs.

If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns as expected, check the selection of the Column delimiter. Otherwise, the menu contains Column 1, Column 2. In this case count the columns in the Raw data field starting at the left.

Select the appropriate column. Check if the correct column has been selected in the **Preview** table.

If data in the **Preview** table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.

Tick the box, if you want to import concentration values. In the menu below, select the file column that contains the concentration values.

If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns

Keep original well position

Import positions

Import sample ID

Import conc. values

as expected, check the selection of the **Column delimiter**. Otherwise, the menu contains **Column 1**, **Column 2**. In this case count the columns in the **Raw data** field starting at the left.

Select the appropriate column. Check if the correct column has been selected in the **Preview** table.

If data in the **Preview** table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.

Note: If the concentration value for a sample is given as N/A in the file, the sample will be imported, but its concentration value will be empty.

Tick the box, if you want to import sample descriptions. In the menu below, select the file column that contains the sample descriptions.

If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns as expected, check the selection of the Column delimiter. Otherwise, the menu contains Column 1, Column2. In this case, count the columns in the Raw data field starting at the left.

Select the appropriate column. Check if the correct column has been selected in the **Preview** table.

If data in the **Preview** table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.

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- 8. Repeat steps 5-7 until the data are correctly displayed in the **Preview** area.
- 9. **Optional:** Save the currently selected parameters for reuse in an import profile by clicking **Save current parameters as profile**, if you would like to load files with the same format for future experiments (in this case go to step 3).
- 10. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 2–9.
- 11. Click **OK** to import the file and to close the dialog.

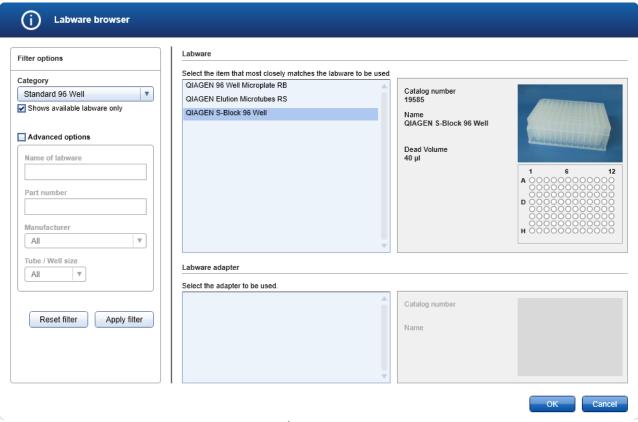
Import sample description

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7.1.3.7 Using the labware browser

The **Labware Browser** is used to select the input and output labware for a run, if you decide not to use the labware proposed by the protocol. It provides access to the labware that is supported by the QIAgility Setup Manager Software. Filter options can be used to restrict the search to labware that has been indicated as **Available in the laboratory** in the **Labware Manager**.

You can access the **Labware Browser** from the **Labware Selection** step of an experiment, by selecting **Choose other labware** from the **Input labware type** or **Output labware type** drop-down menu.



Labware Browser

The **Filter options** area on the left is used to search for a specific labware.

The **Labware** and **Labware adapter** area in the middle shows the search results together with a picture and further details.

To select input or output labware for a run:

In the filter options, select the labware category from the Category drop-down menu. Find a
description of all filter options below.

2. Select the option **Shows available labware only**.

Note: For the run, only labware that has been indicated as **Available in the laboratory** in the **Labware Manager** can be used.

- 3. Use **Apply filter** to show all available labware of the selected category.
- 4. In the middle of the screen, select the desired labware from the list at the top. The software displays a picture and further details about the selected labware on the right.
 - If the desired labware is not listed, either select another available labware, or uncheck the **Shows** available labware only option to search it among all supported labware. You can use the **Advanced** filter options to restrict the search by other criteria.

Note: Before you can use the labware for a run, it must be indicated as **Available in the laboratory** in the **Labware Manager**. If the labware has never been used before on the QIAgility, it must also be calibrated.

5. If the selected labware requires an adapter, the software will list all adapters that can be used with the selected labware in the **Labware adapter** area below. Select the adapter. The software displays a picture and further details about the selected adapter on the right.

Note: If the option **Shows available labware only** has been checked, the software lists only adapters that have been indicated as **Available in the laboratory** in the **Labware Manager**.

If none of the matching adapters has been indicated as **Available in the laboratory** in the **Labware Manager**, the list will be empty and the **OK** button will remain disabled. Either choose another available labware or indicate one of the adapters as **Available in the laboratory** in the **Labware Manager**.

Click OK to confirm the selection and to close the Labware Browser.

Note: The **OK** button will only be enabled if a labware has been selected. In addition, if the labware requires an adapter (see the details area of the selected labware), the **OK** button will become enables after you have selected an adapter from the **Labware adapter** area.

Important: Tubes or plates supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). Therefore, it is important to select the correct plates and tubes.

Filter options

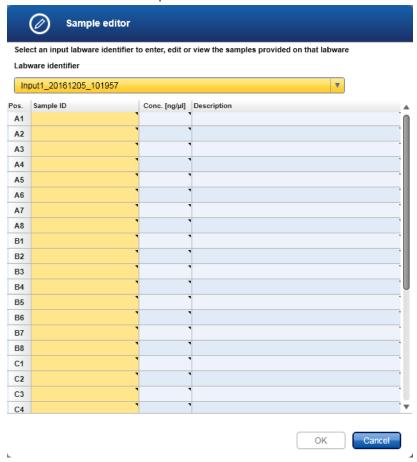
Option	Description
Category	Labware category, such as standard 96-well, Rotor-Gene Q, etc.
Shows available labware only	If checked, the search will be restricted to labware that has been indicated as Available in the laboratory in the Labware Manager .
	Note: The option applies also to the Labware adapter area.
	Uncheck the option if a desired labware is supported in general, but does not appear in the list if the option is checked.
Advanced options	Check to enable the advanced filter options.
	Enter or select details only for those advanced options for that you want to restrict the search. Leave all other options empty/unselected.
Name of labware	Labware name
Part number	Catalog number of the labware
Manufacturer	List of labware manufacturers
Tube/well size	Tube/well size range from $< 0.5 \text{ ml}$ to $> 2 \text{ ml}$
Reset filter	If you click the button, the software clears all filter options to the initial state.
Apply filter	If you click the button, the software lists all labware that matches the search criteria.

7.1.3.8 Editing sample names

If several input labware will be used in a run, sample names can be defined in one dialog for all input labware.

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



Note: For more details how to enter information into a table refer to the section **Entering data**.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

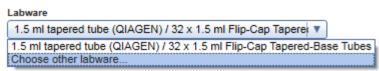
- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.1.3.9 Add input labware dialog

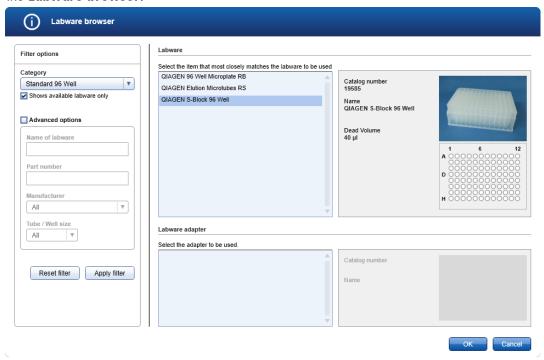
If several input labware will be used in a run, it can be defined in the **Add new input labware dialog**. To define a new input labware:

1. Click on the **Labware type** drop-down menu.

All previously selected labware types will be listed. The Choose other labware option will be listed last.



3. Select your input labware in the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

5. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QlAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

7. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. If you want the software to automatically generate sample names for all positions on the labware, check the option **Auto-generate sample IDs for all samples**.

Note: This option will not be available if the dialog was opened using the **Load sample input from file** or the **Scan barcode** button.

9. Click **OK** to close the dialog.

7.1.3.10 Add output labware dialog

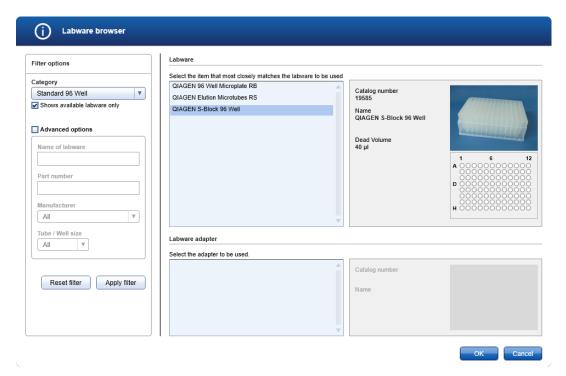
If several output labware will be used in a run, it can be defined in the Add new output labware dialog.

To define new output labware:

- 1. Click on the Labware type drop-down menu.
- All previously selected labware types will be listed. The Choose other labware option will be listed last.



3. Select your output labware. If not listed, click **Choose other labware** to open the **Labware browser**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the output labware, check the option Use cooling block under the Output labware type drop-down menu.



Note: Whenever you select another labware type, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

5. **Optional:** If you would like to indicate that the output labware has been pre-filled, tick the box next to **Output labware is pre-filled with reagents**.



Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report.

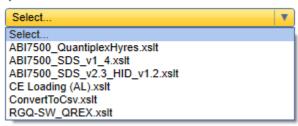
Note: Whenever you select another labware type, this identifier will be automatically generated again by the software.

7. Optional: Click the Print barcode button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.



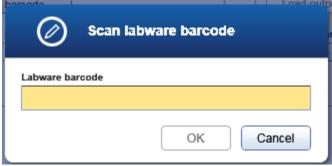


Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

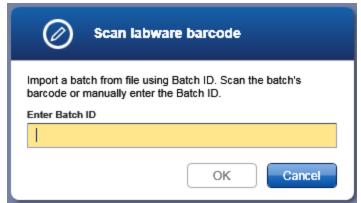
9. Click **OK** to close the dialog.

7.1.3.11 Scan labware barcode dialog

To define samples, the QIAgility Setup Manager Software provides the option to load the sample information based on the barcode on the labware. Click the **Scan labware barcode** button to open the **Scan labware barcode** dialog.



Scan labware barcode dialog used for Gene Expression and Pathogen Detection experiments.



Scan labware barcode dialog used for Sample Pooling experiments.

- Scan the labware barcode. Alternatively, you can enter the labware ID manually.
 Note: The software supports 1-D and 2-D barcodes. 2-D barcodes are supported if they encode a string that is used as labware ID.
- 2. Click **OK** to confirm.
- For input labware, the software will automatically search for a sample information file in the Sample input files directory (defined in the user settings). The software loads the sample information from the sample file.

Note: The software will first search for an *.xml file in QDEF format, which defines the samples for the labware. It is not required that the *.xml file has the labware barcode as its file name. The software looks into the file and compares the labware barcode with the plate ID described in the file. If no file in QDEF format is present, the software will then search for a *.csv file which has the labware barcode as its file name.

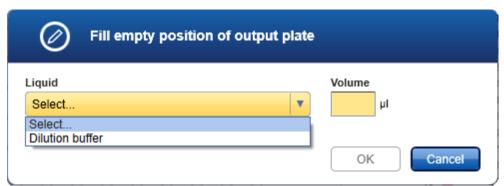
4. For output labware, the software automatically searches for a QDEF format file (*.xml) describing the output labware in the Sample output files directory (defined in the user settings). Based on the information from the loaded file, the software will automatically select the Output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

7.1.3.12 Fill empty output positions dialog

The **Fill empty position of output labware** dialog is used in various workflows of the Generic PCR Plug-in to define output positions which are not used for PCR setup, but will be filled, e.g., with a buffer. The dialog is available from the **Assignment** screen if empty positions of the output labware have been selected by clicking **Fill selected**.

Note: This functionality should only be used after all other positions have been assigned, so that any sample or standard/control assignments are not overwritten.

- 1. Click the **Liquid** menu to select the liquid that shall be used to fill the selected positions. The menu lists all liquids of type Diluent/Water.If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 2. Enter the volume in μ l into the **Volume** field.
- 3. Click **OK** to confirm and to close the dialog.
- 4. The positions on the **Output** panel will be marked with the symbol for Buffer. During the run, the QIAgility will fill the selected positions with the defined amount of the liquid.

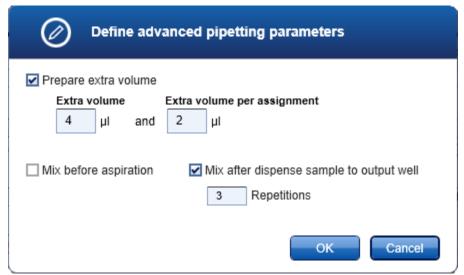


Dialog to select the liquid to fill output positions during run.

To clear a filled position select the position on the Output panel and then click Remove assignment.

7.1.3.13 Advanced sample pipetting parameters dialog

The **Define advanced pipetting parameters** dialog is used in various workflows of the Generic PCR Plugin. The dialog is available from the **Samples/Labware** screen, panel **Sample Pipetting**, by ticking the box **Use custom pipetting parameters** and then clicking **Edit parameters**.



Advanced sample pipetting parameters dialog

- If you tick the box next to **Prepare extra volume**, parameters become visible to define extra volume
 for samples. Extra volumes are set conservatively. Therefore, in most cases displayed loading volume
 is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover
 variations depending on sample assignments later in the Assignment step (e.g., volume loss per tip)
 and is added per replicate.
 - However, if you face any liquid level detection issue increase **Extra volume per assignment** if issues are for higher number of assignments of a sample only. Increase absolute **Extra volume** if issues are not related to the number of assignments of a sample.
- 2. If you tick the box next to **Mix before aspiration**, you can specify the mixing repetitions in the input labware before the sample is aspirated for sample transfer. The box is enabled only if the selected liquid class for sample transfer includes mixing before aspiration.
- 3. If you tick the box next to **Mix after dispense sample to output well** you can specify the mixing repetitions in the output labware after the sample has been dispensed. The box is enabled only if the selected liquid class for sample transfer includes mixing after dispense.
- 4. Click **OK** to confirm your selections and to close the dialog.

7.1.4 Environments

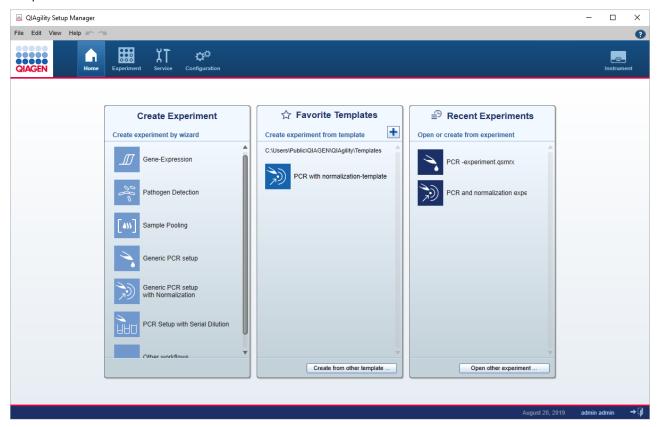
QlAgility Setup Manager Software contains 4 different environments.

You can switch to another environment by clicking the appropriate button from the main toolbar. The icon of the currently active environment is highlighted.

Used to jump to the home screen. From here, you can create or open experiments. (Home) Used to set up experiments. (Experiments) Bundles tasks for maintenance, system check and support of QIAgility. (Service) Bundles the tasks' custom settings, user and labware management, and calibration. (Configuration)

7.1.4.1 Home

The **Home** environment bundles all tasks for creating and opening of experiments and for working with templates.



The **Home** environment is divided in three greas:



Creates an experiment using the wizard. Each installed plug-in comes with its own wizard. The new experiment is empty and does not contain any data. Most data fields are empty, except those that are prefilled by the wizard.

Use this to start an experiment from scratch. For details, refer to Create an empty experiment.



Creates an experiment using a previously saved template. All data fields in the new experiment are populated with existing data from the template. For details, refer to <u>Create an experiment using a template</u>.

All favorite templates are listed in tabular form below the headline. For information regarding how to add a template to the list, refer to <u>Manage favorite templates</u>.

Click the button **Create from other template** to look for a template that is not in the list using the <u>file dialog</u>.



Opens a previously saved experiment. Use this to modify or adapt a saved experiment or alternatively, use it to set up a new experiment that inherits the data from an old experiment. For details, refer to View recent experiments or Create an experiment from an existing experiment, respectively.

All recently saved experiments are listed in tabular form below the headline.

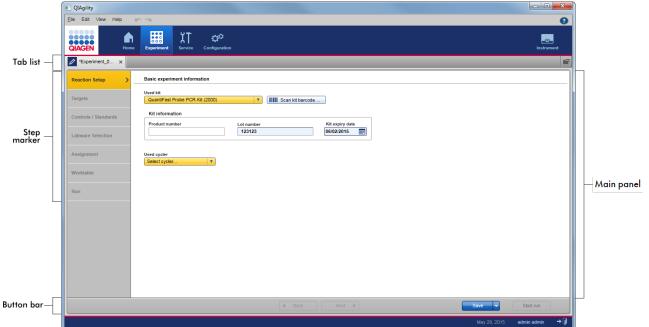
Click the button **Open other experiment** to look for an experiment that is not in the list using the <u>file dialog</u>.

7.1.4.2 Experiments

The **Experiment** environment is the environment in which all user interaction with experiments takes place. This is where experiments are set up and run and reports are created. In order to interact with an experiment, the experiment must be opened. If a new experiment is created, it is open by default.

All changes to an experiment are performed in the computer's memory. In order to make them permanent, it is necessary to save the experiment. Before a run is started, the corresponding experiment must be saved.

The maximum number of experiments that can be opened at one time is 8. Attempting to open more than 8 experiments will result in a warning message.



Experiment environment with one open experiment.

The screen of the **Experiment** environment has four main areas:

Area **Description**

Experiment tab list

The experiment tab control is displayed at the top of the **Experiment** environment. It shows all currently open experiments and offers basic experiment management.

A separate tab is displayed for each open experiment. If an experiment name is too long to be displayed within the available space, the displayed name will be truncated, and ellipses (...) will be shown at the end of the name. In order to display the full name, simply let the mouse pointer remain over the experiment name for a few seconds. A tooltip will display the full name of the experiment.

When you make modifications in any step, an asterisk symbol (*) will be displayed in front of the experiment name. This is a visual reminder for you that there are modifications that have not yet been saved. When you save the experiment, the asterisk symbol disappears.

Clicking on the cross to the right of an experiment name will close the experiment. If any modifications have not been saved, then you will be asked whether the experiment should really be closed. Closing the experiment without saving the modifications will result in loss of the modifications.

The following icon appears on the far right of the tab list: 🖭. This is a shortcut to open an experiment; clicking this displays a file selection dialog that allows you to open an experiment.

Step markers Step markers allow you to navigate between different aspects of one experiment. The step markers control the content of the main panel; each step marker is associated with a certain content of the main panel. To switch to another step, click on the step marker step or use the Back and Next buttons in the button bar.

> Note: It is not required to follow the steps in order, but it is recommended. You can jump any active step at any time.

Main panel

All experiment-specific information is shown and selected in the main panel. The content depends on the selected step marker.

Button bar

The button bar is displayed at the bottom of the **Experiment** environment. It contains buttons for experiment management functions that are not related to a specific step marker, including Back, Next, Save and Start run.

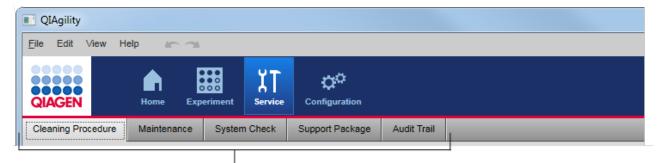
The Start run button is only enabled if all requirements for a new run are met (e.g., all required values have been defined during setup) and if the experiment has not already been run. For details regarding a run, refer to Starting a run.

Selecting the **Save** button will save the current experiment. If the experiment has not yet been named, a dialog will be shown, prompting you to enter a name for the experiment. If the experiment already has a name (for example, if an existing experiment was opened), the existing experiment will be overwritten.

The **Save** button also has a sub-function: **Save experiment as**. Clicking on the small arrow to the right of the button will display a menu with the additional choice. The **Save** experiment/template as file dialog allows you to enter a new experiment name and save the experiment with the new name. Use this option to also create a template from the current experiment by selecting the file extension for a template in the file dialog.

7.1.4.3 Service

The **Service** environment bundles all tasks needed to check and maintain the QIAgility along with tools to facilitate the communication with QIAGEN Technical Services for technical assistance.



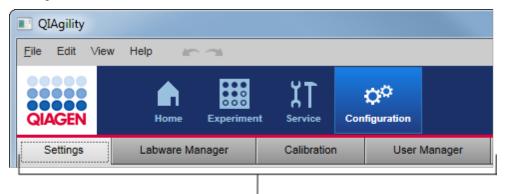
Service environment is organized in five tabls

This environment is organized in five different tabs. You can switch between these tabs by clicking on the respective button in the tab bar.

Tab	Description
Cleaning Procedure	Offers to start the UV lamp as the last step of the <u>cleaning procedure</u> . The UV radiation cleans possible biological contaminations on the worktable.
Maintenance	<u>Maintenance scheduler</u> keeps track of service tasks. Additionally, HEPA filter <u>replacement</u> can be managed here.
System check	Provides several self-diagnostic tools:
	 Checking the connection between computer and instrument and within the instrument itself
	Cycling the pipettor head
	Checking for movement reliability
	Checking the reliability of the liquid-level sensor
Support Package	Create a <u>support package</u> to assist your communication with QIAGEN Technical Services, in case you have an issue.
Audit Trail	Allows you to view the system <u>audit trail</u> . This tab is only available for users with administrator user role.

7.1.4.4 Configuration

The **Configuration** environment bundles all tasks to customize the instrument for your needs. You can set application settings, select the labware available in your laboratory, perform the instrument calibration and manage users.



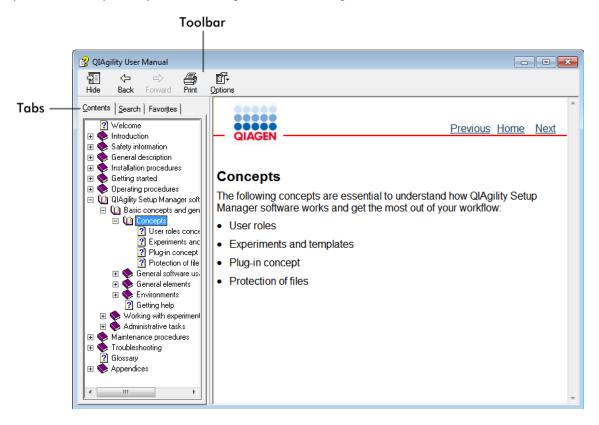
Configuration environment is organized in four tabs

This environment is organized in four different tabs. You can switch between these tabs by clicking on the respective button in the tab bar.

Tab	Description
Settings	Specify <u>general settings</u> including directories for experiments and report customization, set the password policy and e-mail notifications, etc.
Labware Manager	Select the <u>labware</u> (adapter, plates, tubes) available in your laboratory.
Calibration	Perform an <u>instrument calibration</u> , which is essential for accurate and reliable results.
User Manager	Manage users:
	Create and modify users and their roles
	Activate and deactivate users

7.1.5 Getting help

QlAgility Setup Manager Software comes with a detailed help system. The help is provided as both a user manual in PDF format and also as a context-sensitive help system. To access the context-sensitive help, press the **F1** key while you are viewing screens or dialogs.

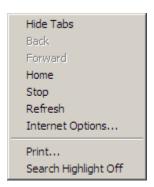


The help file contains two functional areas:

- Toolbar
- Tabs

The toolbar contains the following buttons:

Name	lcon	Description
Hide or Show	Hide	Hides the left-hand side navigation tab. To display the navigation tab again, click Show . This button appears instead of Hide .
Back	⇔ Back	Returns to the previous screen.
Forward	Forward	Returns to the screen displayed before using the Back button.
Print	Print	Provides two options:
		• Print the selected topic.
		Print the selected heading and all subtopics.
		Select one option and confirm with OK or select Cancel to go back.
Options	©ptions	Opens the Options menu with the following entries:



The navigation tab contains the following tabs:

Name	Description
Contents	In the Contents tab the help content can be browsed by topics.
Search	Specific help topics can be found by entering search terms.
Favorites	Shortcuts to individual help topics can be added and managed.

7.2 Working with experiments

The <u>Home</u> environment bundles all tasks for creating and opening experiments and for working with templates. It offers the following functions:

- Creating a new, empty experiment from the Create Experiment section
- Creating a new experiment from a template from the Favorite Template section
- Creating a new experiment from a previously run experiment from the Recent Experiments section

The general workflow is to select a plug-in from the **Home** environment, define the experiment in the <u>Experiment environment</u> and finally <u>start the run</u>. It is not necessary to finish the definition of an experiment in one step. A save function is available at any time, allowing you to save your progress and continue later. To facilitate the experiment definition, you can use <u>templates</u> or reuse previously run experiments.

Refer to the following sections about available experiment setups:

- Working with gene expression experiments
- Working with experiments for pathogen detection
- Working with sample pooling experiments
- Generic PCR experiments
- Generic PCR setup with normalization experiments
- PCR setup with serial dilution experiments

The QIAgility Setup Manager Software provides the following other workflows:

- Normalization only experiments
- Prepare reaction mix experiments
- Prepare and distribute reaction mix experiments
- Prepare standards experiments

7.2.1 Managing experiments and templates

Experiments and templates are managed in the **Home** environment. The following section describes how to work with them.

For details regarding how to create a template and about the template concept, refer to Experiments and templates.

7.2.1.1 Create a template

An experiment can be saved as a **template** for future reuse. Use this function if you plan to enter the same values for experiment setup for several experiments (e.g., for routinely performed experiments or a series of runs). Templates do not inherit the assignment and worktable information from the experiment.

Additionally, the Generic PCR Plug-in offers the option to save a **quick order template**. Quick order templates inherit the sample input positions, assignment and layout on the output labware.

Prepare an experiment as the basis for the template or the quick order template:

- 1. <u>Create a new, empty experiment</u>. Alternatively, <u>create an experiment from an existing experiment</u>. For a quick order template, <u>open the original experiment</u>.
- 2. For a template, enter all values that should be available for reuse in the template.

Note: If you created the experiment from an existing experiment, remove all values that should not be reused. When using the template, it is easier to recognize that some values are still missing than to recognize that some values should be changed.

For a quick order template, define the experiment including sample input positions and assignment, i.e., all wizard steps before the worktable step.

Note: When defining a quick order template, do not use lot numbers/expiry date in the liquid definitions of the original experiment. Instead, enter lot numbers, if needed, into the comment field on the run screen.

Note: When using a quick order template, fewer samples than defined in the original experiment can be run. However, in this case worktable calculation will create different positioning and volumes of reagents.

3. For a quick order template, save the experiment (as experiment) for later reference, because when using the quick order template, the definitions made will not be visible anymore. Select, e.g. the directory that has been defined as default directory for templates.

To save an open experiment as a template or quick order template:

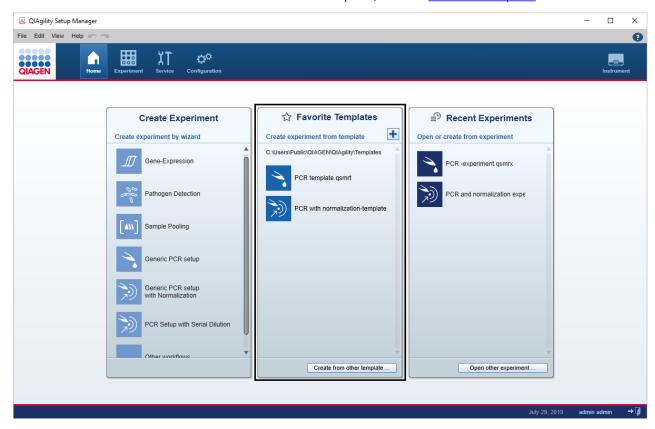
- 1. Click on the small arrow to the right of the **Save** button in the buttof the on bar. From the displayed menu, select **Save experiment as**.
- 2. The Save experiment/template as file dialog opens.
- 3. Select the directory where the template should be saved, e.g. the directory that has been defined as default directory for templates in the settings of the plug-in. Initially, the default directory for experiments is shown.
- 4. Enter a name for the template in the **File name** field.
- 5. From **File type**, for a template select the file extension ending with a **t** (e.g., *.qsmg**t** for a template for use with the Gene Expression Plug-in). To save a quick order template for use with the Generic PCR Plug-in, select the file extension ending with a **qt**.

6. Click Save.

Add the created template to the <u>favorite templates</u> section.

7.2.1.2 Manage favorite templates

The software offers the possibility to manage favorite templates in the **Favorite Templates** list of the **Home** environment. For information about how to create a template, refer to <u>Create a template</u>.



A maximum of 11 templates can be displayed. When this limit is reached, the oldest template will be removed from the list. The template name will be truncated if the available space is exceeded, and ellipses (...) will be shown at the end of the template name. To display the full name, move the mouse pointer over the experiment name and after a few seconds, a tooltip will be displayed showing the full name.

Templates can be added or removed from this list, and <u>new experiments can be created</u> based on these templates. In addition, this section offers the option to create experiments based on templates that are not contained in the list of favorite templates.

Adding a folder with templates

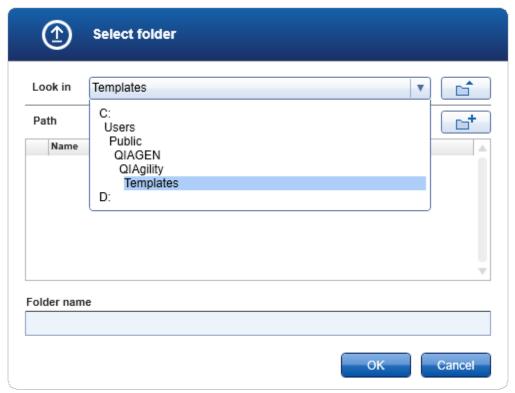
- 1. Click on the **Home** icon in the main toolbar to change to the **Home** environment.
- 2. The **Favorite Templates** list in the middle will display all favorite templates.

3. Click on the plus icon in the list header:

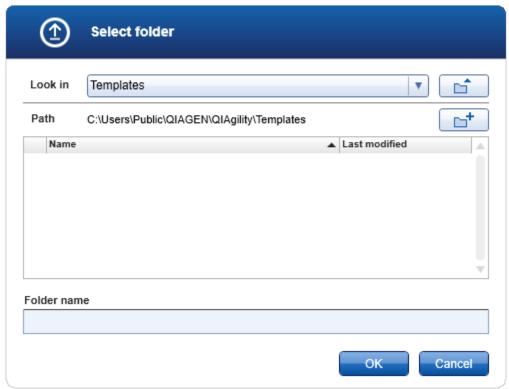


4. Select the folder where your templates are located. The file extension of a template depends on the plug-in that it's compatible with. See the table below for more information about file extensions. Click **OK**.

Plug-in Gene-Expression Pathogen Detection Sample Pooling Generic PCR Generic PCR Quick Order File Extension *.qsmgt *.qsmpt *.qsmpoolit *.qsmrt *.qsmrt *.qsmrt



Folder selection



Selected folder

Note: The **Select folder** dialog does not show the contents of the selected folder. To view the contents of the folder, use your preferred file manager application.

5. Templates from the selected folder will be added to the **Favorite Templates** list.

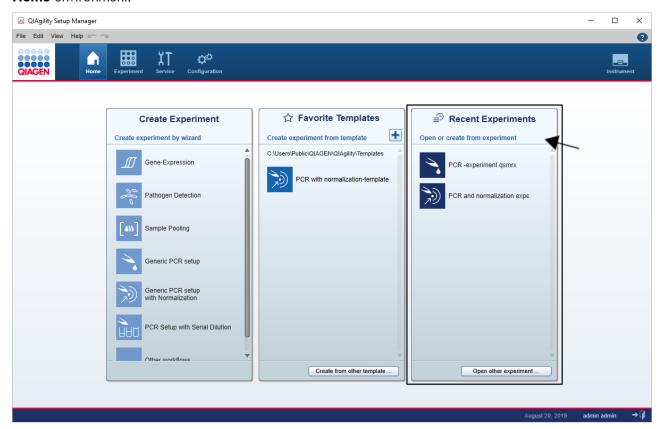
Removing a template

- 1. Click on the **Home** icon in the main toolbar to change to the **Home** environment.
- 2. The **Favorite Templates** list in the middle displays all favorite templates.
- 3. Move the mouse pointer over the template name.
- 4. The context menu icon appears ().
- 5. Click on the icon.
- 6. The context menu is shown.
- 7. Click on the **Remove from favorites** command.
- 8. The selected template is removed from the **Favorite Templates** list.

Note: Removing a template using the steps above will not physically delete or modify the template file. It will only be removed from the **Favorite Templates** list.

7.2.1.3 View recent experiments

When an experiment is saved or run, it is automatically added to the **Recent Experiments** table in the **Home** environment:



The most recent experiment appears at the top of the list. A maximum of 11 experiments can be displayed. When this limit is reached, the oldest experiment will be removed from the list.

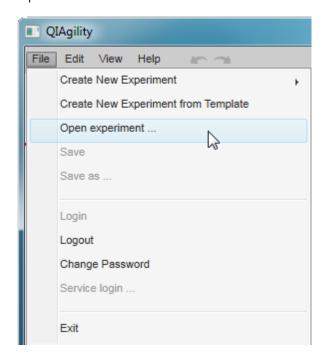
The experiment name will be truncated if the available space is exceeded, and ellipses will be shown at the end of the experiment name. To display the full name, move the mouse pointer over the experiment name, and after a few seconds, a tooltip will appear with the full name.

Opening an existing experiment

- 1. Click on the **Home** icon in the main toolbar to change to the **Home** environment.
- 2. The **Recent Experiments** table on the right displays all recent experiments.

- 3. Click on the experiment name you want to open. Alternatively, position the mouse pointer over the experiment name. Click on the context menu icon that appears (), and select **Open experiment**. **Optional step:** If the experiment is not listed because it has been pushed off the list by newer experiments, click on the Open other experiment button at the bottom and select the experiment from the file dialog.
- 4. The experiment is opened in the **Experiment** environment

 Note: Alternatively, you can use the **Open experiment** command from the **File** menu to open an experiment.



Removing an experiment from the list of recent experiments

Follow these instructions to remove an experiment from the **Recent Experiments** list:

- 1. Click on the **Home** icon in the main toolbar to change to the **Home** environment.
- 2. The **Recent Experiments** table on the right displays all recent experiments.
- 3. Move the mouse pointer over the experiment name you want to remove.
- 4. The context menu icon (is displayed.
- 5. Click on the context menu icon to open the context menu.
- 6. Select the **Remove experiment from recent list** command.
- 7. The selected experiment is removed from the recent experiments list.

Note: The experiment will still be available via the Open other experiment button.

7.2.2 Creating an experiment

You can create an experiment either by

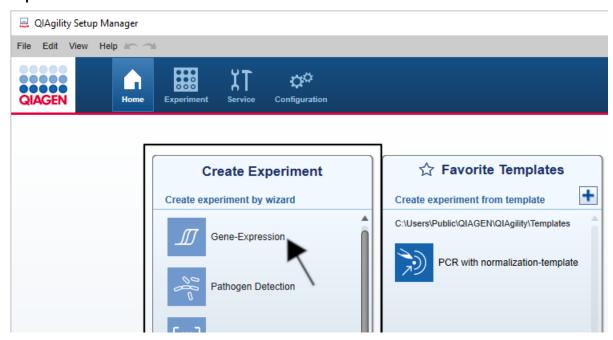
- Creating a <u>new</u>, blank experiment without any data, or
- Creating an experiment with data inherited from a previously saved <u>experiment</u> or <u>template</u>.

7.2.2.1 Create an empty experiment

There are two ways to create an empty experiment:

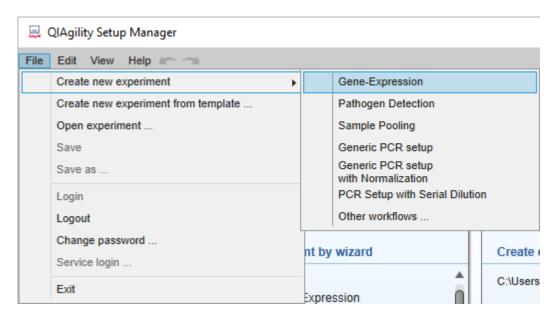
• Using the Home environment

In the **Home** environment, all plug-ins are listed in the **Create Experiment** list. Move the mouse pointer over one of the plug-in entries and click on it. The new experiment is created and displayed in the **Experiment** environment.



• Using the File menu

To create a new experiment using the main menu, select **File** from the main menu and move the mouse pointer over the **Create New Experiment** command. A submenu containing all available plug-ins is shown. Click on the desired plug-in to create a new, blank experiment.

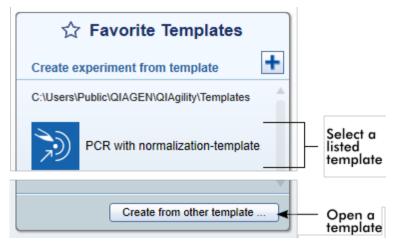


7.2.2.2 Create an experiment using a template

There are two ways to create an experiment based on a template:

• Using the Home environment

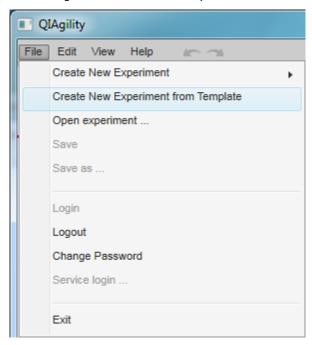
In the **Home** environment, favorite templates are listed in the **Favorite Templates** list. You can either click on one of the listed templates, or if the desired template is not listed, click on the **Create from other template** button at the bottom to open the file dialog that allows you to select a folder that contains the template. In both cases, a new experiment will be created containing all data from the selected template.



For details on how to add a template to the favorite list, refer to Manage favorite templates.

• Using the File menu

To create a new template-based experiment using the main menu, select **File** from the main menu and click on the **Create New Experiment from Template** command. The file open dialog is opened, allowing you to select a template. When you confirm the file dialog, a new experiment will be created containing all data from the template.



For details of the template concept, refer to Experiments and templates.

7.2.2.3 Create an experiment from an existing experiment

There are two ways to use an existing experiment as a starting point for creating a new experiment:

- Like a template: inherit the data of the experiment, but not the sample information. Use this option if you want to provide new sample name only. If you often create an experiment based on the same experiment, consider creating a template and add it to the favorite templates list.
- Make a copy: inherit the data of the experiment, including the sample information. Use this option if you want to rerun the same experiment again with the same sample names and the samples placed in the same positions on the input labware.

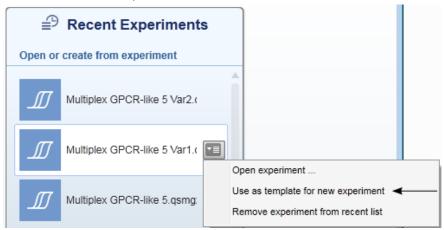
To use the existing experiment like a template, follow this procedure:

1. Change to the **Home** environment.

2. Move the mouse pointer to the desired experiment in the Recent Experiments list.

Note: In case the desired experiment is not listed here, click on the **Open other experiment** button at the bottom to open it once using the file dialog that appears. Now the experiment will be listed in the **Recent Experiments** list. Close it and start again with step 1.

- 3. Hold the mouse pointer over the desired experiment.
- 4. The context menu icon (appears.
- 5. Click on it.
- 6. The context menu is opened.



- 7. Click on **Use as template for new experiment**.
- 8. A new experiment will be created based on the data from the selected experiment (excluding the sample information), and will be displayed in the **Experiment** environment.

To use the existing experiment as a copy, including the sample information, follow this procedure:

- 1. Change to the **Home** environment.
- 2. Click on the desired experiment in the **Recent Experiments** list to open it.
- 3. **Note:** In case the desired experiment is not listed here, click on the **Open other experiment** button at the bottom to open it once using the file dialog that appears.
- 4. The existing experiment will be displayed in the **Experiment** environment.
- 5. Click on the small arrow to the right of the **Save** button in the button bar. From the displayed menu, select **Save experiment as**.
- 6. The Save experiment/template as file dialog opens.
- 7. Select the directory where the new experiment should be saved.
- 8. Enter a name for the new experiment in the **File name** field.

- 9. From **File type**, select the file extension ending with a **x** (e.g., *.qsmg**x** for an experiment for use with the Gene Expression Plug-in).
- 10. Click Save.
- 11. A new experiment will be created based on the data from the existing experiment (including the sample information), and will be displayed in the **Experiment** environment. The existing experiment will close automatically.

7.2.3 Working with gene expression experiments

The Gene Expression Plug-in enables easy and convenient qPCR setup of your gene expression experiments using dedicated lines of QIAGEN kits including QuantiTect, QuantiFast, QuantiNova and Rotor-Gene.

New gene expression experiments are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Reaction Setup** step will initially be enabled. All other steps will become available once the required information has been entered in the **Reaction Setup** step. The coloring of the **Targets** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 7 steps that guide you through the run setup process:

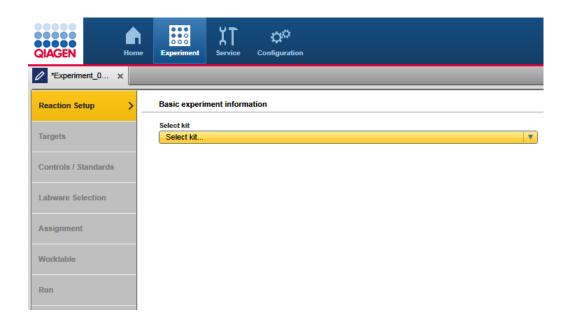
- 1. Reaction setup
- 2. Targets
- 3. Controls/standards
- 4. Labware selection
- 5. Assignment
- 6. Worktable
- 7. Run

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. The file extension for gene expression experiments is *.qsmgx, and for templates *.qsmgt (QlAgility Setup Manager Gene Expression Experiment, and Template, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.3.1 Reaction Setup step

The **Reaction Setup** step is used to make basic selections, such as the kit to be used, the preferred cycler and the volume definitions. When you create a new experiment, the **Reaction Setup** step will be the only active step, and within this step, only the **Used kit** menu will be active. The other controls will initially remain inactive, but will become visible once you have selected the kit to be used in the reactions.



1. Select the kit to be used from the **Select kit** drop-down menu.



Once selected, a kit description is displayed next to the selection menu. When you are using a kit for the first time, we recommend reading it carefully. It contains important information regarding the experiment setup.

2. The **Kit information** and **Used cycler** fields are displayed.



Optionally, enter the Lot number and the Kit expiry date in the corresponding fields for documentation purposes.

- 4. Select the cycler to be used from the **Select cycler and output format** drop-down menu.
- 5. If the selected cycler requires a passive reference, the **Passive reference** option will be enabled. Select this option to use passive reference.

Note: If the selected kit contains a passive reference and the passive reference option is selected, the software will automatically add the passive reference to the additional reagents list (see <u>Target step</u>) along with the recommended volume for your setup.

6. The **Volume definitions** area is displayed. The fields may vary depending on your specific kit and cycler selection.



Note: The default values that appear in the volume fields are the recommended values from the kit handbook for your setup.

7. Enter the volume data in the corresponding fields:

Field	Description
Sample input volume	Enter sample input volume. This is the volume of sample, controls and standards that will be added to the reaction mix.
Total reaction volume	Enter the total reaction volume. This is the volume of the final reaction mix including reaction mix and sample, control or standard.

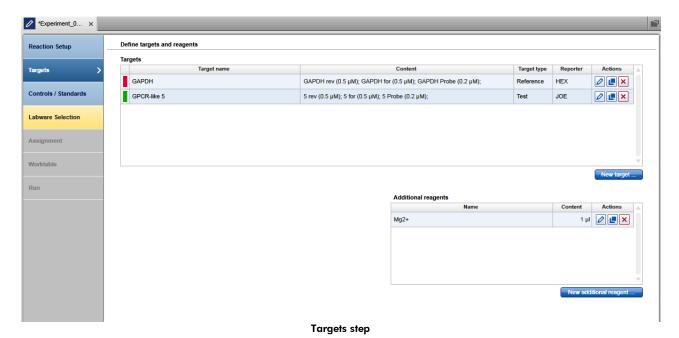
- 8. If your samples are covered with TopElute Fluid, indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
- 9. Check the box next to **Use ready-made reaction mix** to indicate whether you have already prepared the reaction mix containing the kit master mix and all target components. Enter the reaction mix volume per reaction. Later in the worktable step, you will be asked to place this prepared mix onto the worktable.
- 10. Click **Next** to proceed.

7.2.3.2 Targets step

The **Targets** step is used to <u>define primer-probe mixes or single components</u> for the targets. All defined targets and primer-probe mixes are listed in the **Targets** tables. At least one target needs to be defined.

Optionally, <u>additional reagents can be defined</u> to be added to the reaction mix. Defined reagents are listed in the **Additional reagents** table. The QIAgility will add these reagents to the assay points during run, if you select the option **Use ready-made primer-probe mixes** in the <u>Reaction Setup step</u>.

Click **Next** to proceed.



7.2.3.2.1 Defining targets and mixes

You can choose whether the QIAgility should use single components (primers and probes) or ready-made primer-probe mixes.

To define:

- a new primer-probe mix for one or several targets
- or single components for one target

proceed as follows:

- Click on New target below the Targets table.
- 2. The **Add target** dialog will be displayed. Refer to <u>Add target dialog</u> for details.
- 3. New rows for the defined primer-probe mixes or single components for the targets will be added to the **Targets** table.

The buttons in the **Actions** column



of the **Targets** table have the following functions:

Button

Action

To edit a target

Description

The **Edit target** dialog will open, displaying the values that have been entered in the **Add target** dialog.

To duplicate a target

The **Duplicate target** dialog will open, displaying the values that

have been entered for the original target. The target names will

initially display a copy of the target.

To delete a target Deletes the row in the **Targets** table.

7.2.3.2.2 Add target dialog

The **Add target** dialog contains two major options at the top:

Use ready-made Select this option if you intend to load a ready-made primer-probe mix in one

single tube for the run.

You can define several target names. There is also the option to define the

components for documentation purposes.

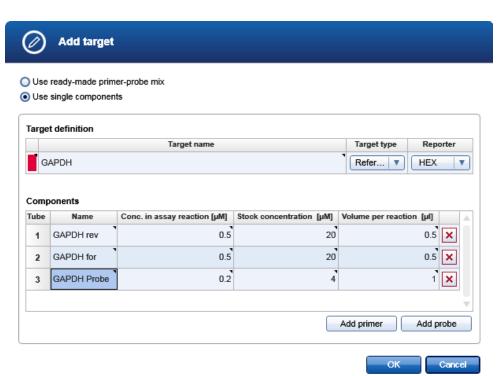
Use singleSelect this option if you intend to load primers and probes in individual tubes for

the run.

components

Define one target name and the components (primers and probes) related to this

target name.



Dialog to load primers and probes in individual tubes for the run.

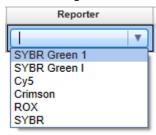
To define single components for one target

- 1. Select the option Use single components.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type Test	Description Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.
	If the kit contains an IC, define a corresponding IC target. Later, when <u>assigning samples and targets</u> to the output labware, either select that your samples already contain the IC or that the QIAgility shall add an IC to the reaction.
	If you use an own IC, you can also define an IC target. If you want to use the IC for selected samples only, the samples on the input labware must already contain the IC when loading them onto the worktable. Alternatively, if the IC shall be used with all the samples in the run and the samples do not yet contain the IC, you can define the IC as an additional reagent. The IC will then be added by the QIAgility to all reaction mixes.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:



Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35

FLUORESCEIN TET TEXASRED

ALEXAFLUOR488 MAX ALEXAFLUOR568

CALFLUOR

GOLD540

YAKIMAYELLOW

Red Blue Crimson

CY5 MARINABLUE QUASAR705

QUASAR670 EDANS ALEXAFLUOR680

ALEXAFLUOR633 BOTHELLBLUE LIGHTCYCLERRED705

LIGHTCYCLERRED640 ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

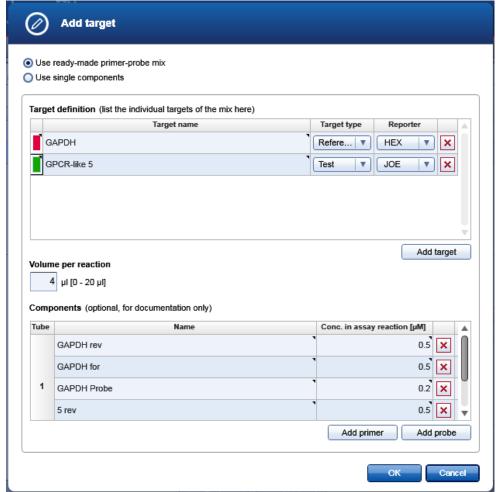
- 5. In the **Components** table, define the primers and the probe (steps 6–10).
- 6. Click on Add primer to add a primer or click on Add probe to add a probe.
- A row for the primer or probe will be added to the Components table and the Name cell will be colored yellow.

Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 8. Enter the name of the primer or probe.
 The QIAgility Setup Manager Software will prompt you at a later step to place a tube with the name of the primer or probe onto the worktable.
- Enter the Volume per reaction of the primer or probe.
 Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the Volume per reaction.
- 10. To define all components, repeat steps 6–10.
- 11. If the single components of the target have been defined, click **OK**. The dialog will close.
- 12. The target will be listed in the **Targets** table.

Note: The software automatically assigns a color to each defined target name. If you would like to select a different color, you can click on the colored rectangle in the first column to open the **Colors** dialog:





Dialog to load a ready-made primer-probe mix in one single tube for the run.

To define a ready-made primer-probe mix

- 1. Select the option Use ready-made primer-probe mix.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.

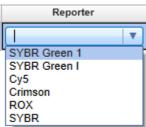
Note: To name a target mix, you can either choose one name that indicates the target mix name, or you can define several different target names. In the software, these targets will always be handled together, because they are part of the same mix. The QlAgility Setup Manager Software will prompt you at a later step to place a tube with the name of the target mix onto the worktable. If you defined several different target names, the software will display a list of the targets for that tube.

3. Click on the menu in the **Target type** column and select a target type.



Target type Test	Description Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.
	If the kit contains an IC, define a corresponding IC target. Later, when <u>assigning</u> <u>samples and targets</u> to the output labware, either select that your samples already contain the IC or that the QIAgility shall add an IC to the reaction.
	If you use an own IC, you can also define an IC target. If you want to use the IC for selected samples only, the samples on the input labware must already contain the IC when loading them onto the worktable. Alternatively, if the IC shall be used with all the samples in the run and the samples do not yet contain the IC, you can define the IC as an additional reagent. The IC will then be added by the QIAgility to all reaction mixes.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:



Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFILIOR	

GOLD540

YAKIMAYELLOW

Red Blue Crimson

CY5 MARINABLUE QUASAR705

QUASAR670 EDANS ALEXAFLUOR680

ALEXAFLUOR633 BOTHELLBLUE LIGHTCYCLERRED705

LIGHTCYCLERRED640 ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- 5. Optional step: To add further target names, click on Add target and repeat the steps 2–5.
 Note: After you have created at least two targets, a Delete icon (x) will be displayed in the last column of each target row. Click on it if you would like to delete a target.
- 6. Enter the volume per reaction of the primer-probe mix in the Volume per reaction field.
- 7. In the **Components** table, you can define the primers and the probe (steps 8–12). **Note:** Steps 8–12 are optional.
- 8. Click on **Add primer** to add a primer or click on **Add probe** to add a probe.
- 9. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon (**×**) will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 10. Enter the name of the primer or probe.
- 11. Enter the **Concentration in the assay reaction** in the corresponding cell.
- 12. **Optional step:** To add further primers or probes, repeat steps 8–11.
- 13. If the target or primer-probe mix has been defined, click **OK**. The dialog will close.
- 14. The primer-probe mix will be listed in the **Targets** table. The targets defined in the dialog share the same **Content** cell.

7.2.3.2.3 Defining additional reagents

Additional reagents can be managed (added, duplicated or deleted) using the **Additional reagents** table at the bottom. The table lists the reagent name and the volume per reaction.

Adding an additional reagent

- 1. To add a new reagent click on **New additional reagent**.
- 2. The **Add additional reagent** dialog will open. For details, refer to section <u>Add additional reagent</u> dialog.
- 3. The reagent will be displayed as an additional row in the **Additional reagents** table.



The buttons in the **Actions** column functions:

of the **Additional reagents** table have the following

Button	Action	Description
0	To edit a reagent	The Edit additional reagent dialog will open, displaying the values that have been entered in the Add additional reagent dialog.
	To duplicate a reagent	The Duplicate additional reagent dialog will open, displaying the values that have been entered for the original reagent. The reagent names will initially display a copy of the reagent.
×	To delete a reagent	Deletes the row in the Additional reagents table.

7.2.3.2.4 Add additional reagent dialog



Dialog to enter an additional reagent

- 1. Enter the reagent name.
- 2. Enter the **Volume per reaction**. Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.
- 3. Click OK.
- 4. The reagent will be displayed as an additional row in the **Additional reagents** table.

Note: The QIAgility will add the additional reagent to the assay points during run.

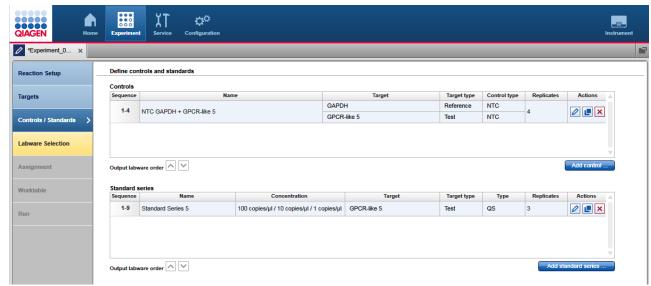
7.2.3.3 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will be opened. For details, refer to <u>Add control</u> dialog or <u>Add standard series dialog</u>, respectively.
- The control will be added to the Controls table, and the standard series will be added to the Standard series table.
- 4. Click **Next** to proceed.



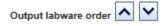
Controls/Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

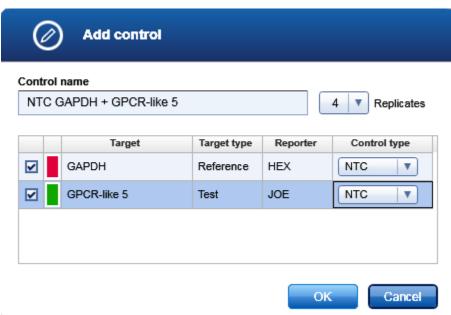
Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output plate will be based on the order indicated in these tables.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.3.3.1 Add control dialog



NTC for a multiplex assay

- 1. Enter a control name in the **Control name** field.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:

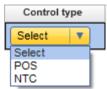


Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

3. The software lists all defined targets and target mixes in the table below. Select at least one of the targets by activating the check box in the first column of the desired target(s).

Note: Selecting more than one target per control will result in a multiplex assay, including all selected targets in one reaction mix.

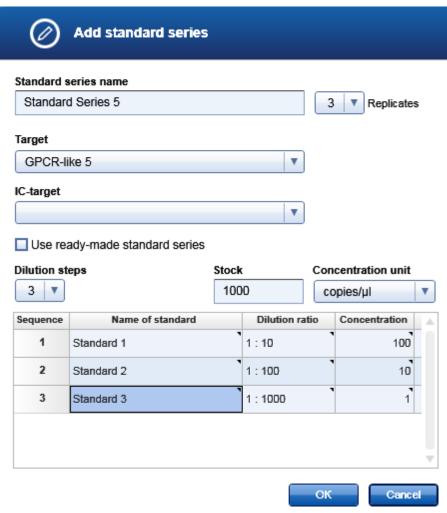
4. Select the control type using the drop-down menu in the **Control type** column:



Option	Description
POS	A positive control will be added to the assay points.
NTC	Water from the kit will be added to the assay points.

- 5. Click **OK** to confirm and close the **Add control** dialog.
- 6. The control will be added to the **Controls** table.

7.2.3.3.2 Add standard series dialog



Dialog for standard series

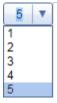
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 3. Select a target from the **Target** drop-down menu.
- 4. Optionally, you can select one IC target from the **IC target** drop-down menu.
- 5. Check the option **Use ready-made standard series**, if you intend to load the ready-made dilutions individually for the run.
 - Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.
- 6. Select the number of dilution steps from the **Dilution steps** menu.

Dilution steps



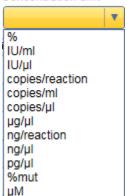
The number of rows in the table at the bottom is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section Entering data.

Note: In order to use the actual stock as the first standard, define the first standard to have the same concentration as the stock (dilution ratio will be 1:1 in this case). If the option **Use ready-made standard series** has not been checked, the QIAgility will directly use the stock as template for the assay points of this standard.

7. If the option **Use ready-made standard series** has not been checked, enter the stock concentration in the **Stock** field and select the concentration unit using the **Concentration unit** drop-down menu.

Concentration unit



8. For each dilution step, enter either a dilution ratio or a concentration. The other value will be calculated automatically by the software. Optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series.

Note: The dilution ratio refers to the stock concentration.

Note: Concentration values lower than 0.01 will be displayed in scientific format, e.g., 1.00E-03 represents 0.001.

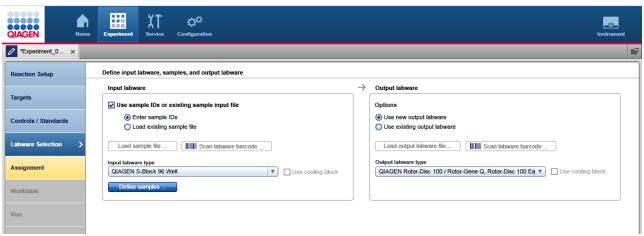
Note: This step is optional if the option Use ready-made standard series has been checked.

- 9. Click **OK** to confirm and close the **Add standard series** dialog.
- 10. The standard series will be added to the **Standard series** table.

7.2.3.4 Labware Selection step

Use the **Labware Selection** screen to select the labware for the input side and the output side and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side.



Labware Selection step

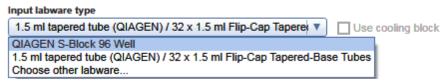
To define the input labware:

- 1. Check the option Use sample IDs or existing sample input file.
- 2. Select either **Enter sample IDs** to define sample IDs manually using the **Sample editor**, and proceed to step 3.

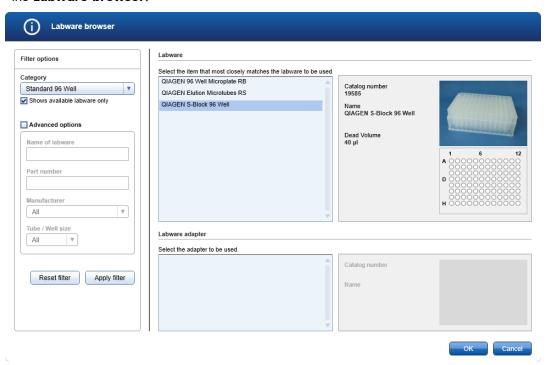
Alternatively, select **Load existing sample file** to use a sample input file, and proceed to step 11.

To select the input labware type:

- 3. Click on the **Input labware type** drop-down menu.
- 4. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.



Select your input labware in the drop-down list. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then also select the adapter below. Click **OK** to close the **Labware browser**.

The selected labware will be displayed and added to the Input labware types drop-down menu

Note: For more details about the **Labware browser**, refer to the section <u>Using the labware browser</u>.

6. Optional step: If you want to use the cooling block for the input labware, check the option Use cooling block next to the Input labware type.

To define samples manually:

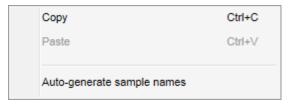
Click **Define samples**.

8. The **Sample editor** will be opened, and the **Sample ID** fields will be colored yellow to indicate that this information is required.



9. Enter each sample ID and optionally, enter the concentration and sample description.

Note: Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells. Press the **SHIFT** key and left-click to select multiple adjacent cells or press the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click to open the context menu and click on **Auto-generate sample names**.



Note: For more details about the sample editor, refer to the section <u>Editing sample names</u>.

10. Click **OK** to close the **Sample editor**. Proceed to step 16.

To define samples by using a sample input file:

11. The Load sample file and Scan labware barcode buttons are enabled, if the option Load existing sample file had been checked.



- 12. Click the **Load sample file** button and select the desired sample file. For details, refer to File dialog.
- 13. Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan labware barcode** button. The **Scan labware barcode** dialog opens. Scan the barcode and confirm by clicking **OK.** For details, refer to <u>Scan labware barcode dialog</u>.
- 14. The software loads the sample information from the sample file and displays the sample editor. Optionally, edit the sample information. Then click **OK** to confirm.
- 15. If the sample information file also contains the labware type, it will automatically be selected in the **Input labware type** drop-down menu.

Note: This is only possible for files in QDEF format.

To define the output labware:

16. You can either **Use an empty output labware** or **Use an existing output labware**. The option **Use empty output labware** is initially selected and can be used for most runs.

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case select the option **Use an existing output labware** and proceed to step 19.

17. Select the output labware from the **Output labware type** menu on the right. It includes all labware suitable for the selected cycler. A fitting adapter is automatically added if marked as available in the labware manager. Proceed to step 22.

Note: If the selection is yellow, you are required to mark the labware or a fitting adapter as available in the **Labware manager**.

Note: If the labware to be used is not listed, select **Choose other labware** to open the **Labware** browser.

18. **Optional step:** If you want to use the cooling block for the output labware, check the option **Use** cooling block next to the **Output labware type**.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

19. The Load output labware file and Scan labware barcode buttons are enabled, if the Use existing output labware option had been selected.

Options

Use empty output labware

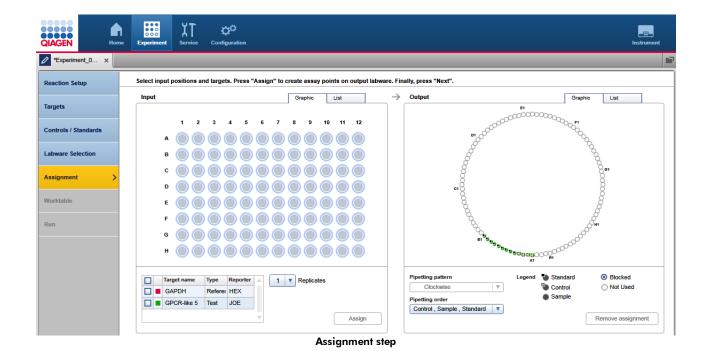
Use existing output labware (partially filled labware from a previous run)

Load output labware file ...

- 20. Click the **Load output labware file** button and select the desired labware file. For details, refer to <u>File</u> dialog.
- 21. Alternatively, click the **Scan labware barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>. Using the information from the loaded file, the software will automatically select the **Output labware type**. In addition, the positions that were used with this labware for a previous run will be blocked.
- 22. Click **Next** to proceed.

7.2.3.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-target combinations to the output labware. Single and multiplex assay points can easily be generated for selected samples.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware. The meaning of the different symbols is described below:

•	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create assay points on the output labware.

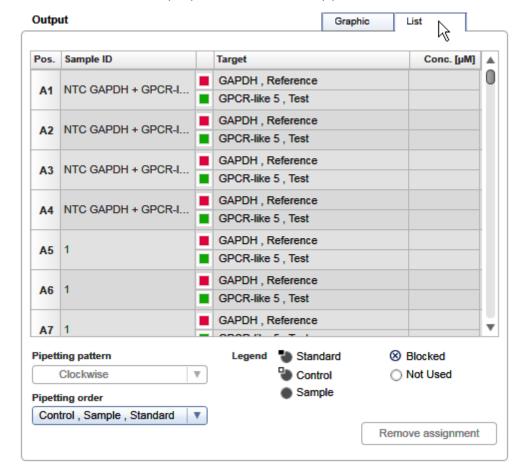
Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

At the bottom of the panel is a table showing all targets defined in the **Targets** step, along with a **Replicates** menu.

In order to assign a sample to an assay point, select the sample position(s) and then the target(s) you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and targets will be assigned to assay points created on the output labware. Each combination of targets assigned to the output labware will result in the setup of a target reaction mix including the target-specific components.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. When entering the screen, the software automatically places all standards and controls from the **Controls/Standard** step. The creation of assay points containing samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the targets. Standards and controls are indicated by an additional small symbol. If you hover with the mouse over one target color of an output position, the software displays the sample ID, the target and the target type for that position.



Use the **List** tab of the output panel to view the assay points that have been created.

The List tab of the Output panel: 4 replicates of the NTC control are placed at A1-A4. Both targets have been assigned to sample 1, with 3 replicates at A5-A7.

The two menus at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column by column) and the **Pipetting order** (e.g., first standards, then controls, then samples). You can also define a new pipetting order.

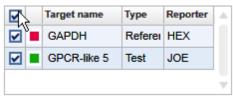
7.2.3.5.1 Assigning samples with targets to the output labware

Proceed as follows:

1. From the **Input** panel, select the sample position you want to assign a target.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.

2. From the targets table below, check the box next to the target you want to assign. You can check multiple targets at the same time in case you want to make multiple target assignments.



All targets selected.

Note: To select all targets, check the box in the table header.

3. If an <u>IC target</u> has been selected, additional options will be displayed.

If the kit contains an IC, use the option **Samples contain IC** if the samples on the input labware already contain the IC. Use **Add IC to reaction** if the QIAgility shall add the IC to the assay points during the run.

The option **Add IC to reaction** is enabled only if the kit contains an IC.

If the kit does not contain an IC, the samples on the input labware must already contain the IC. The option **Samples contain IC** is the only available option. However, but only, if the IC shall be used for all assay points and the samples do not yet contain the IC, you can define the IC as an <u>additional</u> reagent. As every additional reagent, the IC will be added by the QIAgility to all reaction mixes.

4. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



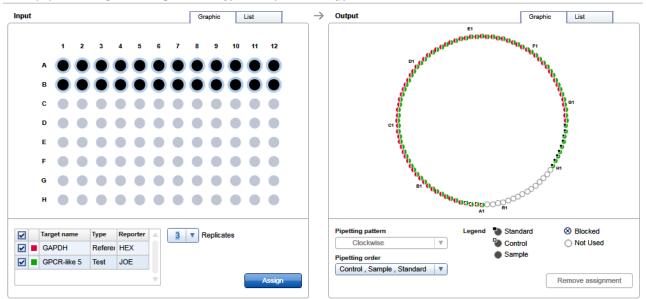
Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 5. Click Assign.
- 6. The software creates assay points on the **Output** panel on the right side of the screen. The selected target(s) are assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the **Pipetting order**.
- 7. Repeat the steps until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- Close the lid.
- 10. Click **Next**. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

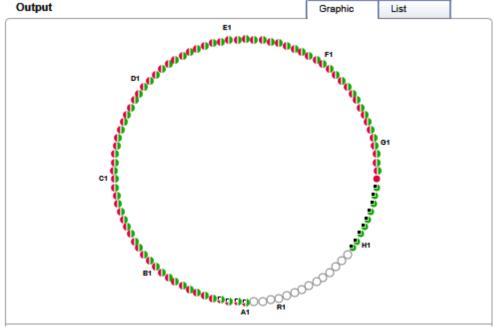
Example:





Both targets have been assigned to the samples of rows A and B, with 3 replicates. Controls have been placed automatically at A1-A4, standards at G5-H1.

In this example, the created assay points for rows A and B have been placed between controls and samples. If you would now select the sample on C1 on the input plate, assign only target GAPDH with one replicate, the software will create one assay point and place it on G5 on the output side. The standards will move automatically to G6-H2:



New assay point placed at G5 (red position).

7.2.3.5.2 Removing target assignment

To remove an assigned target from the output side,

- Select the desired sample position on the **Output** side. Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click on **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side.

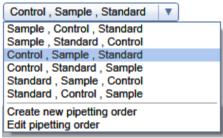
Note: The software removes all replicates of the desired sample(s).

7.2.3.5.3 Creating a new pipetting order

Generally, the pipetting order defines the placement order of the components (samples, controls, standards) on the output side. It can also be used to define blocked positions on the output labware that shall not be used for the run.

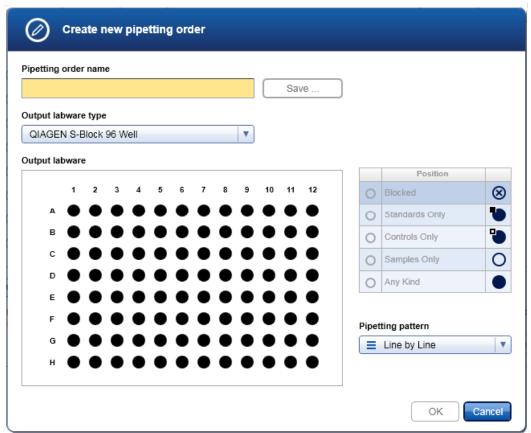
The QlAgility Setup Manager Software comes with a set of predefined pipetting orders, which you find in the **Pipetting order** drop-down menu. At the end of the menu are two commands to **Create a new pipetting order** and to **Edit a pipetting order**. Use this function to define output layouts for reuse. Once defined, they are also listed in the menu, if the same output labware is used.

Pipetting order



- 1. Click on the **Pipetting order** drop-down menu under the output side.
- 2. Click on Create new pipetting order.





- 4. Enter a name for the pipetting order in the **Pipetting order name** field.
- 5. The **Output labware type** menu initially displays the labware that has been selected for the output labware before. This selection needs to be changed only, if you would like to create a pipetting order for another labware. In this case, select the desired labware from the list in the menu, or click on the **Choose other labware** command at the end of the list, in case the desired labware is not listed here. The selected labware will be used as output labware for the run.
- 6. Define the new pipetting order using the labware graphic. All positions are set to **Any Kind** by default:
 - a. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

b. From the **Position** table at the right, click on the button you want to assign to the selected position:

Button	Description
8	Blocked position
	Position for standards
	Position for controls
0	Position for samples
	Any kind

- c. The selected position will be assigned to the selected position. The graphic symbol for the selected positions will be changed accordingly.
- 7. To change the pipetting pattern, select the appropriate command from the **Pipetting pattern** menu (e.g., **Row by Row** or **Column by Column** for rectangular plates).
- 8. Click **Save** to save the pipetting order to a file.
- 9. Click **OK** to close the dialog. The newly created pattern is now available in the **Pipetting order** menu in the **Output panel**.

7.2.3.6 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

The software displays the labware and required minimum volume of reagents to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

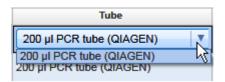
R1 - Reagent block

Pos.	Content	Tube	Req.vol.	Loaded	•
A1	Standard 1	200 μl PCR tube (QIAGEN)	0 μΙ	✓	
A2	Mg2+	200 μl PCR tube (QIAGEN)	111 µl	V	
А3	5 rev	200 μl PCR tube (QIAGEN)	110 µl	V	
A4	5 for	200 μl PCR tube (QIAGEN)	110 µl	V	
A5	5 Probe	200 μl PCR tube (QIAGEN)	110 µl	✓	
A6	GAPDH rev	200 μl PCR tube (QIAGEN)	99 µl	✓	
A7	GAPDH for	200 μl PCR tube (QIAGEN)	99 µl	✓	
A8	GAPDH Probe	200 μl PCR tube (QIAGEN)	99 µl	✓	
B1	Standard Series 5	200 μl PCR tube (QIAGEN)	21 µl	✓	
A	2x Rotor-Gene Multiplex PCR	2.0 ml self standing screw cap tube (969 µl	✓	
В	RNase-Free Water	2.0 ml self standing screw cap tube (285 µl	✓	*

Confirm all

Table of tubes to be loaded to the worktable position R1.

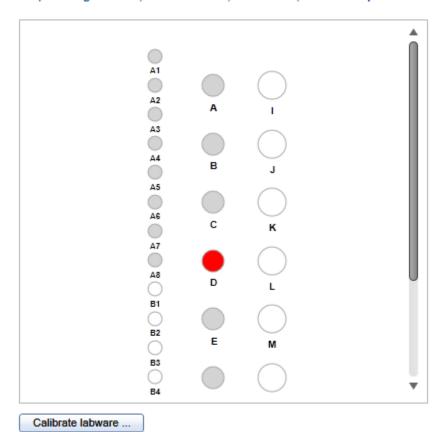
It is possible to change the tube type for a reagent to be loaded.



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that are also marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Tubes from QIAGEN kits are displayed with the same color as their tube caps.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Schematic graphic for R1.

Click the Labware tab to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: QIAGEN S-Block 96 Well

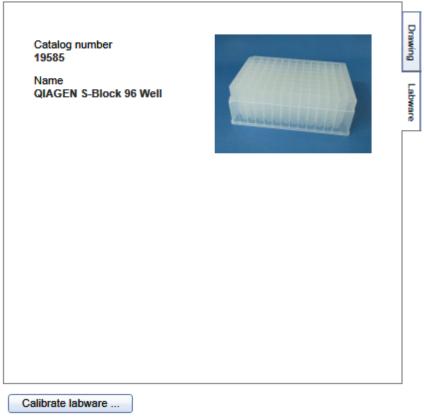
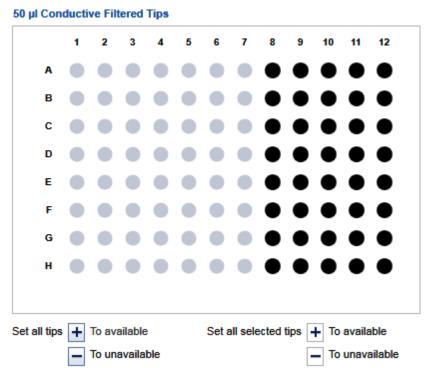


Image and details for input labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



Interactive rack to define tip avilability.

If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate** button.

Preparing the worktable

The worktable preparation starts with the positions for tip racks:

- 1. Open the instrument lid.
- 2. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> holder.

3. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

4. Check that the interactive graphic on the right side of the screen shows the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 5. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 6. Click **Next** to continue with the next worktable position.
- 7. Repeat steps 2–6 for all tip rack positions.

For all other worktable positions:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

8. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps.

Note: Ensure that you have the empty labware available for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility.

- Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 10. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed reagent is provided in the correct tube
 type. The volumes displayed are the minimum volume required, so consider adding extra to
 prevent liquid level detection errors. Load the tube in the correct position of the adapter. For
 orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.
- 11. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.

- 12. Click **Next** to continue with the next worktable position.
- 13. Repeat the steps 8–12 for all worktable positions.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 14. Close the lid.
- 15. Ensure that the tip disposal box is empty.
- 16. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

17. The Run step will be displayed.

7.2.3.7 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only active step of the experiment. All other steps are read only. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

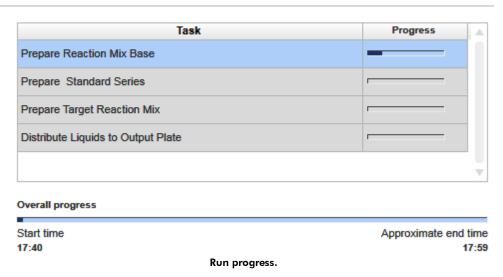
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.3.7.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.3.7.2 Pause/cancel a run

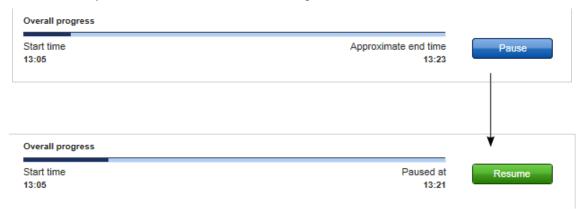
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- Click the Pause button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

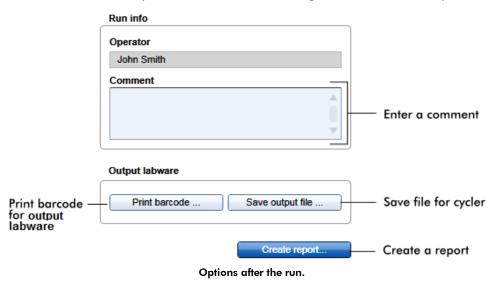
- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

Note: Pauses of a run are listed in the Exceptions/Pauses section of the Advanced report.

7.2.3.7.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen.



Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.
Save output file	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to Customize user settings to define a standard directory.
	The file may serve as sample input file for the Rotor-Gene Q operated with the Q-Rex software. The .xml file can also be used by a LIMS (Laboratory Information Management System). Find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	Note: Depending on the selected cycler, the software also creates a file in the format that is expected by the cycler. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. The input formats of the following cyclers are supported by the first release of the Gene Expression Plug-in: Rotor-Gene Q operated with the Rotor-Gene Software, version 2.3 or higher. Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in the case that the sample input is limited and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Labware Selection step that you are using an existing output labware by selecting the specific option, and load this file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

configure the report options.

7.2.3.8 Report

The QIAgility Setup Manager Software offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The Advanced report contains:

- General information
- Comment
- Kit information
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the name of the kit, the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

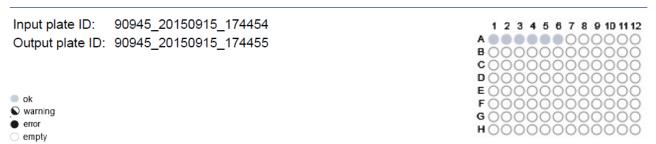
The comment that has been entered on the **Run** screen.

Reaction results

For regular rectangular output plates, the following table and an overview graphic are shown, visualizing each position on the output plate:

Symbol		Description
	ok	The position was processed without any exceptions.
•	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
•	not used	This symbol is only used if an output file has been loaded as a starting point for the output plate (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results



Note: If no input or output file has been loaded, the software automatically creates identifiers for the input and output labware.

A table listing the reaction result for each processed position on the output labware is also shown:

Column	Description
Output	The position on the output labware.
Identifier	Specifies the sample ID or the name of the particular standard or control.
Type	One of Sample, Standard or Control.
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls.
Concentration	For standards, the concentration of the standard is listed. For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty. Always empty for controls or NTC.
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column	Description	
Exception	The event that has been displayed during run.	
Position	Lists the positions that were affected by the exception.	
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.	
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.	
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).	
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as possibly affected by an exception, then you can trust that the output position was completed successfully.	
User response	A short form, which option (button) of the message dialog has been pressed.	

Note: The **Basic** report does not list pauses. If you want to have also pauses listed, use the **Advanced** report, instead.

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

General information

Kit information is not listed here for the **Advanced** report; instead, this information is provided in more detail in a separate section.

Kit information

Kit name, lot number and expiration date. Comment provided with the kit.

Note: The Advanced report does not repeat this information in the General information section.

Reaction results table of the Advanced report

In addition to information provided in the **Basic** report, the **Reaction results** table of the **Advanced** report contains two additional columns:

Output	Identifier	Туре	Input	Actual content	Concentr ation	Final volume µl	Flag
A1	NTC GAPDH + GPCR-like 5	NTC		18 µl Mix1 [GAPDH,GPCR-like 5] + 2 µl RNase- Free Water		20	ok
G4	24	Sample	B12	18 µl Mix1 [GAPDH,GPCR-like 5] + 2 µl 24		20	ok
G5	Standard 1	Standard		18 µl Mix2 [GPCR- like 5] + 2 µl Standard 1	100 copies/µl	20	ok

Column	Description
Actual content	Lists the components. The software creates automatic names for each created reaction mix. The names of the targets used are given in brackets [].
	Examples:
	Output position A1 is the NTC for both targets and contains 18 μ l of the reaction mix Mix1 for GAPDH and GPCR-like 5, and 2 μ l RNase-free water.
	Output position G4 contains 18 μ l of the reaction mix Mix1 for GAPDH and GPCR-like 5, and 2 μ l of sample 24 which was on input position B12.
	Output position G5 is the first standard of the standard series and contains 18 μ l of the reaction mix Mix2 for target GPCR-like 5 and 2 μ l of the created first standard.
Final volume	Lists the final volume.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description	
Exception	The event or pause that has been displayed during run.	
Position/Duration	For an event:	
	Lists the positions that were affected by the exception.	
	First, the position is listed for which the exception occurred. After "Destination", all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.	
	The $@$ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.	
	Example:	
	"M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).	
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.	
	For a pause: the duration of the pause.	
User response	A short form, which option (button) of the message dialog has been pressed.	

Sample information

Lists the sample information as it was entered in the sample editor or loaded by the sample input file, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Detailed run information of the Advanced report

In addition to the run task list included in the **Basic** report, the assay setup information is also listed:

Assay Setup

Reaction mix [Target(s)]	Content
Mix1 [GAPDH,GPCR-like 5]	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 76 μl RNase- Free Water + 76 μl GAPDH rev + 76 μl GAPDH for + 76 μl 5 rev + 76 μl 5 for + 76 μl GAPDH Probe + 76 μl 5 Probe
Mix2 [GPCR-like 5]	850 µl 2x Rotor-Gene Multiplex PCR Master Mix + 85 µl Mg+ + 36 µl RNase- Free Water + 9 µl 5 rev + 9 µl 5 for + 9 µl 5 Probe

This provides detailed information about each mix that was automatically created by the QIAgility. Information about standard series is also provided if they were used in the run.

Advanced instrument information

This section lists not only the series numbers of the QIAgility and the pipettor head, but also information about HEPA filter usage, service due dates and volume calibration.

7.2.4 Working with experiments for pathogen detection

The Pathogen Detection Plug-in enables easy and convenient qPCR setup of your pathogen testing experiments using dedicated lines of QIAGEN kits including QuantiTect, QuantiFast and QuantiNova.

New experiments for pathogen detection are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Reaction Setup** step will initially be enabled. All other steps will become available once the required information has been entered in the **Reaction Setup** step. The coloring of the **Targets** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 8 steps that guide you through the run setup process:

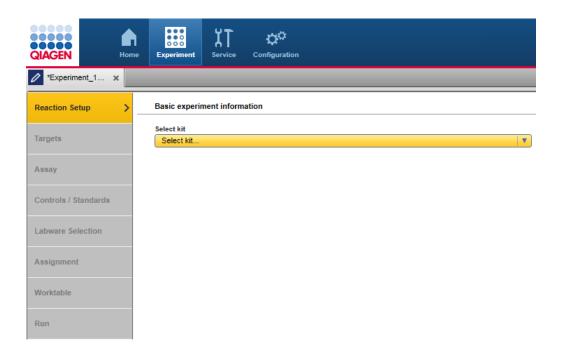
- 1. Reaction setup
- 2. Targets
- 3. Assay
- 4. Controls/standards
- 5. Labware selection
- 6. Assignment
- 7. Worktable
- 8. Run

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. The file extension for pathogen detection experiments is *.qsmpx, and for templates *.qsmpt (QlAgility Setup Manager Pathogen detection Experiment, and Template, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.4.1 Reaction Setup step

The **Reaction Setup** step is used to make basic selections, such as the kit to be used, the preferred cycler and the volume definitions. When you create a new experiment, the **Reaction Setup** step will be the only active step, and within this step, only the **Select kit** menu will be active. The other controls will initially remain inactive, but will become visible once you have selected the kit to be used in the reactions.



1. Select the kit to be used from the **Select kit** drop-down menu.



2. The **Kit information** and **Select cycler** fields are displayed.



3. Optionally, enter the **Lot number** and the **Kit expiry date** in the corresponding fields for documentation purposes.

- 4. Select the cycler to be used from the **Select cycler and output format** drop-down menu.
- 5. If the selected cycler requires a passive reference, the **Passive reference** option will be enabled. Select this option to use passive reference.

Note: If the selected kit contains one or more passive references, and the passive reference option is selected, the software will automatically add the passive reference according to the cycler you select to the additional reagents list (see <u>Target step</u>) along with the recommended volume for your setup.

6. The **Volume definitions** area is displayed. The fields may vary depending on your specific kit and cycler selection.



Note: The default values that appear in the volume fields are the recommended values from the kit handbook for your setup.

7. Enter the volume data in the corresponding fields:

Field	Description
Sample input volume	Enter sample input volume. This is the volume of sample, controls and standards that will be added to the assay mix.
Total reaction volume	Enter the total reaction volume. This is the volume of the final reaction mix including assay mix and sample, control or standard.

8. Check the box next to **Use ready-made assay mixes** to confirm that you have already prepared the assay mixes containing all the components. The assay mix must include the target components, the passive reference (if required by your cycler), and all other reagents. Enter the assay mix volume per reaction.

Later in the worktable step, you will be asked to place these already prepared mixes onto the worktable. During the run, the QlAgility will use the mixes directly for the assay points and add only water if the assay mix volume per reaction plus the sample input volume is less than than the total reaction volume you specified.

9. Click **Next** to proceed.

7.2.4.2 Targets step

The **Targets** step is used to define targets, internal controls and, optionally, additional reagents.

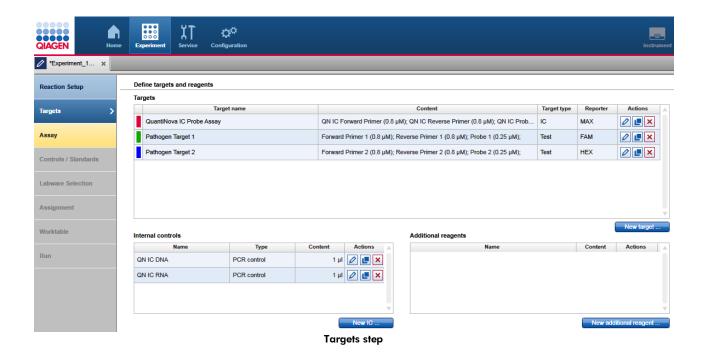
<u>Define primer-probe mixes or single components</u> for the targets. All defined targets and primer-probe mixes are listed in the **Targets** tables. At least one target needs to be defined.

<u>Define internal controls</u> (PCR or process controls) that will be used. All defined internal controls are listed in the **Internal controls** table.

If the selected kit contains an internal control (IC), the corresponding IC target and internal control(s) are already predefined.

Optionally, <u>additional reagents</u> can <u>be defined</u> to be added to all reaction mixes. Defined reagents are listed in the **Additional reagents** table. If the selected kit contains one or more passive references, and the passive reference option is selected, the software will automatically add the passive reference according to the cycler you selected to the additional reagents list with the recommended volume for your setup. If you selected the option **Use ready-made assay mixes**, the definitions in the **Additional reagents** table are for documentation purposes, only. Ensure that you include all listed reagents already in the ready-made assay mixes, because the QIAgility will not add them to the assay points during run.

Click Next to proceed.



7.2.4.2.1 Defining targets and mixes

You can choose whether the QIAgility should use single components (primers and probes) or ready-made primer-probe mixes.

To define:

- a new primer-probe mix for one or several targets
- or single components for one target

proceed as follows:

- 1. Click on **New target** below the **Targets** table.
- 2. The **Add target** dialog will be displayed. Refer to <u>Add target dialog</u> for details.
- 3. New rows for the defined primer-probe mixes or single components for the targets will be added to the **Targets** table.



The buttons in the **Actions** column of the **Targets** table have the following functions:

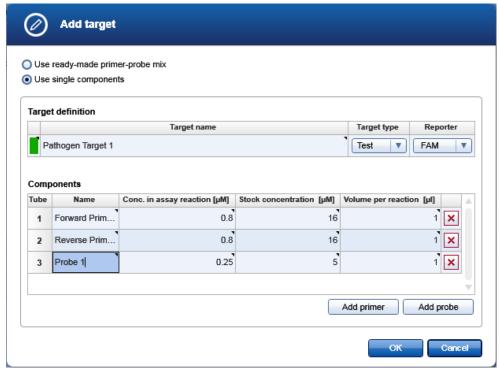
Button	Action	Description
0	To edit a target	The Edit target dialog will open, displaying the values that have been entered in the Add target dialog.
<u>e</u>	To duplicate a target	The Duplicate target dialog will open, displaying the values that have been entered for the original target. The target names will initially display a copy of the target.
×	To delete a target	Deletes the row in the Targets table.

Add target dialog 7.2.4.2.2

The **Add target** dialog contains two major options at the top:

Use ready-made	Select this option if you intend to load a ready-made primer-probe mix in one single tube for the run.
primer-probe mix	You can define several target names. There is also the option to define the components for documentation purposes.
Use single components	Select this option if you intend to load primers and probes in individual tubes for the run.
	Define one target name and the components (primers and probes) related to this target name.

Note: If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, the options are not displayed. Proceed with the steps **To define a ready-made primer-probe mix**, below.



Dialog to load primers and probes in individual tubes for the run.

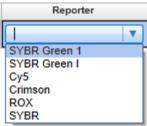
To define single components for one target

- 1. Select the option **Use single components**.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type	Description
Test	Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:



Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, save the output file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- 5. In the **Components** table, define the primers and the probe (steps 6–10).
- 6. Click on **Add primer** to add a primer or click on **Add probe** to add a probe.
- 7. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

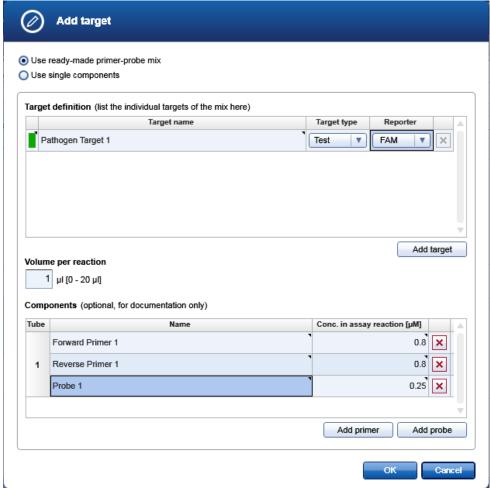
Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 8. Enter the name of the primer or probe.

 The QIAgility Setup Manager Software will prompt you in later steps to place a tube with the name of the primer or probe onto the worktable.
- Enter the Volume per reaction of the primer or probe.
 Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the Volume per reaction.
- 10. To define all components, repeat steps 6–10.
- 11. If the single components of the target have been defined, click **OK**. The dialog will close.
- 12. The target will be listed in the **Targets** table.

Note: The software automatically assigns a color to each defined target name. If you would like to select a different color, you can click on the colored rectangle in the first column to open the **Colors** dialog:





Dialog to load a ready-made primer-probe mix in one single tube for the run.

To define a ready-made primer-probe mix

- 1. Select the option Use ready-made primer-probe mix.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.

Note: To name a target mix, you can either choose one name that indicates the target mix name, or you can define several different target names. In the software, these targets will always be handled together, because they are part of the same target mix. The QIAgility Setup Manager Software will prompt you in a later step to place a tube with the name of the target mix onto the worktable. If you defined several different target names, the software will display a list of the targets for that tube.

3. Click on the menu in the **Target type** column and select a target type:



Target type	Description
Test	Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:



Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705

LIGHTCYCLERRED640

ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- Optional step: To add further target names, click on Add target and repeat the steps 2-5.
 Note: After you have created at least two targets, a Delete icon (x) will be displayed in the last column of each target row. Click on it if you would like to delete a target.
- Enter the volume per reaction of the primer-probe mix in the Volume per reaction field.
 Note: If you selected the option Use ready-made assay mixes in the Reaction Setup step, this field is not available.
- 7. In the **Components** table, you can define the primers and the probe (steps 8–12). **Note:** Steps 8–12 are optional.
- 8. Click on **Add primer** to add a primer or click on **Add probe** to add a probe.
- 9. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 10. Enter the name of the primer or probe.
- 11. Enter the **Concentration in the assay reaction** in the corresponding cell.
- 12. **Optional step:** To add further primers or probes, repeat steps 8–11.

- 13. If the target or primer-probe mix has been defined, click **OK**. The dialog will close.
- 14. The primer-probe mix will be listed in the **Targets** table. The targets defined in the dialog share the same **Content** cell.

7.2.4.2.3 Defining internal controls

The QIAgility Setup Manager Software supports two kind of internal controls, the **PCR Internal Control** (PCR IC) and the **Process Internal Control** (Process IC).

Use PCR Internal Control to control for successful amplification and to exclude, for example, the
presence of PCR inhibitors. The PCR IC assumes that you include an internal control DNA/RNA as a
reagent and that you define the corresponding internal control target.

On the <u>Assay step</u>, define an assay that combines the PCR IC with the internal control target and the actual test target.

On the <u>Assignment step</u>, assign the defined assay to the samples. The QlAgility Setup Manager Software will prompt you later in the <u>Worktable step</u> to load the tube with the internal control DNA/RNA. During the run, the QlAgility will add it directly to the reaction mix of that assay.

Note: The use of PCR ICs will result in an internal control signal detection in the no-template control (NTC) reactions.

• Use **Process IC** to control the whole workflow, from DNA/RNA purification to amplification. This option requires that you add the Process IC during sample lysis.

On the <u>Assay step</u>, define an assay that combines the Process IC with the Internal Control target and the actual test target.

On the Assignment step, assign the defined assay to all samples that contain the Process IC.

Note: No internal control signal will be detected in the NTC reactions, but will be detected in the sample reactions.

Note: Make sure that the corresponding target is defined in the **Targets** table.

To define a new internal control:

- 1. Click **New IC** below the **Internal controls** table.
- 2. The Add internal control dialog will be displayed. Refer to Add internal control dialog for details.
- 3. New rows for the defined internal controls will be added to the **Internal controls** table.

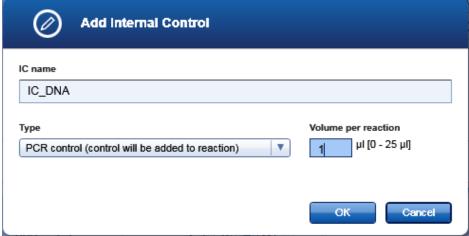


The buttons in the **Actions** column

of the Internal controls table have the following functions:

Button	Action	Description
0	To edit an internal control	The Edit internal control dialog will open, displaying the values that have been entered in the Add internal control dialog.
E	To duplicate an internal control	The Duplicate internal control dialog will open, displaying the values that have been entered for the original internal control. The internal control name will initially display a copy.
×	To delete an internal control	Deletes the row in the Internal controls table.

7.2.4.2.4 Add internal control dialog

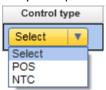


Dialog to define an internal control.

To define an internal control

1. Enter a name for the internal control. The **IC name** field will be colored yellow as a reminder that this entry is required.

2. Click on the **Type** menu and select a type. The menu will be colored yellow as a reminder that this entry is required.



Target type	Description
PCR control	Use this internal control type to test for successful amplification.
	The QIAgility Setup Manager Software will prompt you in a later step to place a tube with the IC name onto the worktable. During the run, the QIAgility will add it to the reaction mix.
Process control	Use this internal control type to control the whole workflow, from DNA/RNA purification to amplification.
	You must have already added the Process IC during the sample lysis step.

For details about internal control types, refer to Defining internal controls.

- 3. If you selected **PCR control**, enter the **Volume per reaction** of the internal control.
- 4. Click **OK**. The dialog will close.
- 5. The internal control will be listed in the **Internal controls** tables.

7.2.4.2.5 Defining additional reagents

Additional reagents can be managed (added, duplicated or deleted) using the **Additional reagents** table at the bottom. The table lists the reagent name and the volume per reaction. During the run, the QIAgility will add all defined additional reagents to all assay mixes.

Adding an additional reagent

- 1. To add a new reagent click on **New additional reagent**.
- 2. The **Add additional reagent** dialog will open. For details, refer to section <u>Add additional reagent</u> dialog.
- 3. The reagent will be displayed as an additional row in the **Additional reagents** table.

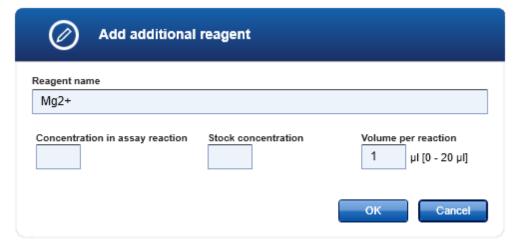
Actions

The buttons in the **Actions** column functions:

of the **Additional reagents** table have the following

Button	Action	Description
0	To edit a reagent	The Edit additional reagent dialog will open, displaying the values that have been entered in the Add additional reagent dialog.
	To duplicate a reagent	The Duplicate additional reagent dialog will open, displaying the values that have been entered for the original reagent. The reagent names will initially display a copy of the reagent.
×	To delete a reagent	Deletes the row in the Additional reagents table.

7.2.4.2.6 Add additional reagent dialog



Dialog to enter an additional reagent

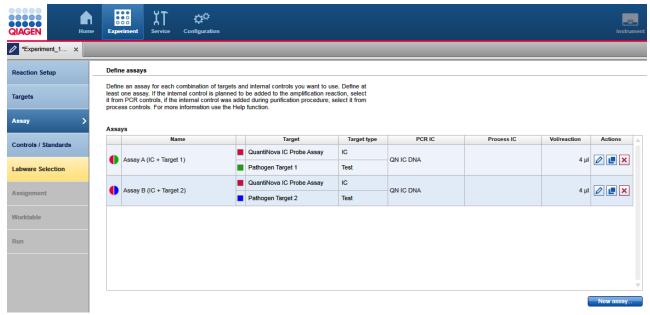
- 1. Enter the reagent name.
 - The QlAgility Setup Manager Software will prompt you in a later step to place a tube with the reagent name onto the worktable.
- 2. Enter the **Volume per reaction**. Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.
- 3. Click **OK**.
- 4. The reagent will be displayed as an additional row in the **Additional reagents** table.

Note: If you selected the option **Use ready-made assay mixes**, the definitions of **Additional reagents** are for documentation purpose only. Ensure that you include all listed reagents already in the ready-made assay mixes, because the QIAgility will not add them to the assay points during run.

7.2.4.3 Assay step

The **Assay** step is used to <u>define one or more assays</u>, based on the defined targets and internal controls from the previous <u>Targets step</u>. All defined assays are listed in the **Assays** table. At least one assay must be defined.

Click Next to proceed.



Assay step

Note: If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, the **Volume per reaction** cell will be empty.

7.2.4.3.1 Defining assays

An assay consists of one or more targets that were defined in the previous <u>Targets step</u>. An assay may also include one PCR IC and several Process ICs. If you want to use several combinations of targets and internal controls, several assays need to be defined: one for each combination of targets and internal controls, without taking into account the assignment of the samples. Samples can be defined and assigned in later steps (<u>Labware selection</u> and <u>Assignment</u> step).

Note: An assay allows simultaneous amplification of all defined targets within one reaction. However, for optimal performance of a multiplex assay, or if an internal control assay is used, special considerations need to be made with respect to primer and probe design and quality. Follow the guidelines provided in the kit handbook of the kit you are using for more information.

It is possible to define more assays than you actually need for one run. This can be helpful if you intend to prepare a general template for several test variants. For more information about templates and experiments, refer to the Experiments and templates concept.

To define a new assay, proceed as follows:

- 1. Click on **New assay** below the **Assays** table.
- 2. The **Add assay** dialog will be displayed. Refer to <u>Add assay dialog</u> for details.
- 3. New rows for the defined assays will be added to the **Assays** table.

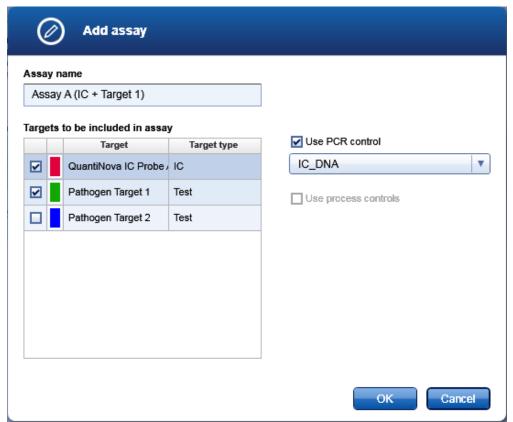


The buttons in the **Actions** column

of the **Assays** table have the following functions:

Button	Action	Description
0	To edit an assay	The Edit assay dialog will open, displaying the values that have been entered in the Add assay dialog.
	To duplicate an assay	The Duplicate assay dialog will open, displaying the values that have been entered for the original assay. The assay name will initially display a copy.
×	To delete an assay	Deletes the row in the Assays table.

7.2.4.3.2 Add assay dialog



Dialog to combine targets and internal controls into assays

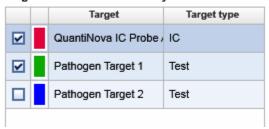
To define an assay:

1. Enter an assay name. The **Assay name** field will be colored yellow as a reminder that this entry is required.

If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, the QIAgility Setup Manager Software will prompt you in a later step to place a tube with the assay name onto the worktable.

2. Select the targets that will be combined in the assay.

Targets to be included in assay



To select a target or target mix, check the box next to the target color(s). The checked targets or target mixes will become part of the assay. Later in the <u>Assignment step</u>, when assays are assigned to samples, these targets will be assigned together to the samples, because they are part of the assay.

3. Optional step: If a PCR IC will be used in the assay, check the box next to Use PCR control.

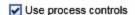


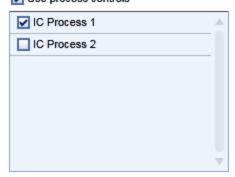


Then select the particular PCR IC from the PCR ICs that were defined in the previous **Targets** step. Only one PCR IC can be used in a particular assay.

Note: If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, you can use this option for documentation purposes. Ensure that you include the PCR IC already in the ready-made assay mix, because the QIAgility will not add it during run.

4. Optional step: If a Process IC will be used in the assay, check the box next to Use Process control.





Check the box next to the name of the Process IC that should be used in the assay. Several Process ICs can be used within one assay. Make sure that the Process IC was already added during sample lysis.

- 5. Click **OK**. The dialog will close.
- 6. The assay will be listed in the **Assays** table.

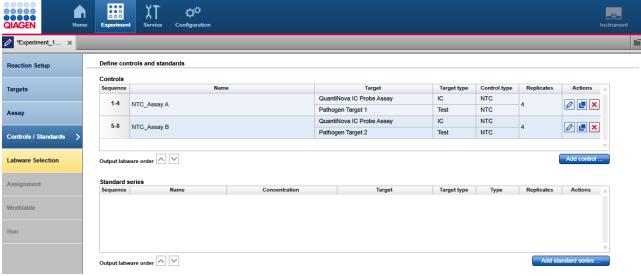
7.2.4.4 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Adding a control or standard series

- Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will be opened. For details, refer to <u>Add control</u> dialog or <u>Add standard series dialog</u>, respectively.
- The control will be added to the Controls table, and the standard series will be added to the Standard series table.
- 4. Click **Next** to proceed.



Controls/Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
<u></u>	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

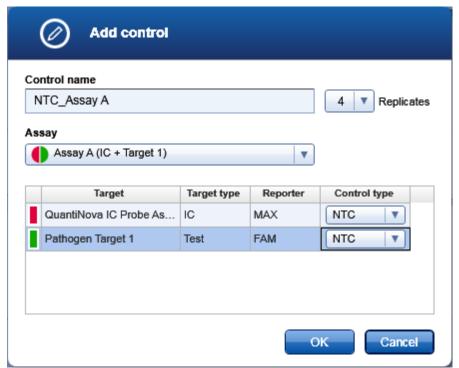
Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output plate will be based on the order indicated in these tables.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.4.4.1 Add control dialog

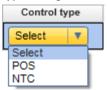


NTC for Assay A

- 1. Enter a control name in the **Control name** field.
- Select the number of replicates, using the Replicates drop-down menu next to the Control name field.
 Note: The replicates are listed in an editable menu. If the number of replicates you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section Entering data.
- 3. Select an assay from the Assay menu:



4. The software lists the defined targets and target mixes of the assay in the table below. Select the control type using the drop-down menu in the **Control type** column:

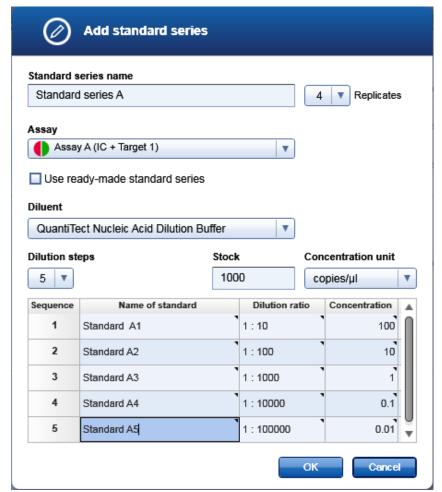


Option	Description
POS	A positive control will be added to the assay points.
NTC	No-template control. Water from the kit will be added to the assay points.

Note: The use of PCR ICs will result in an Internal Control signal detection in the NTC reactions.

- 5. Click **OK** to confirm and close the **Add control** dialog.
- 6. The control will be added to the **Controls** table.

7.2.4.4.2 Add standard series dialog



Dialog for standard series

- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select the number of replicates, using the **Replicates** drop-down menu next to the **Standard series** name field.

Note: The replicates are listed in an editable menu. If the number of replicates you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering data</u>.

- 3. Select an assay from the **Assay** drop-down menu.
- Check the option Use ready-made standard series, if you intend to load the ready-made dilutions individually for the run.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

- 5. **Optional:** if the option **Use ready-made standard series** has not been checked, select in the **Diluent** drop-down menu which diluent the QIAqility should use to create the dilutions.
- Select the number of dilution steps from the **Dilution steps** menu.

 Dilution steps



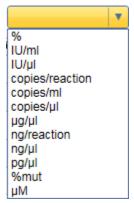
The number of rows in the table at the bottom is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> data.

Note: In order to use the actual stock as the first standard, define the first standard to have the same concentration as the stock (dilution ratio will be 1:1 in this case). If the option **Use ready-made standard series** has not been checked, the QIAgility will directly use the stock as template for the assay points of this standard.

7. If the option **Use ready-made standard series** has not been checked, enter the stock concentration in the **Stock** field and select the concentration unit using the **Concentration unit** drop-down menu.

Concentration unit



8. For each dilution step, enter either a dilution ratio or a concentration. The other value will be calculated automatically by the software. Optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series.

Note: The dilution ratio refers to the stock concentration.

Note: Concentration values lower than 0.01 will be displayed in scientific format, e.g., 1.00E-03 represents 0.001.

Note: This step is optional if the option Use ready-made standard series has been checked.

- 9. Click **OK** to confirm and close the **Add standard series** dialog.
- 10. The standard series will be added to the **Standard series** table.

7.2.4.5 Labware Selection step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

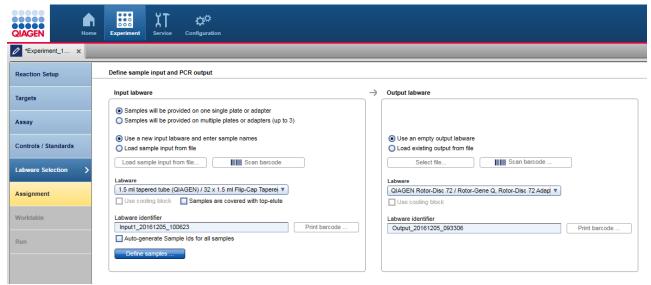
The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side.

Proceed as follows:

Select the input labware and define the samples.
 If the samples will be on a single plate or adapter, check the option Samples will be provided on one single plate or adapter on the left side of the screen and follow the steps in <u>Defining one input labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters (up to 3)** and follow the steps in <u>Defining up to 3 input labware</u>.

- 2. Define the output labware.
- 3. Click **Next** to proceed.

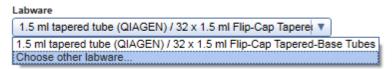


Labware Selection step

7.2.4.5.1 Defining one input labware

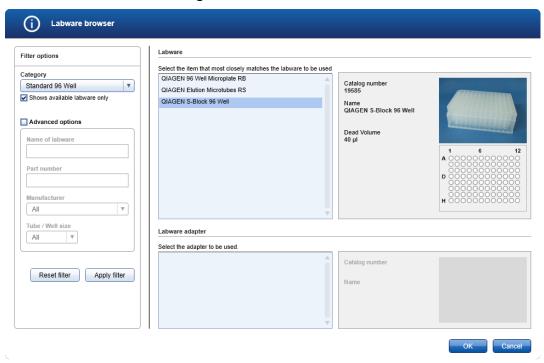
If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

- Select Use a new input labware and enter sample names and proceed to step 2.
 Alternatively, select Load sample input from file to use a sample input file and proceed to step 11.
- 2. Click on the **Labware** drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.



4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Optional step: If you would like to use the cooling block for the input labware, check the option Use cooling block next to Labware.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

6. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QlAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Editing sample names.
- 10. Proceed with step 2 in Labware Selection step.

To define samples by using a sample input file, follow these steps:

11. If the option Load sample input from file has been checked, the Load sample input from file and Scan barcode buttons will be enabled.

Use a new input labware and enter sample names Load sample input from file			
Load sample input from file	Scan barcode		

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

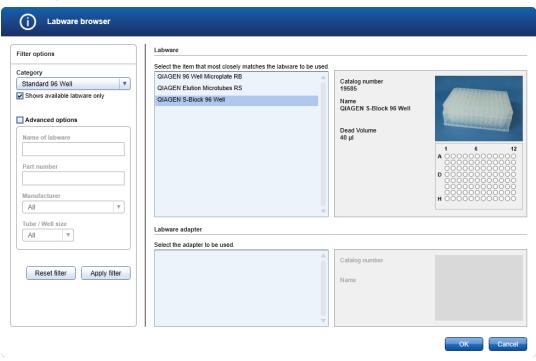
The software will load the sample information from the sample file.

13. If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the Labware drop-down menu. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the **Labware** browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

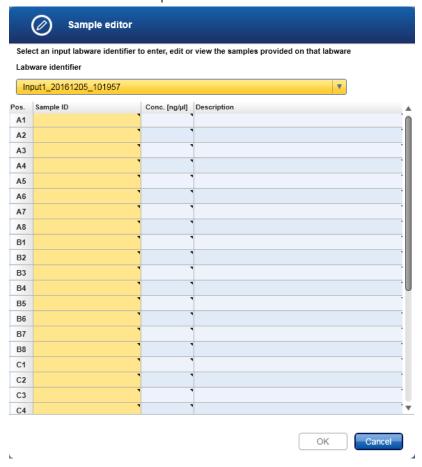
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- 16. Optional step: Edit the sample information. If the sample editor does not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor. For details about the sample editor refer to Editing sample names.
- 17. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QlAgility will pipet the samples accordingly.
 - Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.
 - **Note:** Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.
- 19. Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in Labware Selection step.

7.2.4.5.2 Editing sample names

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



Note: For more details how to enter information into a table refer to the section **Entering data**.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.4.5.3 Defining up to three input labware

If the option Samples will be provided on multiple plates or adapters (up to 3) has been selected, you can define up to three input labware types.

For each input labware, you can decide whether to load the sample names from a file or to define the input labware manually. All defined input labware will be listed in the **Sample input labware** table.

1. Click the **Add new** button to define the labware manually. The **Add new input labware** dialog opens. Proceed with step 2.

Alternatively, click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode</u> dialog.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case proceed with step 2. Otherwise, proceed with step 3.

- 2. Follow the steps in Add input labware dialog.
- 3. Repeat steps 1 and 2 until all input labware have been defined.
- 4. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Note: This step is optional if the sample names were loaded from a file or automatically generated.

5. Proceed with step 2 in Labware Selection step.

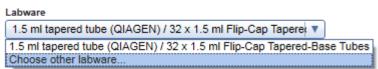
The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

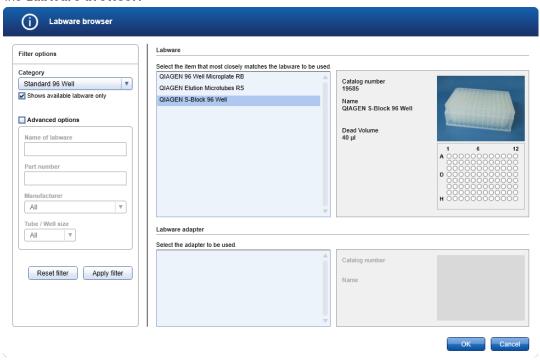
7.2.4.5.4 Add input labware type dialog

To define a new input labware:

- 1. Click on the **Labware type** drop-down menu.
- All previously selected labware types will be listed. The Choose other labware option will be listed last.



Select your input labware in the drop-down list. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the input labware, check the option **Use** cooling block next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

5. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QlAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

7. Optional: Click the Print barcode button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. If you want the software to automatically generate sample names for all positions on the labware, check the option **Auto-generate sample IDs for all samples**.

Note: This option will not be available if the dialog was opened using the **Load sample input from file** or the **Scan barcode** button.

9. Click **OK** to close the dialog.

7.2.4.5.5 Defining output labware

To define the output labware:

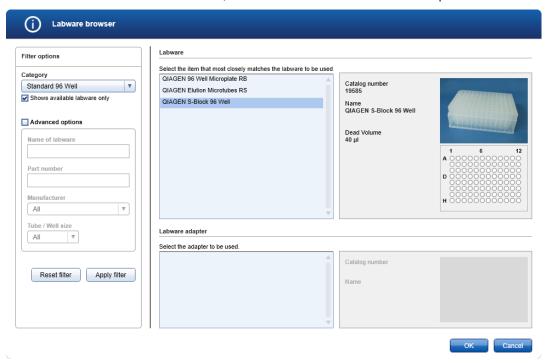
1. You can either **Use an empty output labware** or **Load existing output from file**. The option **Use an empty output labware** will be selected by default and can be used for most runs.

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case select the option **Loading an existing output from file**, and proceed to step 6.

 Select the output labware from the Labware menu on the right. This menu includes all labware suitable for the selected cycler. An appropriate adapter is automatically added if one has been marked as available in the labware manager.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

If the labware to be used is not listed, select Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Labware**.
- 4. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.

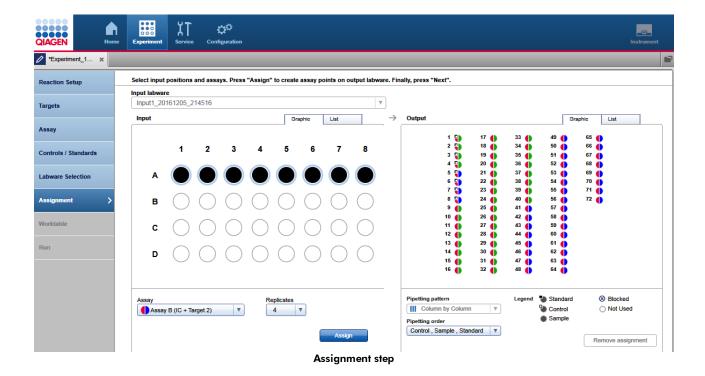
Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

- The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.
- 7. Click the **Select file** button and select the desired labware file. For details, refer to File dialog.
- 8. Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>. Using the information from the loaded file, the software will automatically select the **Labware**. In addition, the positions that were used with this labware for a previous run will be blocked.
- 9. Click **Next** to proceed.

7.2.4.6 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-assay combinations to the output labware.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create assay points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this

sequence is used when assigning selected samples to the output labware.

At the bottom of the panel is a menu that lists all assays defined in the **Assays** step, along with a **Replicates** menu.

In order to assign a sample for assay setup, select the sample position(s) and then the assay you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the assay will be assigned to assay points created on the output labware. Each assay assigned to the output labware will result in the setup of an assay mix. Follow the procedure described in Assigning samples with assays to the output plate.

If the option Samples will be provided on multiple plates or adapters has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. When entering the screen, the software automatically places all standards and controls from the **Controls/Standard** step. The creation of assay points containing samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the targets. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over one target color of an output position, the software displays the sample ID, the target and the target type for that position. Assay points containing samples can be removed. For details, refer to Removing assay assignments.

Output Graphic List Pos. Sample ID Assay Conc. [µM] NTC_Assay A Assay A (IC + Target 1), Control 1 NTC_Assay A Assay A (IC + Target 1), Control 2 NTC_Assay A Assay A (IC + Target 1), Control 3 Assay A (IC + Target 1), Control NTC_Assay A NTC_Assay B Assay B (IC + Target 2), Control 5 NTC_Assay B Assay B (IC + Target 2), Control 6 NTC_Assay B Assay B (IC + Target 2), Control 7 NTC_Assay B Assay B (IC + Target 2), Control Sample 1 Assay A (IC + Target 1) 9 Sample 1 Assay A (IC + Target 1) 10 Sample 1 Assay A (IC + Target 1) 11 Sample 1 Assay A (IC + Target 1) Pipetting pattern Blocked Legend Standard Column by Column ∇ Not Used Control Sample Pipetting order Control, Sample, Standard

Use the **List** tab of the output panel to view the assay points that have been created.

The List tab of the Output panel. Four replicates of the NTC controls for Assay A and B are placed at 1-8. Assay A has been assigned to sample 1, with four replicates at 9-12.

The two menus at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column by column) and the **Pipetting order** (e.g., first standards, then controls and then samples). You can also define a <u>new pipetting order</u>.

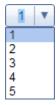
Remove assignment

7.2.4.6.1 Assigning samples with assays to the output labware

Proceed as follows:

 From the Input labware menu on the left side of the screen, select an input labware that was defined in the previous Labware selection step. The software will display its defined samples below in the Input panel.

- 2. From the **Input** panel, select the sample position for which you would like to assign an assay.
 - **Note:** To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.
- 3. From the **Assay** menu below, select the assay you want to assign.
- 4. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



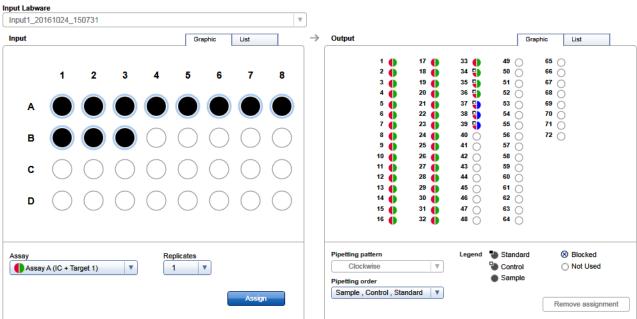
Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 5. Click Assign.
- 6. The software creates assay points on the **Output** panel on the right side of the screen. The selected assay is assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the **Pipetting order**.
- 7. Repeat the steps until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 9. Close the lid.
- 10. Click **Next**. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

Example:

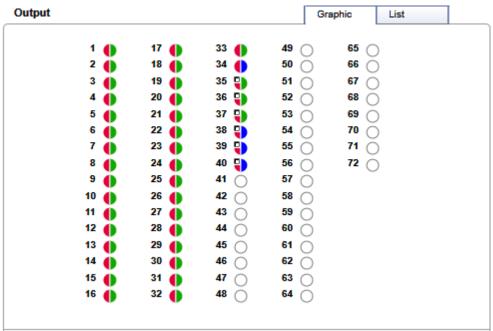
Select input positions and assays. Press "Assign" to create assay points on output labware. Finally, press "Next".



Assay A (IC + Target 1) has been assigned to all samples, in replicates of 3. Controls have been placed automatically at 34–39.

Standards were not defined.

In this example, the assay points created for all samples with the assay "A (IC + Target 1)" have been placed on the output side before the controls. If you now select the sample in position B1 of the input labware and assign assay "B (IC + Target 2)" with one replicate, the software will create one assay point and place it in position 34 on the output side. The controls will move automatically to positions 35–40:



New assay point placed at position 34 (red/blue position).

7.2.4.6.2 Removing assay assignments

To remove an assigned assay from the output side, follow these steps:

- Select the desired sample position on the **Output** side. Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side.

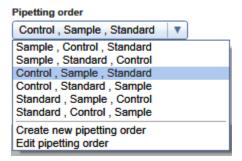
Note: The software will remove all replicates of the desired sample(s).

7.2.4.6.3 Creating a new pipetting order

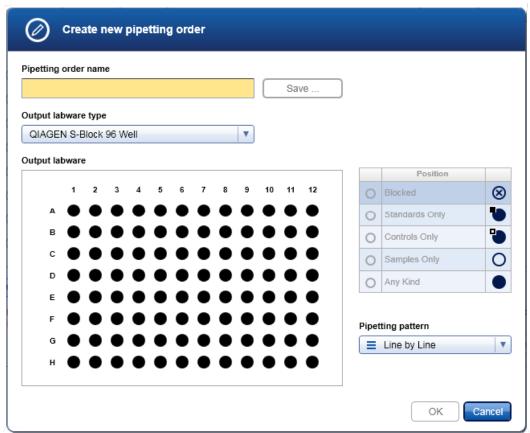
Generally, the pipetting order defines the placement order of the components (samples, controls, standards) on the output side. It can also be used to define blocked positions on the output labware that shall not be used for the run.

The QlAgility Setup Manager Software comes with a set of predefined pipetting orders, which you find in the **Pipetting order** drop-down menu. At the end of the menu are two commands to **Create a new pipetting order** and to **Edit a pipetting order**. Use this function to define output layouts for reuse. Once defined, they

are also listed in the menu, if the same output labware is used.



- 1. Click on the **Pipetting order** drop-down menu under the output side.
- 2. Click on Create new pipetting order.
- 3. The Create new pipetting order dialog is shown:



- 4. Enter a name for the pipetting order in the **Pipetting order name** field.
- 5. The **Output labware type** menu initially displays the labware that has been selected for the output labware before. This selection needs to be changed only, if you would like to create a pipetting order for another labware. In this case, select the desired labware from the list in the menu, or click on the

Choose other labware command at the end of the list, in case the desired labware is not listed here. The selected labware will be used as output labware for the run.

- 6. Define the new pipetting order using the labware graphic. All positions are set to **Any Kind** by default:
 - a. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

b. From the **Position** table at the right, click on the button you want to assign to the selected position:

Button	Description
8	Blocked position
	Position for standards
	Position for controls
0	Position for samples
	Any kind

- c. The selected position will be assigned to the selected position. The graphic symbol for the selected positions will be changed accordingly.
- 7. To change the pipetting pattern, select the appropriate command from the **Pipetting pattern** menu (e.g., **Row by Row** or **Column by Column** for rectangular plates).
- 8. Click **Save** to save the pipetting order to a file.
- 9. Click **OK** to close the dialog. The newly created pattern is now available in the **Pipetting order** menu in the **Output panel**.

7.2.4.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.



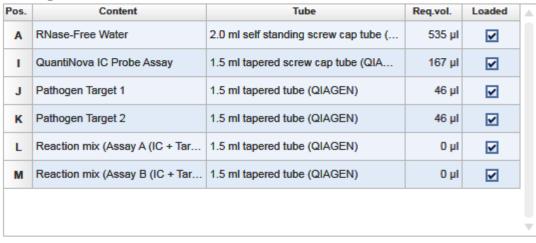
Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

The software displays the labware and required minimum volume of reagents to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

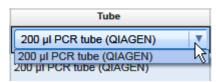
R1 - Reagent block



Confirm all

Table of tubes to be loaded to the worktable position R1.

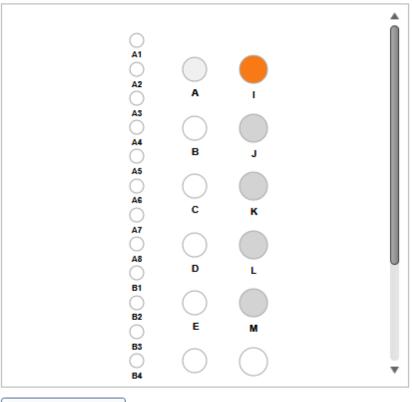
It is possible to change the tube type for a reagent to be loaded.



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Tubes from QIAGEN kits are displayed with the same color as their tube caps.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Calibrate labware ...

Schematic graphic for R1.

Click the **Labware** tab to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

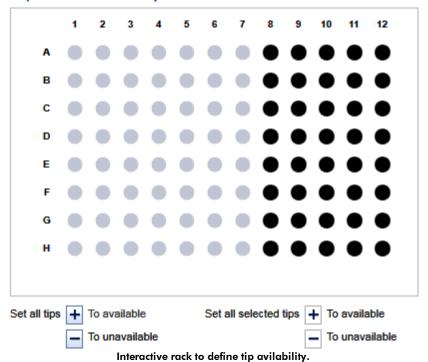
Labware: 1.5 ml tapered tube (QIAGEN)
Adapter: 32 x 1.5 ml Flip-Cap Tapered-Base Tubes



Image and details for input labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

The worktable preparation starts with the positions for tip racks:

- 1. Open the instrument lid.
- 2. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

3. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

4. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 5. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm** all to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 6. Click **Next** to continue with the next worktable position.
- 7. Repeat steps 2–6 for all tip rack positions.

For all other worktable positions:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

8. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps.

Note: Ensure that you have the empty labware available for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility.

- Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 10. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed reagent is provided in the correct tube type. The volumes displayed are the minimum volume required, so consider adding extra to prevent liquid level detection errors. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.

- 11. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 12. Click **Next** to continue with the next worktable position.
- 13. Repeat steps 8–12 for all worktable positions.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 14. Close the lid.
- 15. Ensure that the tip disposal box is empty.
- 16. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

17. The Run step will be displayed.

7.2.4.8 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only available step of the experiment. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

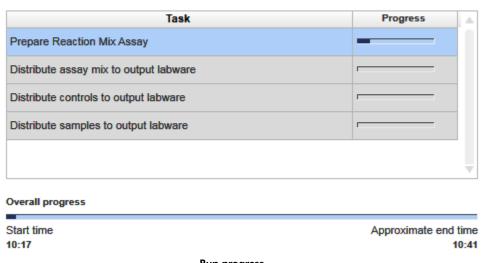
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.4.8.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Run progress.

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.4.8.2 Pause/cancel a run

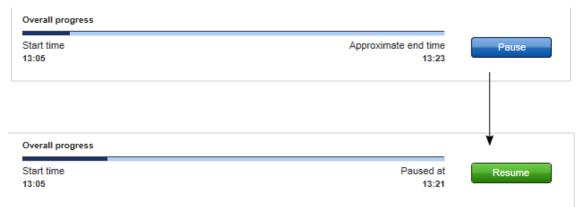
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

Note: Pauses of a run are listed in the Exceptions/Pauses section of the Advanced report.

7.2.4.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen.



Options after the run.

Option	Description				
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.				
Print barcode	You can print a barcode for the output labware.				
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.				
Save output file	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.				
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>Customize user settings</u> to define a standard directory.				
	The file may serve as sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by a LIMS (Laboratory Information Management System). Find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.				
	Note: Depending on the selected cycler, the software also creates a file in the format that is expected by the cycler. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. The input formats of the following cyclers are supported by the first release of the Pathogen Detection Plug-in: Rotor-Gene Q operated with the Rotor-Gene Software, version 2.3 or higher. Contact QIAGEN Technical Services if you need another file format.				
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Labware Selection step that you are Loading an existing output from file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.				
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to				

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QlAgility.

configure the report options.

7.2.4.9 Report

The QIAgility Setup Manager Software offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The Advanced report contains:

- General information
- Comment
- Kit information
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the name of the kit, the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

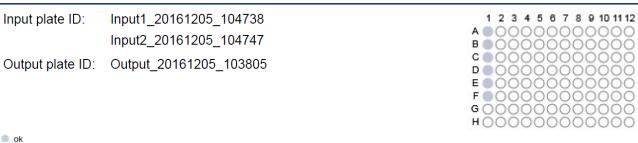
The comment that has been entered on the **Run** screen.

Reaction results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol		Description
	ok	The position was processed without any exceptions.
•	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
•	not used	This symbol is only used if an output file has been loaded as a starting point for the output plate (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results



OK

warning error

error

For your reference, the labware identifiers for input and output labware will be listed even if the graphic is not shown. If multiple input labwares were used for the run, the labware identifiers will be listed in the same order in which they were defined on the **Labware selection** step.

A table listing the reaction result for each processed position on the output labware is shown:

Column	Description
Output	The position on the output labware.
Identifier	Specifies the sample ID or the name of the particular standard or control.
Туре	Sample, Standard or for controls, the Control type.
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls. If multiple input labwares were used for the run, the position is enhanced by a short input labware reference (e.g., A1/I1 refers to position A1 of the 1st Input labware, where "I" indicates Input, and "1" indicates the 1st input labware in the identifier list).
Concentration	For standards, the concentration of the standard is listed. For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty. Always empty for controls or NTC.
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column	Description			
Exception	The event that has been displayed during run.			
Position	Lists the positions that were affected by the exception.			
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.			
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.			
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).			
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.			
	Example: If the "not enough liquid" liquid level detection error occurs in the assay mix tube while serving the last output position with an assay mix, all output positions that were designated to contain this assay will be listed here as potentially affected. However, most of these positions have already been served successfully and will be flagged OK in the reaction results table. You can trust an 'OK' flag in the reaction results table.			
User response	A short form, which option (button) of the message dialog has been pressed.			

Note: The **Basic** report does not list pauses. If you want to have also pauses listed, use the **Advanced** report, instead.

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

General information

Kit information is not listed here for the **Advanced** report; instead, this information is provided in more detail in a separate section.

Kit information

Kit name, lot number and expiration date. Comment provided with the kit.

Note: The Advanced report does not repeat this information in the General information section.

Reaction results table of the Advanced report

In addition to information provided in the **Basic** report, the **Reaction results** table of the **Advanced** report contains two additional columns:

Output	Identifier	Туре	Input	Actual content	Concentration	Final vol. µl	Flag
A1	Sample 1	Sample	A1/I1	15 µl Reaction mix (Assay A (IC + Target 1)) + 5 µl Sample 1		20	ok
B1	Sample 2	Sample	A2/I1	15 µl Reaction mix (Assay A (IC + Target 1)) + 5 µl Sample 2		20	ok
C1	Sample 3	Sample	A3/I1	15 µl Reaction mix (Assay A (IC + Target 1)) + 5 µl Sample 3		20	ok

Column	Description
Actual content	Lists the components. The software refers the assay name for each reaction mix created.
	Example:
	Output position A1 is the NTC for assay "A (IC + Target 1)" and contains 15 μ l of the reaction mix for assay "A (IC + Target 1)" and 5 μ l of RNase-free water.
Final volume	Lists the final volume.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description		
Exception	The event or pause that has been displayed during run.		
Position/Duration	For an event:		
	Lists the positions that were affected by the exception.		
	First, the position is listed for which the exception occurred. After "Destination", all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.		
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.		
	Example:		
	"M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which potentially impacted the output positions D1–D8 (row-wise).		
	Note : If an output position is flagged OK in the reaction results table even though it is listed here as potentially affected by an exception, then you can trust that this output position has been completed successfully.		
	For a pause: the duration of the pause.		
User response	A short form, which option (button) of the message dialog has been pressed.		

Sample information

Lists the sample information for each input labware as it was entered in the sample editor, loaded by the sample input file, or automatically generated by the software, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Detailed run information of the Advanced report

In addition to the run task list included in the **Basic** report, the assay setup information is also listed:

Assay Setup

Reaction mix [Target(s)]	Content
MIXT [GAPDH,GPCR-like	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 76 μl RNase- Free Water + 76 μl GAPDH rev + 76 μl GAPDH for + 76 μl 5 rev + 76 μl 5 for + 76 μl GAPDH Probe + 76 μl 5 Probe
Mix2 [GPCR-like 5]	850 µl 2x Rotor-Gene Multiplex PCR Master Mix + 85 µl Mg+ + 36 µl RNase- Free Water + 9 µl 5 rev + 9 µl 5 for + 9 µl 5 Probe

This provides detailed information about each mix that was automatically created by the QIAgility.

The selected cycler and information about standard series is also provided if they were used in the run.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QIAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.5 Working with sample pooling experiments

The Sample Pooling Plug-in enables easy and convenient setup of your sample pooling experiments. The output from this experiment can be used to perform a manual or automated PCR setup using a plug-in of your choice.

New experiments for sample pooling are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Sample Batches** step will initially be enabled. All other steps will become available once the required information has been entered in the **Sample Batches** step. The coloring of the **Pooling Setup** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 4 steps that guide you through the run setup process:

- 1. Sample batches
- 2. Pooling setup
- 3. Worktable
- 4. Run

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. The file extension for sample pooling experiments is *.qsmpoolix, and for templates *.qsmpoolit (QIAgility Setup Manager Sample Pooling Experiment and Template, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.5.1 Sample Batches step

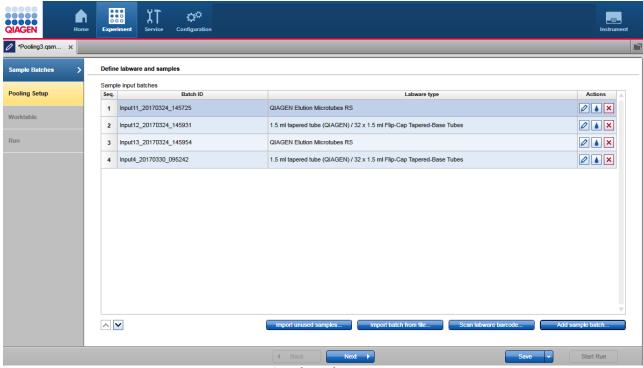
Use the **Sample Batches** screen to select the labware type for sample input and define the corresponding sample information. Several sample input batches can be defined for a pooling run. The QIAgility Setup Manager Software will plan reloading of input labware during the run, if appropriate.

A sample input batch is defined by a unique batch identifier, the labware type and sample names.

Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

Tagging samples is independent of the pool size. The pool size will be defined under Pooling Setup step.

Note: The batch identifier (Batch ID) will be used in the report and in the sample output file.



Sample Batches step

For each sample input batch, you can decide whether to load the sample names from a file or to define them manually. All defined sample batches will be listed in the **Sample input batches** table.

 Click the Add sample batch button to define the labware manually. The Add new sample batch dialog opens. It offers the option to automatically generate sample names and pooling tag. Follow the steps in Add new sample batch dialog.

Alternatively, click the **Import batch from file** button or the **Scan labware barcode** button to import sample information from a file. Follow the steps in Importing a sample batch from file.

Alternatively, if you want to continue with unused samples from a previous run, click the **Import unused samples** button and select the desired sample file. The software will load the information from the file and open the **Import unused samples** dialog. Follow the steps in <u>Importing unused</u> samples from previous runs.

Note: In order to work with unused samples from a previous run, the 'unused samples' file should have been saved after that run. This file can then be used as a starting point for your current run.

- 2. If sample names have not been imported from a file or automatically generated, define them now. The button will be colored yellow to indicate that the sample names still need to be defined.

 Click the button to enter sample names and pooling tags manually, or to change automatically generated sample names and pooling tags individually. For details, refer to Editing samples and tags.

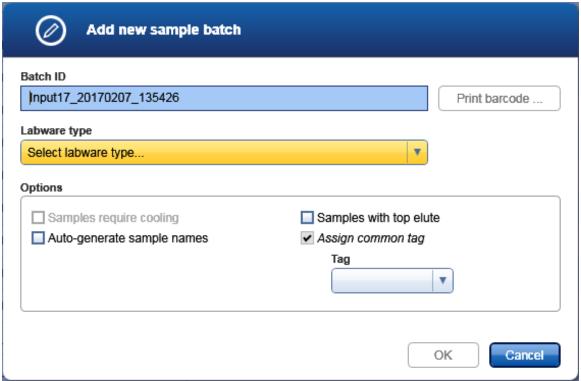
 Alternatively, click the button if you want the sample names or a pooling tag to be generated automatically for all positions.
- Repeat steps 1 and 2 until all sample input batches have been defined.
 Note: If more sample input batches have been defined than can be placed on the worktable, the QIAgility Setup Manager Software prompts you later during the run to update the worktable with samples and tips.
- 4. Later, during pool planning and run, the software will use the order of sample input batches as defined on this screen. Select a table row and use the buttons below the table to optimize the order of input batches as per your need. Finally, click **Next** to proceed to the <u>Pooling Setup step</u>.

The buttons in the right column of the **Sample input batches** table have the following functions:

Button	Action To edit a sample input batch	Description The Edit sample batch dialog will open, displaying the values that have been entered in the Add new sample batch dialog. It offers the option to automatically generate sample names and/or to assign a common pooling tag for all samples of the sample input batch.
		Note: If you select the option to automatically (re)generate sample names or pooling tag, existing sample names or pooling tags will be overwritten.
٨	To edit sample names and pooling tags	The Sample editor will open, displaying the sample names and pooling tags that have already been entered or automatically generated. Sample names and tags can be entered or changed individually.
×	To delete a sample input batch	Deletes the row in the Sample input batches table.

7.2.5.1.1 Add new sample batch dialog

The Add new sample batch dialog allows simple, manual setup of a sample batch.



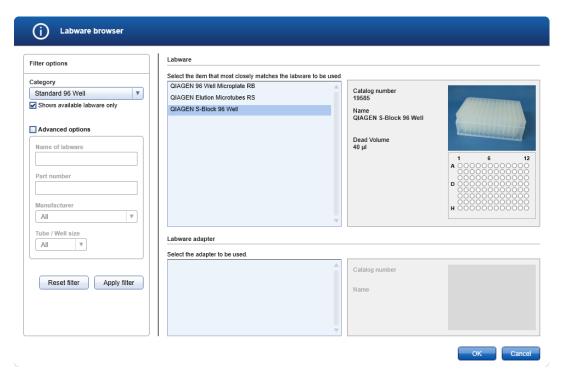
Add new sample batch dialog

1. Optional:

If you would like to change the batch identifier that was automatically generated by the software, you can edit it in the **Batch ID** field.

Note: This identifier will be used in the report and in the sample output file.

2. Select the labware type of your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**. Initially, the drop-down list is colored yellow to indicate that this information is required.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

3. Optional:

If you would like to use the cooling block for the input labware, check the option **Samples require** cooling.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will become disabled.

4. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

- 5. If you would like the QIAgility Setup Manager Software to generate sample names automatically, check the option **Auto-generate sample names**. You can define a prefix with which every sample name will start. Sample names will be generated for all positions on the labware. It is possible to edit sample names individually or change sample names later under <u>Sample Batches step</u>, step 2.
- **6.** If you would like to assign a common pooling tag to all samples automatically, check the option **Assign common tag**. Select a tag from the **Tag** drop-down menu.

Note: The **Tag** menu is an editable menu. If you want to use another tag or if the menu is still empty, just type the tag into the field. For details about editable menus, refer to Entering data.

It is possible to change tags later or assign tags individually to samples from the **Sample Batches** screen by clicking the button in the right column corresponding to the input batch.

Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

- 7. Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 8. Click **OK**. The defined sample batch will be added to the **Sample input batches** table.

Continue with step 2 in Sample Batches step.

7.2.5.1.2 Importing a sample batch from file

To import a sample input batch from file:

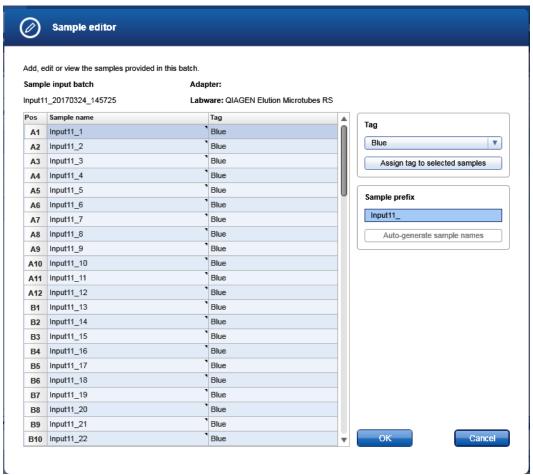
a) Click the Import batch from file button and select the desired sample file. For details, refer to <u>File</u> dialog.

The software will load the sample information from the sample file. If the sample information file also contains the labware type, it will automatically be selected for the sample batch. In case of a *.csv or *.txt file, the file name will be used as identifier for the sample batch. In case of a *.xml file, the plate ID described in the file will be used as identifier for the sample batch.

- b) Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan labware barcode** button. The **Scan labware barcode** dialog opens.
- Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>. The software will load the sample information from the sample file.
- 2. If the sample file does not contain the labware type, the **Add new sample batch** dialog opens. Follow the steps in <u>Add input labware dialog</u>.

Continue with step 2 in Sample Batches step.

7.2.5.1.3 Editing samples and tags



Dialog to edit samples and tags

To define samples manually and to assign pooling tags:

- 1. In the **Sample Batches** step, click the **b**utton in the right column of the **Sample input batches** table corresponding to the input batch. The **Sample editor** will be opened. The sample batch is indicated at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample** names for all samples, the **Sample ID** fields will be colored yellow to indicate that this information is required.
- 3. Enter each sample ID.

Note: Alternatively, you can automatically generate sample IDs. Select the relevant cells. Press the **SHIFT** key and left-click to select multiple adjacent cells, or press the **CTRL** key and left-click to select multiple non-adjacent cells. Optionally, define a prefix with which the generated sample names shall start in the **Sample prefix** field. Click the **Auto-generate sample names** button to generate sample names for the selected samples.

Note: For more details about the sample editor, refer to the section Working with tables.

4. Optionally, assign pooling tags to selected samples. Select the relevant sample cells. Press the **SHIFT** key and left-click to select multiple adjacent cells, or press the **CTRL** key and left-click to select multiple non-adjacent cells. Select a tag from the **Tag** drop-down menu. The **Tag** menu is an editable menu. If the Tag menu is still empty or if you want to use a tag that is not listed, just type the tag name into the field. For details about editable menus, refer to Entering data.

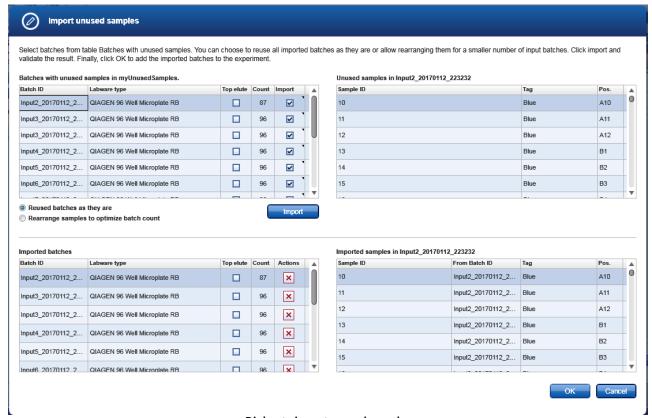
Note: Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

5. Click **OK** to close the **Sample editor**.

7.2.5.1.4 Importing unused samples from previous runs

Save the information about unused samples of any given run in a file by clicking the **Save unused samples** button on the **Run** screen. In order to work with the unused samples from an earlier run, simply load the 'unused samples' file and use it as a starting point. The software loads the information from the file and opens the **Import unused samples** dialog.

The upper part of the dialog shows the information about sample batches containing unused samples as loaded from the file. You can then select which of the sample batches shall be imported for use in your current run and they will appear in the lower part of the dialog.



Dialog to import unused samples

- Select the sample batches from the file that shall be used in this run. To do so, check the box in the column **Import** in the corresponding rows of the **Batches with unused samples** table at the top left. To view the content of a sample batch before importing, click in the sample batch's row in the table **Batches with unused samples** at the left to see the list of its unused samples in a table at the right.
 - **Hint:** If you plan to use up all the unused samples in the next runs, you can mark to import all sample batches from the file at once, even though not all samples can be pooled in the immediate run. After the current run is finished, you can again export a file, this time containing fewer unused samples. You can repeat the import and save files step until all the initially unused samples have been pooled. This feature allows to define a large number of samples to be pooled in a single step, although several runs are needed to complete the pooling.
- If the samples on a sample batch have been indicated in the previous run to be covered with TopElute Fluid, the corresponding check box in the **Batches with unused samples** table is checked. The QIAgility will pipet the samples accordingly.
- 3. Select one of the import options.
 - Use the option **Reuse batches as they are** if adapters shall be reused without rearranging sample

Use the option **Rearrange samples to optimize batch count** if you want to reduce the number of input batches for the run. If possible, the software will try to fill up adapters.

Note: The software will not rearrange samples that are placed on plates, i.e., plates will always be reused as they are independent of the selected option. For adapters with tubes, the software will fill up adapters only with samples that have the same tube type.

4. Click the **Import** button. The software takes the sample input batches that have been marked for import from the **Batches with unused samples** table at the top left.

Note: At least one of the **Import** check boxes needs to be checked in the **Batches with unused samples** table at the top left. Otherwise, the **Import** button is disabled.

5. The software lists the sample batches in the order they will be imported into the **Imported sample** batches table below the **Import** button. If the option **Rearrange samples to optimize batch count** has been enabled, the number of batches can be reduced.

To view the content of a sample batch after rearrangement, click in the sample batch's row in the table **Imported sample batches** at the left to see the list of its unused samples in the table at the right.

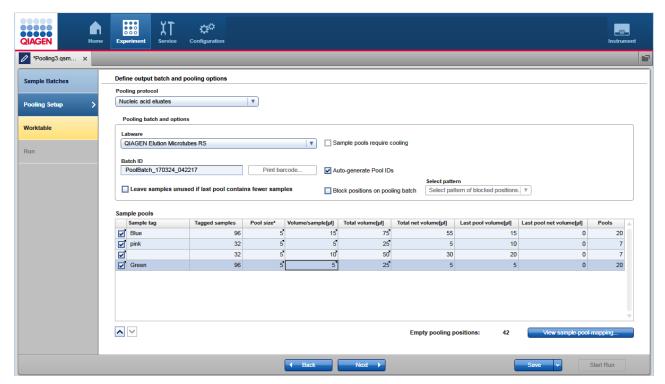
Note: If you click the **Import** button again, all previously listed batches in the **Imported sample** batches table will be replaced by this new action.

Click OK to import the sample batches as listed in the Imported sample batches table.
 Otherwise, click Cancel to discard the whole action to import sample batches from the selected file of unused samples.

Continue with step 2 in Sample Batches step.

7.2.5.2 Pooling Setup step

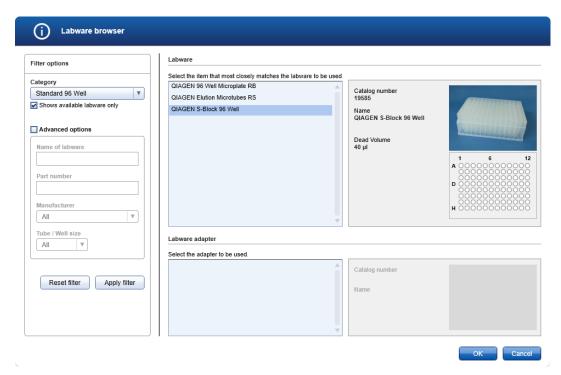
Use the **Pooling setup** screen to select the pooling protocol, the labware type for the output labware, and to define the sample pools itself.



Pooling Setup step

Firstly, select the pooling protocol and define the pooling batch (output labware):

- 1. Select the protocol from the **Pooling protocol** drop-down list that fits best for your samples. Initially, the drop-down list is colored yellow to indicate that this information is required.
- 2. Select the **Labware** for the pooling batch (i.e., the output labware of the run) from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**. Initially, the drop-down list is colored yellow to indicate that this information is required.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Optional:

If the sample pools on the output labware require cooling, indicate this using the corresponding check box next to the **Labware**.

4. Optional:

If you would like to change the batch identifier that was automatically generated by the software for the pooling batch, you can edit it in the **Batch ID** field.

Note: This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

5. **Optional:**

Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been selected in the **Global Settings** tab of the **Configuration** environment.

6. Optional:

Check the box next to **Auto-generate Pool IDs**, if the software shall generate pool identifiers automatically. Pool IDs will be used in the report and in the sample output file to name the positions of

the pooling batch (on the output labware). Otherwise, pool IDs need to be defined later in the **Review** sample-pool-mapping dialog.

Note: The Auto-generate Pool IDs option is the most convenient way to define pool IDs.

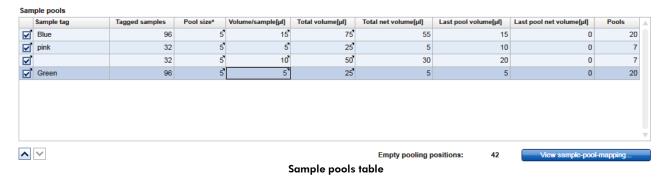
7. **Optional:**

Select the option **Leave samples unused if last pool contains fewer samples**, if you want to create pools always with the complete pool size.

8. Optional:

If certain positions on the pooling batch shall not be used during the run, check the option **Block positions on pooling batch** and select a pattern from the drop-down list next to it. Select **Manage patterns** to <u>define a new pattern</u>.

The **Sample pools** table defines the sample pools along the tags that have been defined with the samples. The table lists all sample tags and the number of samples that have been tagged.



For each row in the **Sample pools** table:

Select the **Tag** if pools shall be created in this run by checking the box next to the sample tag name. If
the tag has been selected, proceed with the next step to define the pools for it, otherwise proceed with
step 12.

Note: At least one tag needs to be selected. The background of the boxes is yellow if no tag has been selected to indicate that a selection is required.

Note: If samples have been defined without an assigned tag, a row will be displayed with an empty sample tag name. Those samples will be treated as a separate tag group.

- 10. Enter the **Pool size** for the tag into the corresponding cell of the table, i.e., the number of samples that shall be combined together into one pool. The cell is initially colored yellow to indicate that this information is required.
- 11. Enter either the **Volume per sample** that shall be added to the pool or the **Total volume** of the resulting pool. If one value has been entered, the software will automatically calculate the other value. Both cells are initially colored yellow to indicate that the values are required.

Note: A minimum volume per sample is required, depending on the selected pooling protocol. The **Volume per sample** field is colored yellow, otherwise. Hover the mouse over the field to get a tool tip displayed with the minimum value. The **Total volume** field is colored yellow if the pool volume exceeds the labware capacity of the output labware.

The **Sample pools** table contains additional information about the pools in the other columns:

Table column	Description
Total net volume	If the pools shall serve as input for a further QIAgility run, this is the actual input volume that will be available in the next run. Depending on the dead volume of the chosen labware, the total net volume can be lower than the actual physical volume.
	Adjust the volumes if necessary to meet the required input volumes of the next run.
Last pool volume	The volume that will be available in the last pool. This volume can be lower than the total volume of a complete pool if the last pool will contain fewer samples. You can prevent the creation of incomplete last pools by selecting the option Leave samples unused if last pool contains fewer samples .
Last pool net volume	If the pools shall serve as input for a further QIAgility run, this is the actual input volume of the last pool that will be available in the next run. Depending on the dead volume of the chosen labware, the last pool net volume can be lower than the actual physical volume.
	Adjust the volumes if necessary to meet the required input volumes of the next run. Alternatively, you can prevent the creation of incomplete last pools by selecting the option Leave samples unused if last pool contains fewer samples .
Pools	The number of pools that will be created for the tag.

The number of **Empty pooling positions** on the output labware is displayed below the table.

12. Repeat steps 9–11 until all sample pools for the run have been defined.

The software plans pools according to the definitions in the **Sample pools** table. The following criteria are taken into consideration:

The order of the selected tags in the **Sample pools** table. Click a tag name in the table and use the buttons below the table to optimize the order of tags as per your need.

The software uses the order of sample input batches as defined in the Sample Batches step.

Samples are taken from the sample input batches according to the default pipetting order for sample batches as defined in the user settings for sample pooling.

13. If the **View sample-pool-mapping** button is colored yellow, click it to define pool IDs in the upcoming dialog. This step is optional if the option **Auto-generate Pool IDs** has been selected. You can click the button at any time to see a list of the planned pooling batch (output labware), or to define/refine pool IDs individually. Refer to <u>Sample-pool mapping dialog</u> for details.

Note: Pool IDs must be defined in order to proceed to the next Worktable step.

14. If the required pool definition is completed, the **Next** button gets enabled.

- 15. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 16. Close the lid.
- 17. Click Next.

Note: The software will show a message if more samples have been defined than the number of samples that will be pooled in this run, or if the number of defined sample pools exceeds the number of available positions on the output labware. Once the run is completed, you can save a file with unused samples to use it as a starting point for the next run.

If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

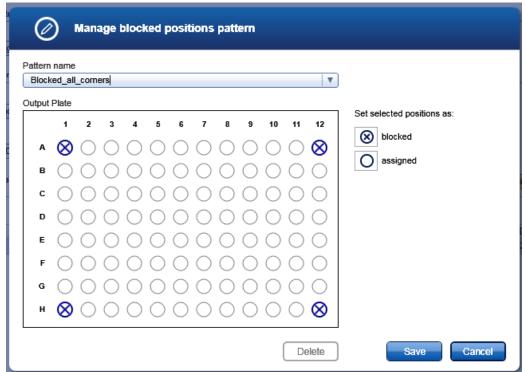
Proceed with the instructions described in Worktable step.

7.2.5.2.1 Defining a pattern for blocked positions

Use the **Manage blocked positions pattern** dialog to define blocked positions. The dialog shows a graphical representation of the selected output labware.

The dialog can be opened by selecting **Manage patterns** from the drop-down list next to the option **Block positions on pooling batch** on the **Pooling Setup** screen.

Note: The option **Block positions on pooling batch** needs to be selected in order to enable the drop-down list.



Example of a pattern with blocked positions.

To define a new pattern:

- Select New from the Pattern name drop-down list and enter a name for the pattern. This is an
 editable menu. For details, refer to <u>Entering data</u>. The pattern name will be displayed in the Pooling
 Setup step.
- 2. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions. Use the mouse to select a rectangular area.

3. On the right, click on the button you want to assign to the selected position:

Button Description Blocked position. Position that can be used during the run.

- 4. The graphic symbol for the selected positions will be changed accordingly.
- 5. Click **Save** to finish the definition and to close the dialog. The newly created pattern is now selected in the Pooling Setup step.

Note: The pattern will be available in the drop-down list of blocked positions patterns in the **Pooling Setup** step whenever this output labware has been selected.

To refine a pattern:

- Select the pattern from the drop-down list in the **Pooling Setup** screen.
 Note: The list contains all patterns that have been defined for the currently selected output labware.
- 2. Select Manage patterns from the drop-down list in the Pooling Setup screen.
- 3. The **Manage blocked positions pattern** dialog opens. The pattern chosen in step 1 is selected in the **Pattern name** drop-down list.
- 4. Select the position in the graphic whose assignment you want to change.
- 5. On the right, click on the button you want to assign to the selected position.
- 6. The graphic symbol for the selected positions will be changed accordingly.
- 7. Click **Save** to finish the definition and close the dialog. Otherwise, click **Cancel** to leave the dialog without changing the selected pattern.

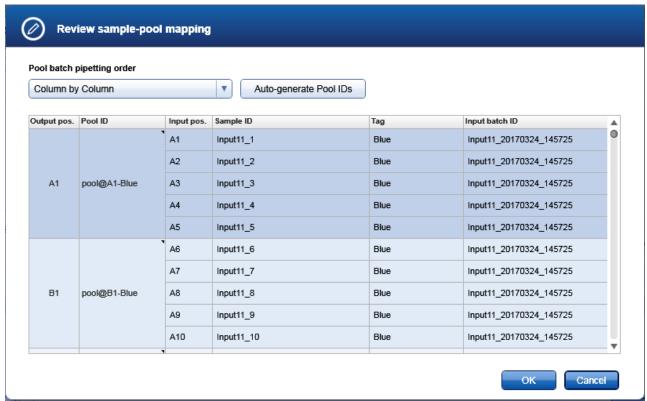
To delete a pattern:

- 1. Select Manage patterns from the drop-down list in the Pooling Setup screen.
- 2. The Manage blocked positions pattern dialog opens.
- 3. Select the **Pattern name** from the drop-down list which you want to delete.
- 4. Click the **Delete** button to delete the selected pattern. The pattern is deleted.
- 5. Click **Cancel** to leave the dialog.

7.2.5.2.2 Sample-pool mapping dialog

The **sample-pool mapping** dialog lists the sample pools on the pool batch (output labware) as currently planned for the run. For each output position, the list of samples to be pooled together is displayed along with the position on the sample input batch, the sample ID, the tag, and the input batch ID.

The definition of pool IDs is required in order to start the run. Pool IDs are displayed on the report. If pool IDs have not yet been generated or defined, the pool ID fields are colored yellow.



Review sample-pool mapping dialog with automatically generated pool IDs

1. Optional:

Select the pipetting order for the pool batch. Initially, the **Pool batch pipetting order** drop-down list displays the default pipetting order for pool batches as defined in the user settings.

Note: Each time the pipetting order is changed, pool IDs will be generated automatically by the software.

2. If not yet defined, define pool IDs.

Click the **Auto-generate pool IDs** button if the software shall create pool IDs automatically. The ID will contain the position on the pooling batch (output labware) and the tag.

Note: The **Auto-generate Pool IDs** option on the **Pooling Setup** screen is the most convenient way to define pool IDs.

Alternatively, enter the pool IDs into the Pool ID cells individually.

Note: Each time the pipetting order is changed, pool IDs will be generated automatically by the software.

Note: Each time the setup is changed on the **Pooling Setup** screen, pool IDs are removed. The software regenerates them automatically if the option **Auto-generate Pool IDs** is selected on the **Pooling Setup** screen.

3. Optional:

Review the list of pools.

4. Click **OK** to acknowledge the entered or generated pool IDs and leave the dialog. Otherwise, click **Cancel** to leave the dialog and to discard all changes.

Note: The OK button is enabled only if all pool IDs have been defined.

7.2.5.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. If more sample input batches have been defined or more tips are required than can be placed on the worktable, the QIAgility Setup Manager Software will prompt you during the run to reload the worktable with sample batches and tips.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.



- Worktable selector
- B1 (black border): worktable position for which the loading details are displayed.
- B1, B2, C1 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.
- A1, A2, C2 (blue): worktable positions that have already been loaded.
- M1, R1 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

The software displays the labware and required minimum volume of sample to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

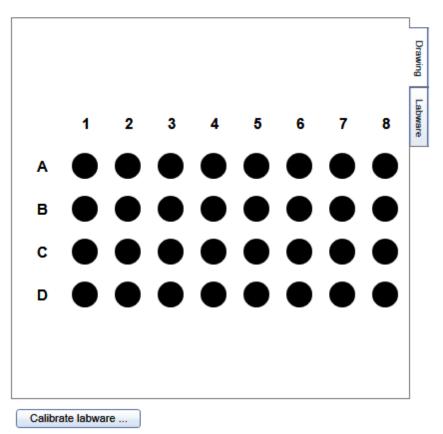


Table of samples to be loaded to the worktable position B1.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Click on the **Labware** tab to get an image and details about the required labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

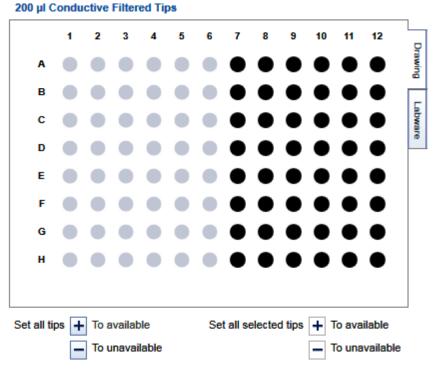
Adapter: 32 x 1.5 ml Flip-Cap Tapered-Base Tubes Labware: 1.5 ml tapered tube (QIAGEN)



Schematic graphic for B1.

If labware required for the run has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



Interactive rack to define tip avilability.

Preparing the worktable

- 1. Open the instrument lid.
- 2. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that a tip rack or labware has not yet been calibrated on the worktable position required for the run. It must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for the current run and that has not yet been calibrated on the required worktable positions and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

Note: Ensure that you have the empty labware available for the calibration. Tip racks of both tip type will be required for labware calibration.

Note: Sample pooling runs use only one tip type. However, labware calibration requires both tip types. Ensure to correctly follow the tip loading instructions in the calibration wizard.

Note: The calibration worklist also considers all labware that will be reloaded later during the run that have not yet been calibrated on the planned worktable positions.

Note: If tip racks have not been calibrated for all required worktable positions, they will be added to the calibration worklist too.

After the calibration steps have been completed, the worktable screen will be displayed again.

Now, the worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips are sufficient. If not, load additional tips. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 4. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 5. Click **Next** to continue with the next worktable position.
- 6. Repeat steps 3–6 for all tip rack positions.

For sample batches and pool batch:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

- 7. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 8. Perform the following steps for each row in the table:
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.
- 9. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 10. Click **Next** to continue with the next worktable position.
- 11. Repeat steps 8–12 for all worktable positions.

Note: If calibration has been performed between steps 3–12, ensure to load (or reload) all worktable positions as described in the steps.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 12. Close the lid.
- 13. Ensure that the tip disposal box is empty.
- 14. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

Note: If more sample input batches have been defined or more tips are required than can be placed on the worktable, a message is displayed to inform you that the QIAgility Setup Manager Software will prompt you during the run to update the worktable with samples and tips.

15. The Run step will be displayed.

7.2.5.4 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only available step of the experiment. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

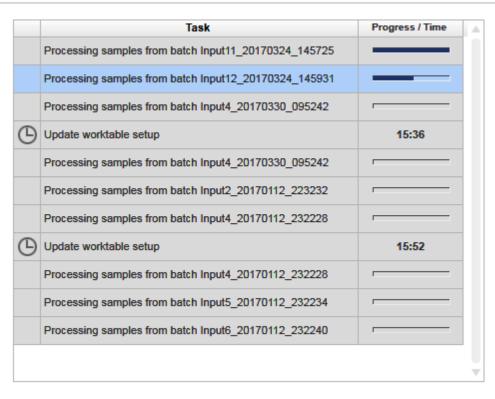
The run can be paused in between, if more samples or tips are needed than was initially placed on the worktable.

7.2.5.4.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups. The task list contains tasks to <u>update the worktable</u> with samples and tips during the run, if more sample input batches have been defined or tips are required than was initially placed on the worktable. The **Progress/Time** column lists the estimated time when the worktable update will be required.

The instrument icon in the main toolbar as well as the progress indicator on the run step show the overall run progress.

Run progress



Overall progress

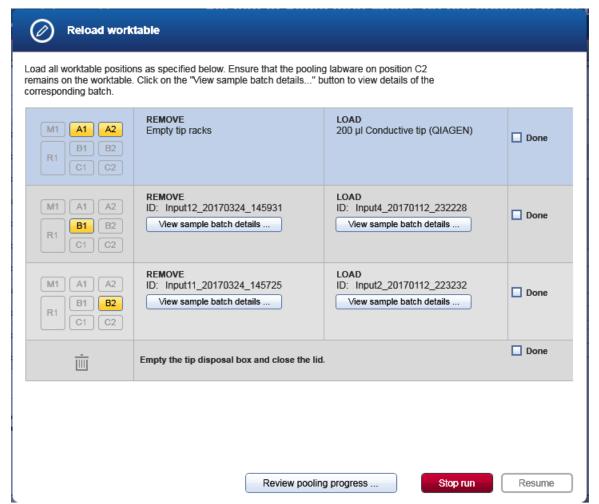
Start time Approximate end time
15:25 Run progress.

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be a part of the report. You may enter the comment during or after the run.

7.2.5.4.2 Reloading worktable during run

If samples or tips need to be reloaded during the run, the **Reload worktable** dialog is displayed. The instructions in the dialog are different for each experiment. The QIAgility Setup Manager Software optimizes these tasks to reduce the number of required reloads during the run.



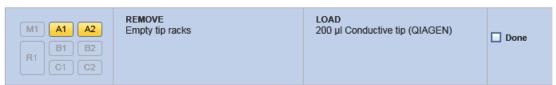
Relaod worktable dialog with instructions.

Each instruction is displayed in a row. The columns display from left to right: the affected worktable position(s), the item(s) that shall be removed and the item(s) that shall be loaded. Perform the actions described and check the "Done" boxes. Once all preparation steps are completed and confirmed, the Resume button will become active.

Follow the instructions listed in the dialog.

For **tip racks**:

1. Remove empty tip racks from the worktable positions highlighted on the left.



Instruction to remove empty tip racks from worktable positions A1 and A2 and to load full 200 μ l tip racks, instead.

2. Load full tip racks of the required tip type to the worktable positions.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> holder.

Note: Full tip racks need to be loaded during worktable reloading.

To confirm that the tip racks have been loaded, check the box next to **Done**.
 The color of the worktable positions changes to blue to indicate that they have already been loaded.

For sample input batches:

4. Remove the sample input batch indicated in the second column from the highlighted worktable position.



Instruction to remove sample batch with ID Input12_20170324_145931 from worktable position B1.

You can click the **View sample batch details** button to get the sample list displayed.

Note: If no batch needs to be removed from the worktable position, the instruction displays "No unload needed."

5. Load the sample input batch indicated in the third column.



Instruction to load sample batch with ID Input 20170112 232228 to worktable position B1.

Ensure that the samples are loaded using the correct labware type. Hover with the mouse over the batch ID to see a tool tip with labware information.

Check that the samples are placed in the correct positions. You can click the **View sample batch details** button to get the sample list displayed.

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

Note: If no batch needs to be loaded, e.g., because only a finished sample batch can be removed, the instruction displays "No load needed."

- To confirm that the sample batches have been removed and loaded, check the box next to **Done**.
 Note: If the loading has been confirmed in the **View sample batch** dialog, the box is already checked.
- 7. Repeat steps 1–6 until all tips and sample batch instructions have been completed.
- 8. Optional:

To review the current pooling progress, click the **Review pooling progress** button. The <u>Review pooling progress</u> dialog opens. After closing the dialog, the **Reload worktable** dialog is displayed again.

To **continue** the run:

- 9. Ensure that the tip disposal box is empty.
- 10. Close the lid. To confirm, check the box next to **Done**.



Instruction to empty the tip box and to close the lid.

11. Click the **Resume** button to continue the run.

Note: If the completion of any instruction has not yet been confirmed, the run cannot be continued, and the **Resume** button will remain deactivated. The software will indicate the affected instruction with a yellow worktable position in the respective instruction row.

You can stop the run by clicking the **Stop** button. In this case, the run will be stopped. Pools may not have been completed. Click the **Review pooling progress** button to review the status of the pools before stopping the run. All samples that have not yet been added to a pool are treated as unused samples. After the run, a file of unused samples can be saved as a starting point for the next run.

7.2.5.4.3 View sample batch dialog

The **sample batch** dialog lists the samples of the sample batch. It lists information about unused samples for batches that shall be removed. For batches that shall be loaded, it provides the option to check the sample positions on the labware and to confirm sample loading.

Column	Description
Pos	The samples position on the sample batch.
Sample name	The name of the sample.
Tag	The tag that has been assigned to the sample during sample batch definition.

Req. Vol. $[\mu I]$ The required minimum amount of sample to be loaded.

Unused Only for sample batches that shall be removed. Information if the sample has already

been pooled or not.

If the sample has already been pooled, the box is unchecked. If the sample has not

been pooled, the box is checked.

Loaded Only for sample batches that shall be loaded.

Similar to the **Loaded** check boxes during worktable setup. Check the box to confirm the loading of the sample position individually. Alternatively, click the **Confirm all**

button below the table to confirm loading of all samples with one click.

For sample batches that shall be loaded:

1. Check that the samples are placed in the positions as displayed in the table.

2. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.

3. Click **OK** to close the dialog. The **Reload worktable** dialog is displayed again and the **Done** check box for this instruction is checked.

Alternatively, click the **Cancel** button to return to the **Reload worktable** dialog and confirm the loading there.

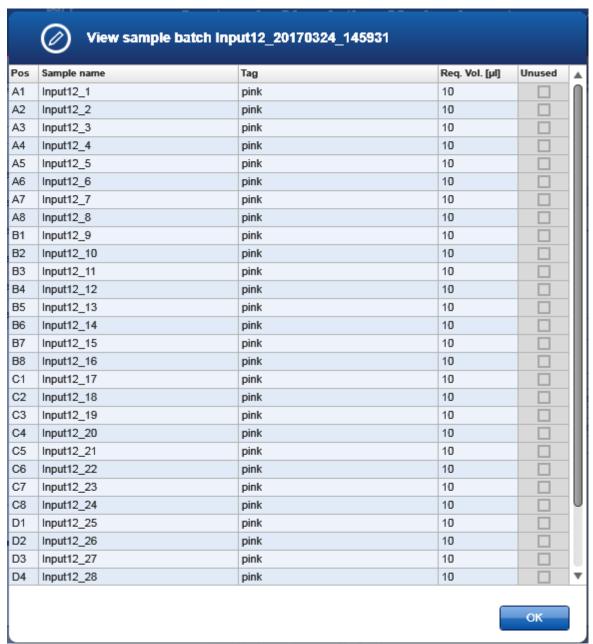
Note: If the loading of any position has not yet been confirmed, the OK button will remain deactivated.



Sample batch that shall be loaded.

For sample batches that shall be removed:

- 1. View the sample list.
- 2. Click **OK** to return to the **Reload worktable** dialog.



Sample batch that shall be removed from the worktable.

7.2.5.4.4 Reviewing pooling progress during run

While reloading the worktable, the current pooling progress can be reviewed. The table shows a row for each position on the pooling batch with the completion status and samples contained in the pool.

Outpu	Pool ID	Complete	Input pos.	Sample ID	Tag	Input batch ID	Result	
			H7	Input11_91	Blue	Input11_20170324_145725	ОК	
			Н8	Input11_92	Blue	Input11_20170324_145725	ОК	
C3	pool@C3-Blue	100%	Н9	Input11_93	Blue	Input11_20170324_145725	OK	
			H10	Input11_94	Blue	Input11_20170324_145725	OK	
			H11	Input11_95	Blue	Input11_20170324_145725	OK	
			H12	Input11_96	Blue	Input11_20170324_145725	OK	
			A10	10	Blue	Input2_20170112_223232	-	
D3	pool@D3-Blue	20%	A11	11	Blue	Input2_20170112_223232	-	
			A12	12	Blue	Input2_20170112_223232	-	
			B1	13	Blue	Input2_20170112_223232	-	
	pool@E3-Blue	ue 0%	B2	14	Blue	Input2_20170112_223232	-	
			В3	15	Blue	Input2_20170112_223232	-	
E3			B4	16	Blue	Input2_20170112_223232	-	
			B5	17	Blue	Input2_20170112_223232	-	
			B6	18	Blue	Input2_20170112_223232	-	

Different stages of sample pooling (completed, started and not started).

Column	Description
Output pos.	The position on the pooling batch (output labware).
Pool ID	The name of the pool on that position on the pooling batch.
Complete	Completion status of the pool. Example: If a pool shall contain 5 samples and only one sample has been added so far, the completion status would be 20%.
Input pos.	The position of the sample on the original sample batch. Each sample that shall be added to the pool is displayed in a small row.
Sample ID	The name of the sample.
Tag	The tag that has been assigned to the sample during sample batch definition.

Input batch ID

The identifier of the sample's input batch.

Result

OK, if the sample has been added to the pool, otherwise -

Click on the **OK** button to leave the dialog and to return to the <u>Reload worktable dialog</u>.

7.2.5.4.5 Pause/cancel a run

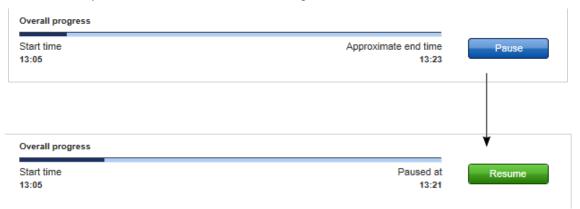
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the Pause button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the Pause button will change its label to Resume.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click

OK to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.

3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

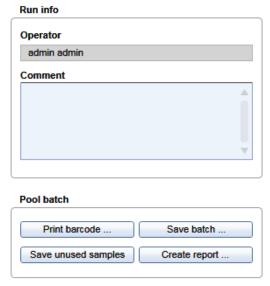
All samples that have not been added to a pool are treated as unused samples. After the run, a file of unused samples can be saved as a starting point for the next run.

Note: Pauses of a run are listed in the **Exceptions/Pauses** section of the report.

7.2.5.4.6 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Print barcode	You can print a barcode for the pooling batch.
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.
Save batch	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the pool ID and the samples they originate from. The file may serve as sample input file for the next run of PCR setup using the QlAgility instrument operated with the QlAgility Setup Manager Software. The .xml file can also be used by LIMS (Laboratory Information Management System).
	A file dialog opens to select directory and file name of the .xml file. Refer to Customize user settings to define a standard directory.
	Note: Depending on the <u>user settings</u> , the software also creates a file in another format that is expected by LIMS, for example. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. Contact QIAGEN Technical Services if you need another file format.
Save unused samples	A file will be saved with information about the samples that have not been pooled during this run.
	A file dialog opens to select directory and file name of the .csv file. Refer to <u>Customize user settings</u> to define a standard directory.
	Note: Use this option also in case the pool batch is limited, and one pooling run is not sufficient to completely pool the defined samples. In such a situation, you can perform more than one run to completely pool the defined samples. Save the unused sample file after the first run. To set up the second run properly, click in the Sample Batches step on the Import unused samples button and load the file from the first run as a starting point for the second run. If there are unused samples remaining even after the second run, save the unused samples file after the second run and import this again as a starting point for the third run. Continue this step until all the samples that were initially defined in the Sample Batches step have been pooled.
Create report	A $\underline{\text{report}}$ can be created for documentation purposes. Refer to the $\underline{\text{user settings}}$ to configure the report options.

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.

3. Clean the worktable as described in section Cleaning the QlAgility.

7.2.5.5 Report

The report contains the following sections:

- General information
- Comment
- Pooling setup
- Pooling results
- Exceptions/Pauses
- Detailed run information
- Advanced instrument information

General information

Some general information is provided at the beginning the report. This includes information such as the name of the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

The comment that has been entered on the **Run** screen.

Pooling setup

Indicates the pooling protocol, pool batch ID and labware. Tags, number of samples with a certain tag, pool sizes, volumes per sample, volumes per pool and number of pools are also indicated.

Pooling results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol		Description
	ok	The position was processed without any exceptions.
•	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
8	blocked	The position was marked as blocked in the Pooling Setup step and has not been used during the run.

A table listing the pooling result for each processed position on the output labware is shown:

Column	Description
Output Pos	The position on the output labware.
Pool ID	Specifies the pool ID.
Input Pos	The corresponding position on the sample input batch.
Sample ID	Specifies the sample ID.
Tag	The tag that has been assigned to the sample.
Input batch ID	The batch ID of the sample's input batch.
Result	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic.

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions/Pauses

This section lists all events but also any pauses that may have occurred during the run in a table:

Column	Description			
Exception The event or pause that has been displayed during run.				
Position/Duration	For an event:			
	Lists the positions that were affected by the exception.			
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.			
	The $@$ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.			
	Example:			
	"B1 @ A1 Destination: output labware @ D1": The exception occurred on the worktable position B1 at position A1, which potentially impacted the output position D1.			
	Note: If an output position is flagged OK in the pooling results table even though it is listed here as potentially affected by an exception, then you can trust that this output position has been completed successfully.			
	For a pause: the duration of the pause.			
User response	A short form, which option (button) of the message dialog has been pressed.			

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QlAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.6 Generic PCR experiments

The Generic PCR Plug-in enables a kit-independent, easy and convenient, qPCR setup.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections Working with experiments for pathogen detection and Working with gene expression experiments, respectively. For generic PCR setup experiments including sample normalization refer to section Generic PCR setup with normalization experiments. For PCR setup with serial dilution experiments, refer to the PCR setup with serial dilution experiments section.

New experiments for generic PCR setup are created by clicking **Generic PCR setup** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will be enabled initially. Subsequent steps will become available once the required information has been entered in the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 7 steps that guide you through the run setup process:

- 1. Liquids
- 2. Mixes
- 3. Controls/standards
- 4. Samples/Labware
- 5. Assignment
- 6. Worktable
- 7. Run

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

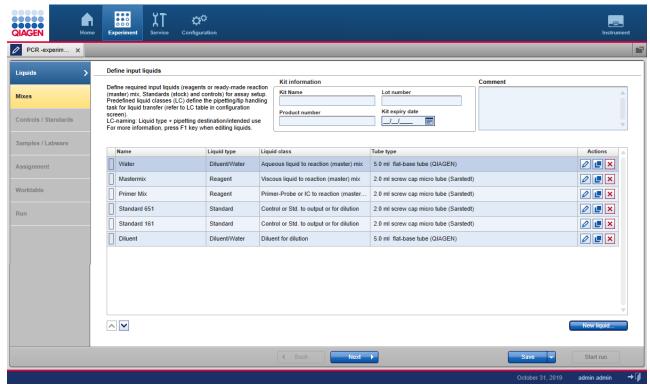
7.2.6.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will, e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.



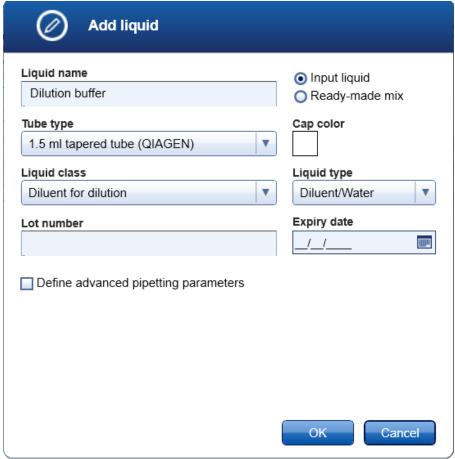
The buttons in the **Actions** column

of the table have the following functions:

Button	Action	Description
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
<u>e</u>	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.6.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.



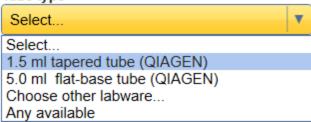
Add liquid dialog

- Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed. If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

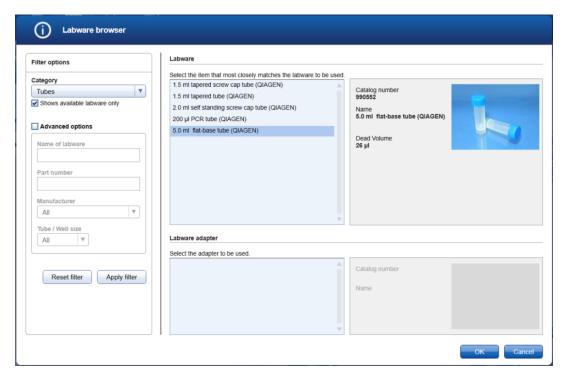
Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types
used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the
respective entry.

Tube type



Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the <u>Labware Manager</u>, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

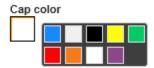
The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the **Labware browser**, refer to the section <u>Using the labware browser</u>.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

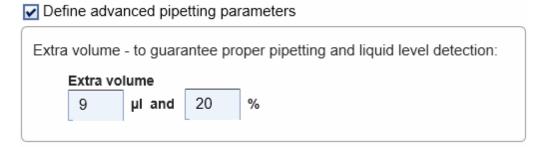
6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.



In the dialog, click OK to confirm your values and to close the dialog.
 Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.6.1.2 Liquid classes

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

The liquid classes that are available for experiments for **Generic PCR setup**, **Generic PCR setup** with **Normalization**, **PCR setup** with **serial dilution** or experiments having **Other workflows**, are listed in a table in the settings section of the QIAgility Setup Manager Software. Click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step.

Column	Description
Name	The name of the liquid class. This name is listed in the dialog were a liquid class has to be selected.
Version	The version of the liquid class.
	Liquid classes will be improved over time and new versions of the QIAgility Setup Manager Software may come with newer versions of liquid classes. These new

versions of liquid classes will apply to new experiments. If you use existing templates or experiments that have been created before, they still use the older versions of liquid classes, as a snapshot of the liquid classes is saved within the experiments and templates. This ensures that existing templates run as before, after a software update. If you would like the new versions of liquid classes to be applied to your existing templates, open the templates, and for every liquid defined reselect the liquid class. Then, save the template again.

Description

Information for which liquids the liquid class should be used.

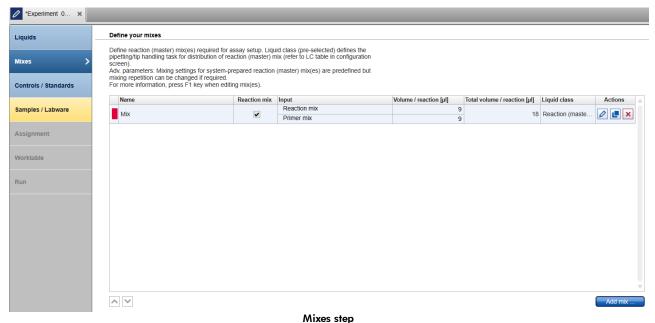
All pre-defined liquid classes make use of the liquid level sensor. For more information refer to <u>Liquid-level</u> tracking.

7.2.6.2 Mixes step

The Mixes step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.



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Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in Add new mix dialog input.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in <u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the **Mixes** screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click **Next** in the button bar to proceed to the **Controls/Standards** step, described in <u>Define</u> <u>controls and standards step</u>.

Note: The **Next** button is disabled, if a row in the table is highlighted yellow. User prepared reaction (master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit() the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.



The buttons in the **Actions** column

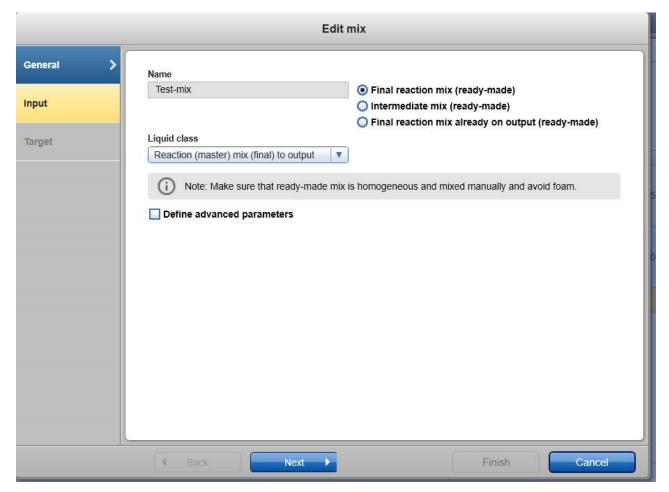
of the table have the following functions:

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.
	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

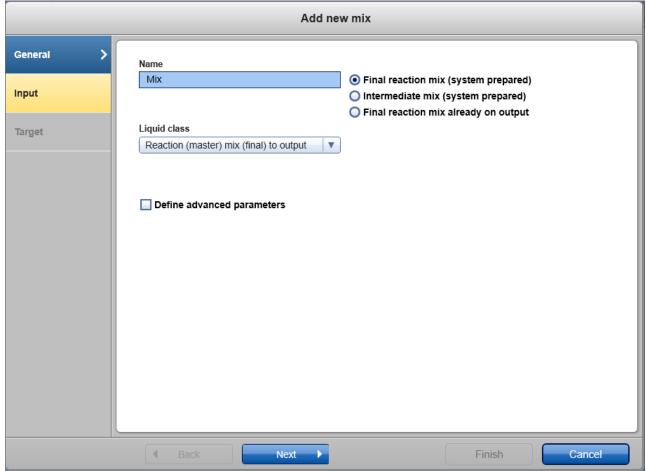
7.2.6.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.



Edit mix dialog



Add new mix dialog - General

 Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option

Description

Final reaction mix (ready-made)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QlAgility. In the later Assignment step, the mix will be available for selection. Duringthe run, the QlAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.

Intermediate mix (readymade)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QlAgility. During the run, the QlAgility will pipette the mix into the tube, where the more specific mix is created.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.

Final reaction mix already on output (ready-made) Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.

If the option is selected, the Input screen of the dialog will be skipped.

Final reaction mix (system prepared)

Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.

Intermediate mix (system prepared)

Use this option if this mix will be created by the QlAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QlAgility. Then the QlAgility will pipette the mix into the tube where the more specific mix is created.

Final reaction mix already on output

Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

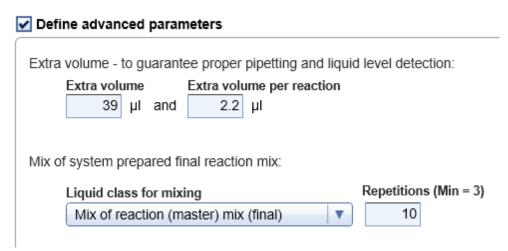
Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QlAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.



For ready-made reaction (master) mixes the QlAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QlAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

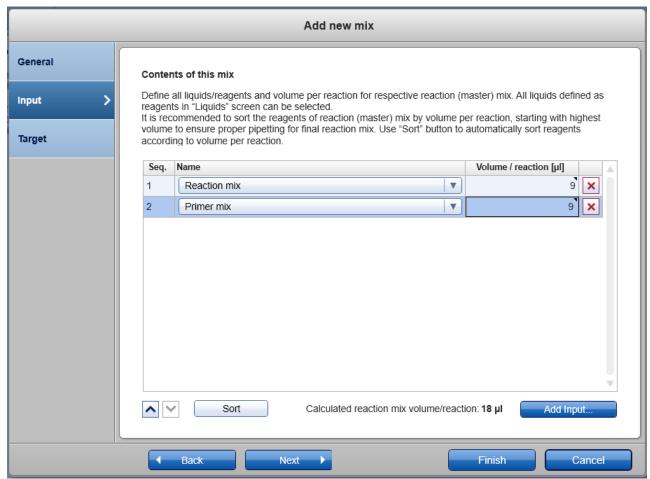
During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in Mixes step.

7.2.6.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.



Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

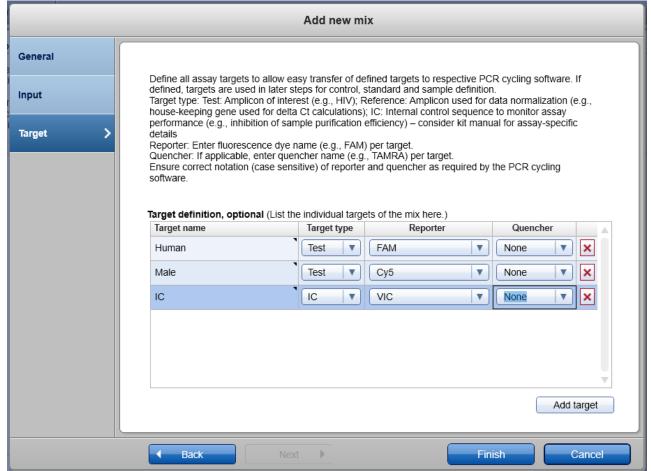
- 3. Define the volume per reaction for the input liquid. Left-click the Volume/reaction field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.
 Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.6.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.



Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type Description

Test Amplicon of interest (e.g., HIV)

Reference Amplicon used for data normalization (e.g., house-keeping gene used for delta

CT calculations)

IC Internal control sequence to monitor assay performance (e.g., inhibition of

sample purification efficiency) – consider kit manual for assay specific details)

 Optional step: Click on the menu in the Reporter column and select an appropriate reporter dye for the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.



Green Yellow Orange FAM JOE ROX

SYBRGREEN1 VIC CALFLUORRED610

SYBRGREENI HEX CY35

FLUORESCEIN TET TEXASRED

ALEXAFLUOR488 MAX ALEXAFLUOR568

CALFLUOR

GOLD540

YAKIMAYELLOW

Red Blue Crimson

CY5 MARINABLUE QUASAR705

QUASAR670 EDANS ALEXAFLUOR680

ALEXAFLUOR633 BOTHELLBLUE LIGHTCYCLERRED705

LIGHTCYCLERRED640 ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, save the output file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in Mixes step.

7.2.6.3 Controls/Standards step

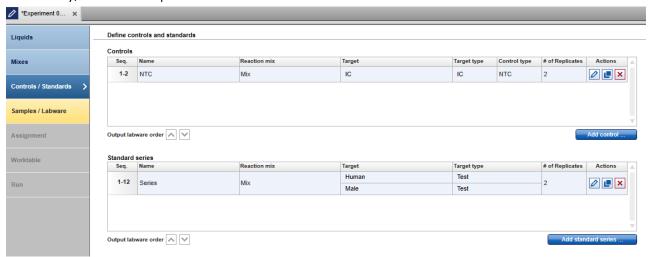
This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add standard series</u>, respectively.
- The control will be added to the Controls table, and the standard series will be added to the Standard series table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.



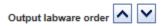
Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.6.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.



Add control dialog

 Enter a control name in the Control name field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- 2. Select the input liquid from the **Liquid** menu. The menu lists all liquids that have been defined with liquid type **Pos./neg. control** or **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix** (ready-made), **Final reaction mix** (system prepared), **Final reaction mix in output** (ready-made) or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type



Option	Description
PC	Is a positive control for the intended assay.
NTC	Is a non-template control for the intended assay.
NC	Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

6. If the PC control type has been chosen, you can specify a custom volume for the control. Tick the **Use** specific volume box, type in a value in the **Volume** field, and select a diluent from the **Diluent** (used to fill up to sample volume) drop-down menu.

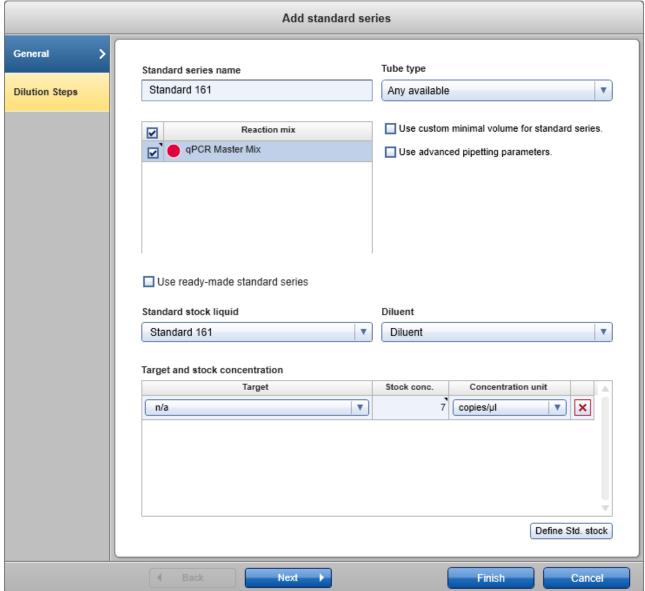


Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the Controls table.

7.2.6.3.2 Add standard series

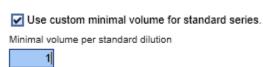
In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.



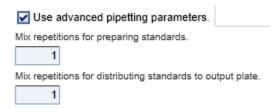
Add standard series dialog - General

- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.



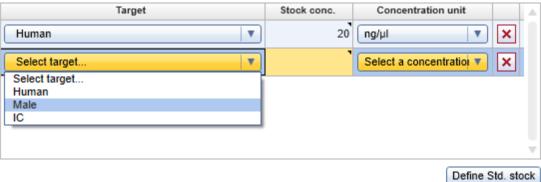
5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



concentration, enter concentration value 0.

- 6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.
 - Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.
- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 8. If the option Use ready-made standard series has not been checked, select the appropriate diluent from the Diluent menu. The menu lists all liquids that have been defined with liquid type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only. If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock

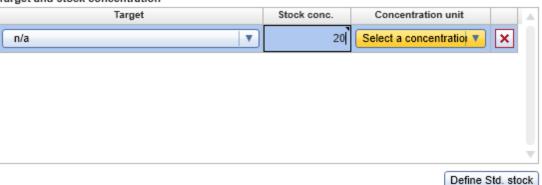
Target and stock concentration



Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration



Defining common stock, if no targets have been defined for the mix.

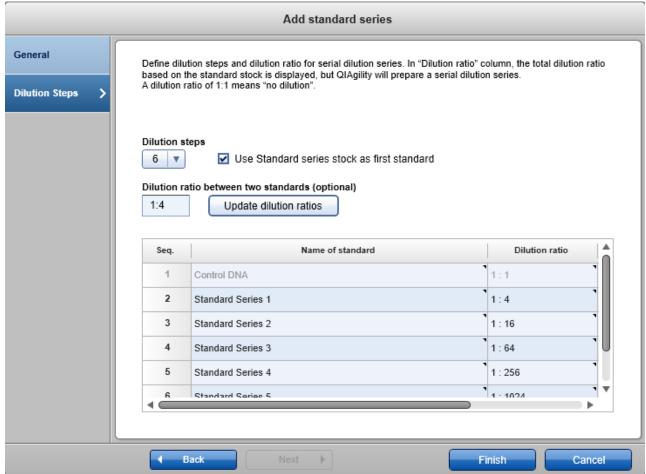
Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column	Description
Target	Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration.
	This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

7.2.6.3.3 Add standard series dilutions

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.



Add standard series dialog - Dilution steps

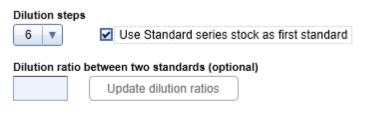
1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section Entering data.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.





Option "Use standard series stock as first standard" selected.

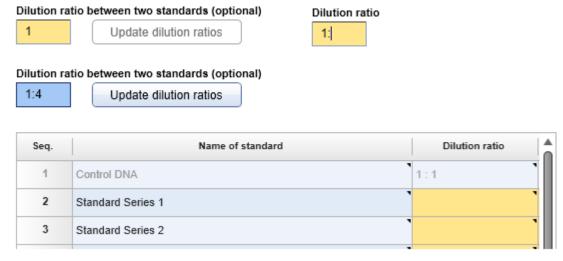
For each dilution step, if the option Use ready-made standard series has been checked in the
previous General screen, select the appropriate liquid from the corresponding menu in the Name of
standard cell.



Selecting the appropriate liquid for each standard for a user prepared standard series.

If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

4. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the **Dilution ratio** field above the dilution steps and then click **Update dilution ratios** to apply it to the series.



Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

1:4 Update dilution ratios

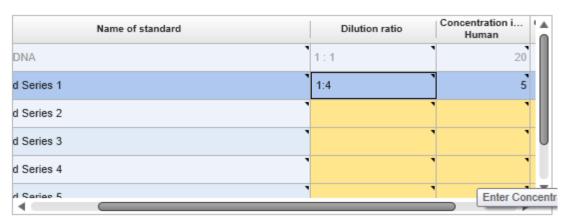
Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1:16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

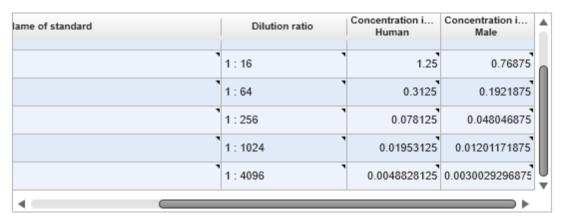
Update dilution ratios



Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.



Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

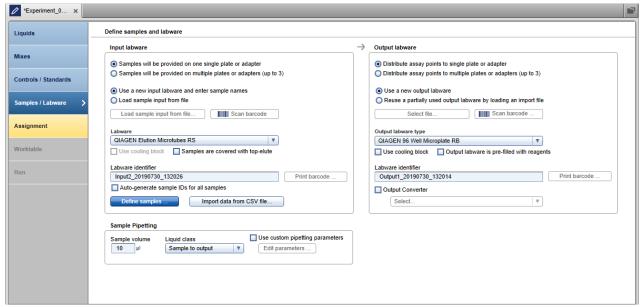
Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in Controls/Standards step.

7.2.6.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.



Samples / Labware step

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

Proceed as follows:

- 1. Select the input labware and define the samples.
 - If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input</u> labware.
 - If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to three input labware</u>.
- 2. Select the output labware.
 - If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define the output labware</u>.
 - If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to three output labware</u>.
- 3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.



- 4. The liquid class used for sample transfer is predefined by default.

 If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to Advanced sample pipetting.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the Assignment step.

7.2.6.4.1 Define an input labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

- If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.
 - Use a new input labware and enter sample names
 - O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the **Labware** drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

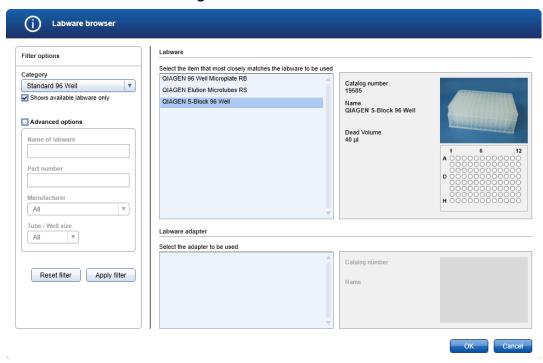
Labware

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered ▼

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware...

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section <u>Using the labware browser</u>.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use** cooling block next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

6. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. Optional step: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples		
Define samples	Import data from CSV file	

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in Samples/Labware step.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.



Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

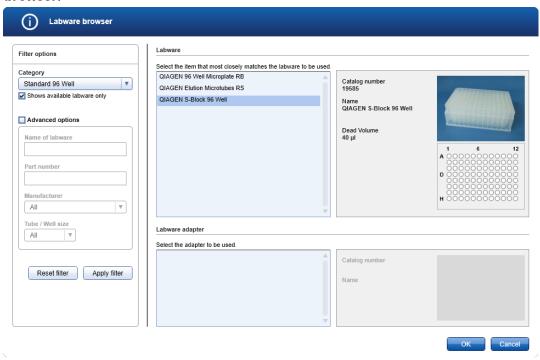
The software will load the sample information from the sample file.

13. If the sample information file also contains the labware type, it will automatically be selected in the **Labware** drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the Labware drop-down menu. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

16. Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

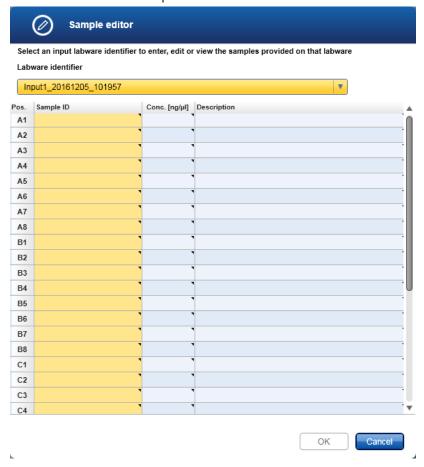
For details about the sample editor, refer to Editing sample names.

- 17. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 - Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.
 - **Note:** Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.
- 19. Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in Samples/Labware step.

7.2.6.4.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



Note: For more details how to enter information into a table refer to the section **Entering data**.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the Labware identifier field will no longer be colored yellow, and the OK button will be enabled. If the Labware identifier field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

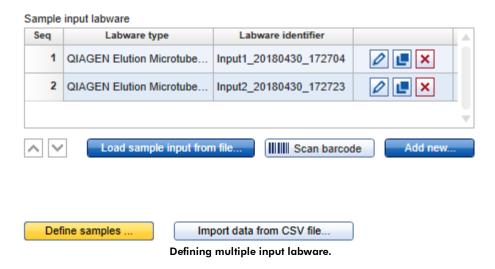
7.2.6.4.3 Defining up to three input labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option Samples will be provided on multiple plates or adapters (up to 3) has been selected, you can define up to three input labware types.

Note: The first loaded input plate will be located in the C1 worktable position.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.



1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

Click the Load sample input from file button and select the desired sample file. For details, refer to
 <u>File dialog</u>. Click Open to close the file dialog. The software will load the sample information from the
 selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode</u> dialog.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The **Add new input labware** dialog opens. Follow the steps in <u>Add input labware dialog</u>.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1-4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to **Editing sample names**.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in Samples/Labware step.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
<u></u>	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.6.4.4 Define output labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

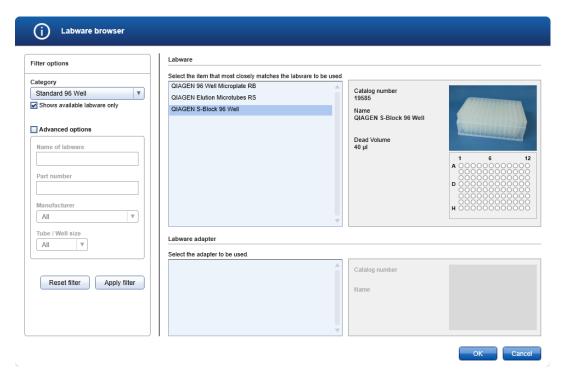
If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- 1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.





In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the **Labware** browser, refer to the section <u>Using the labware browser</u>.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

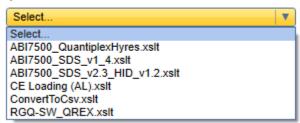
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. Optional: Click the Print barcode button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. Optional: If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to Output converter to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, None is the only entry in the list.





Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in Samples/Labware step.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.



10. Click the Select file button and select the desired labware file. For details, refer to <u>File dialog</u>. Alternatively, click the Scan barcode button. In the Scan labware barcode dialog, scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.6.4.5 Define up to three output labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option **Distribute assay points to multiple plates or adapters (up to 3)** has been selected, you can define up to three output labware types by following the steps below.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.

Output labware Distribute assay points to single plate or adapter Distribute assay points to multiple plates or adapters (up to 3) Output labware Seq Labware type Labware identifier QIAGEN S-Block 96 Well Output1 20190729 141... Eppendorf twin.tec PCR Pl.. Output2 20190729 141... QIAGEN S-Block 96 Well Output3_20190729_141... Load output labware from file. Scan barcode Add new.

Defining multiple output labware

- 1. If you would like to add output labware manually, click the **Add new** button and proceed to step 4. If you would like to load an output labware file, proceed to step 2.
 - If you would like to provide output labware information by scanning a barcode, proceed to step 3.
- Click the Load output labware from file button and select the desired file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the output information from the selected file.
 - If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.
- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.
 - If the labware is to be used with the cooling block, the box in the about column will be ticked. If the

labware is pre-filled with reagents, the box in the document of column will be ticked. To change these settings, edit the labware.

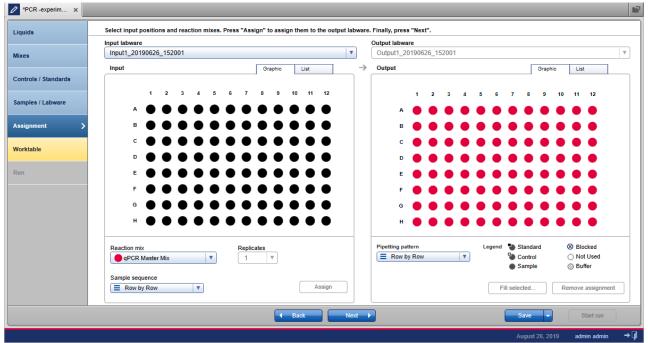
- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the Samples/Labware step.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.

7.2.6.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option Samples will be provided on multiple plates or adapters has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at

the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

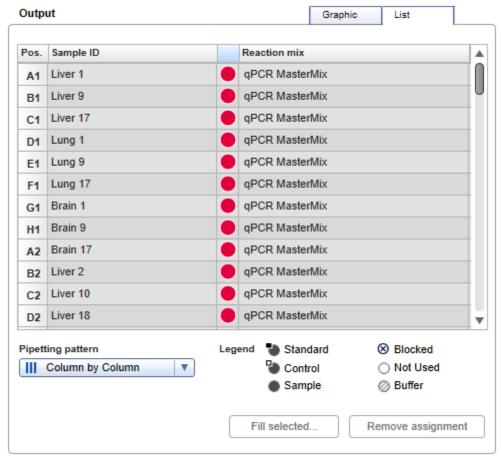
Assay points can be removed. For details, refer to Removing assay assignments.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

It is possible to define blocked positions on the output labware by selecting the position(s) and then rightclicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the assay points that have been created.



The List tab of the Output panel

7.2.6.5.1 Assigning samples with reaction mixes to the output labware

Proceed as follows:

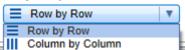
- From the Input labware menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous Labware selection step. The software will display its defined samples below in the Input panel.
- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.





Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click Assign.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate,

the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

- 8. Repeat the steps above until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 9. Close the lid.
- 10. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.6.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the Output side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or CTRL and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

2. Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.

Note: The software will remove all replicates of the desired sample(s).

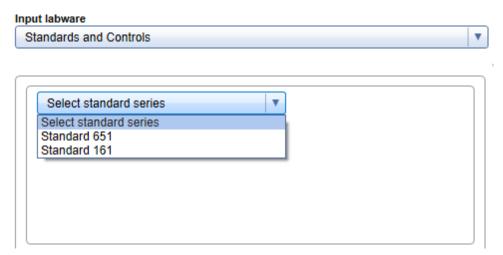
7.2.6.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

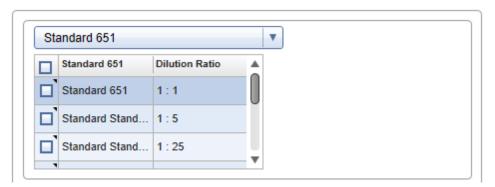
Proceed as follows:

- 1. Select **Standards and Controls** from the **Input labware** drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** dropdown list.



A table with dilution steps and dilution ratios will appear.



- 3. Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.

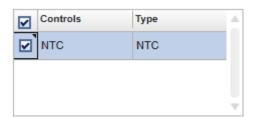


5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 6. Click Assign.
- Tick the box next to one or more controls in the following table. To select all controls, tick the box in the table header.



8. Select a mix from the Reaction mix drop-down list.



9. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 10. Click Assign.
- 11. Repeat steps 2 10 as needed, until you have defined all the positions for the planned run.
- 7.2.6.6 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

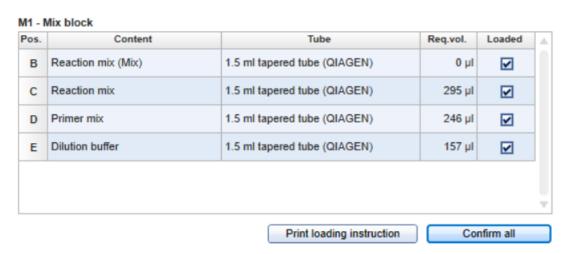
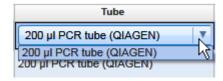


Table of tubes to be loaded to the worktable position M1.

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).

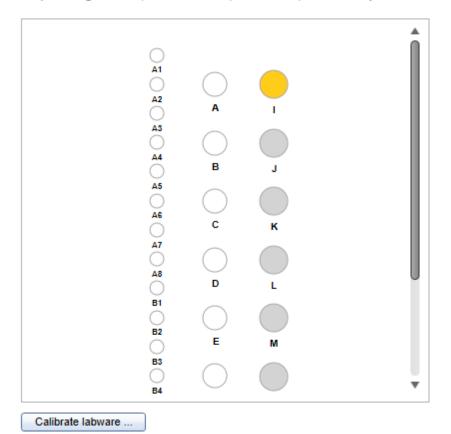


Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a

replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the Labware tab on the right to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

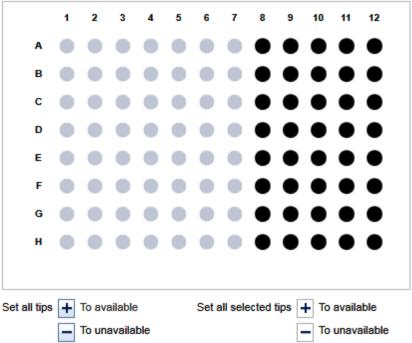
Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to Loading tips.

50 µl Conductive Filtered Tips



Interactive rack to define tip avilability.

If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

 Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in Insert tips to tip rack

holder.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

 Optional step: A yellow Calibrate labware button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- 19. Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for Generic PCR experiments.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.6.7 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

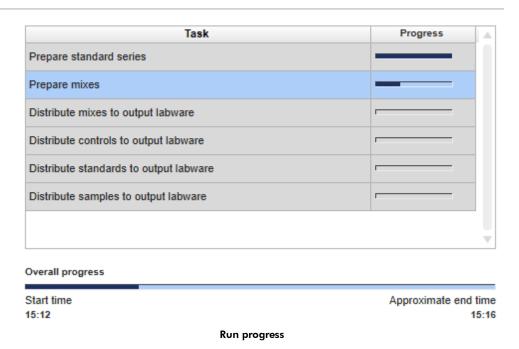
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.6.7.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.6.7.2 Pausing or canceling the run

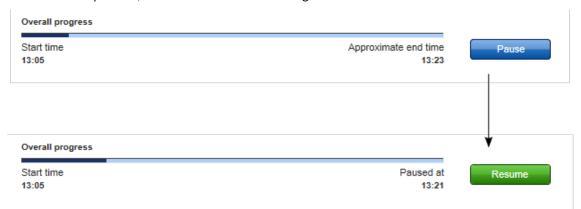
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- Click the Pause button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

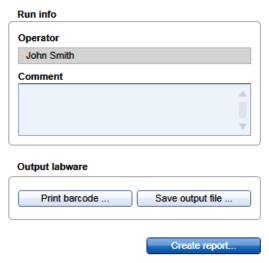
To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.6.7.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.qiagen.com/goto/QlAgility . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QlAGEN\QlAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml, the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.

Print barcode

3. Clean the worktable as described in section Cleaning the QIAgility.

configure the report options.

You can print a barcode for the output labware.

Settings tab of the **Configuration** environment.

Note: The option is available only if a printer has been defined in the Global

7.2.6.8 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the experiment file and the output file (if any has been saved), the instrument serial number, the software and plug-in version that has been used for the run and the date, time and duration of the run.

Comment

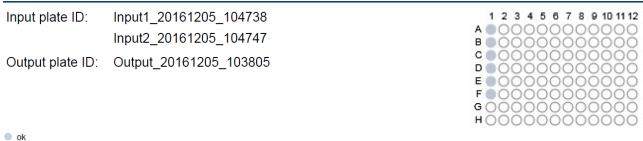
The comment that has been entered on the **Run** screen.

Reaction results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol		Description
	ok	The position was processed without any exceptions.
•	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
•	not used	This symbol is only used if an output file has been loaded as a starting point for the output labware (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results



- ok ■ warning
- error
- error
- empty

For your reference, the labware identifiers for input and output labware will be listed even if the graphic is not shown. If multiple input labwares were used for the run, the labware identifiers will be listed in the same order in which they were defined on the **Labware selection** step.

A table listing the reaction result for each processed position on the output labware is shown:

Column	Description
Output	The position on the output labware.
Identifier	Specifies the sample ID or the name of the particular standard or control.
Туре	Sample, Standard or for controls, the Control type.
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls. If multiple input labwares were used for the run, the position is enhanced by a short input labware reference (e.g., A1/I1 refers to position A1 of the 1st Input labware, where "I" indicates Input, and "1" indicates the 1st input labware in the identifier list).
Actual content	Lists the components, i.e., the liquids that have been transferred into the position during the run.
Initial sample conc. /Std conc.	For samples, the initial concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty.
	For standards, the concentration of the standard is listed. If standards have been defined individually per target, the concentrations are enhanced by a short target reference (e.g., (1) 5 ng/ μ l refers to the 1st target). A target legend is provided below the table.
	Always empty for controls or NTC.
xNA content	For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty.
	For standards, the concentration of the standard is listed.
	Always empty for controls or NTC.
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column Description

Exception The event that has been displayed during the run.

Position Lists the positions that were affected by the exception.

First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.

The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.

Example:

"M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).

Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.

Example:

If the "not enough liquid" liquid level detection error occurs in the final reaction (master) mix tube while serving the last output position with the mix, all output positions that were designated to contain this final reaction (master) mix will be listed here as potentially affected. However, most of these positions have already been served successfully and will be flagged OK in the reaction results table. You can trust an 'OK' flag in the reaction results table.

User response A short form, which option (button) of the message dialog has been pressed.

Note: The **Basic report** does not list pauses. If you want to have also pauses listed, use the **Advanced report**, instead.

Detailed run information

This section lists experiment definitions and the task list of the run.

Defined mixes:

Column	Description	
Name [Targets]	Name of the mix. If defined, targets are listed additionally.	
Input	Lists the content of the mix per reaction.	
Total volume	The total volume of the mix, given in μ l.	
Liquid class	The liquid class used to distribute the mix, including version number.	
Advanced pipetting parameters	The advanced pipetting parameters that have been used for the mix: extra volumes, liquid class for mixing incl. version number, mix repetitions after the last input liquid has been added.	
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.	

Input liquids:

Column	Description	
Name	Name of the liquid.	
Lot number/Expiry date	Lot number and expiry date, if defined for the liquid.	
Liquid class	The liquid class used to distribute the liquid, including version number.	
Advanced pipetting	The extra volumes that have been used for the liquid.	
parameters	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.	

The definitions for sample pipetting:

Column	Description	
Volume	Sample volume used for the run.	
Liquid class	The liquid class used to distribute samples to the output labware, including version number.	
Advanced pipetting parameters	The advanced pipetting parameters that have been used for samples: extra volumes, mix repetitions after the sample has been distributed.	
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.	

Standard series, if defined in the experiment:

Column	Description	
Name [Mix]	Name of the standard series and the related reaction (master) mix.	
Standards	Name of the standard and the concentration, if defined the related target is listed.	
Advanced pipetting parameters	The advanced pipetting parameters that have been used for samples: extra volumes, mix repetitions after the sample has been distributed.	
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.	

Finally, this section lists all tasks of the run as they were displayed in the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description
Exception	The event or pause that has been displayed during run.
Position/Duration	For an event:
	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.
	For a pause: the duration of the pause.
User response	A short form, which option (button) of the message dialog has been pressed.

Sample information

Lists the sample information for each input labware as it was entered in the sample editor, loaded by the sample input file, or automatically generated by the software, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Note: For tip racks of the same tip type, the overall required, and the overall available number of tips is shown, similar to the information in the worktable screens.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QIAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.6.9 Samples only experiments

The Generic PCR Plug-in allows to setup a run to distribute samples to the output labware, only.

Such experiments are created by clicking **Generic PCR setup** from the **Home** screen and then defined in the **Experiment** environment of the software.

The experiment wizard has 7 steps that guide you through the run setup process. Use the **Next** and **Back** buttons at the bottom to navigate between steps. The setup uses the screens for <u>generic PCR setup</u>. We recommend that you familiarize yourself with the features of the generic PCR setup first.

To setup a run to distribute samples to the output labware, only:

- Skip the Liquids step by clicking Next to proceed to the Mixes step.
 Note: However, in the case that you need to define liquids for the run, follow the steps described in Liquids step.
- 2. In the Mixes step add at least one mix. In general, follow the instructions described in Mixes step, however, in the Add mix dialog select the option Final reaction mix already on output from the General screen, to indicate that the mix will not be created nor distributed. The Target screen of the Add mix dialog is optional in order to allow easy transfer of defined assay targets to respective PCR cycling software.
 Click Next to proceed.
- 3. Skip the Controls/Standards step by clicking Next to proceed to the Samples/Labware step.

Note: It is possible to use standards and controls in this workflow, too. In this case, define the needed liquids in the Liquids step. For details on how to define standards and controls refer to Controls/Standards step.

4. In the Samples/Labware step follow the instructions described in <u>Samples/Labware step</u>. For the output labware, the option Output labware is prefilled with reagents is preselected. Keep this option selected, because pipetting behavior will be adjusted accordingly. Click Next to proceed.

Note: It is recommended to use sample volumes of at least 5 μ l, as it is technically not reliable to transfer less than 5 μ l volume into an empty labware.

- 5. In the **Assignment** step follow the instructions described in <u>Assignment step</u>.
- 6. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 7. Close the lid.
- 8. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize. **Note:** While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.
- 9. In the **Worktable** step, follow the loading instructions and the instructions to start the run described in <u>Worktable step</u>.
- 10. Refer to Run for information about the Run step. After run, follow the instructions in Run completion.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.7 Generic PCR setup with normalization experiments

The Generic PCR Plug-in enables easy and convenient qPCR setup including sample normalization.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections Working with experiments for pathogen detection and Working with gene expression experiments, respectively. For generic PCR setup experiments without sample normalization refer to section Generic PCR experiments. For PCR setup with serial dilution experiments, refer to the PCR setup with serial dilution experiments section. It is also possible to set up a run for sample normalization only. In this case, refer to section Normalization only experiments.

Samples can be normalized based on mass or concentration. Ensure that the correct selection has been made in the <u>User settings for Generic PCR experiments</u> before the experiment is created. Changing the setting will take effect for new experiments, only.

New experiments for generic PCR setup, including sample normalization, are created by clicking **Generic PCR setup with Normalization** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and **Mixes** step will be enabled,

initially. Subsequent steps will become available, once the required information has been entered into the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 8 steps that guide you through the run setup process:

- 1. <u>Liquids</u>
- 2. Mixes
- 3. Controls/standards
- 4. Samples/Labware
- 5. Assignment
- 6. Normalization
- 7. Worktable
- 8. Run

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

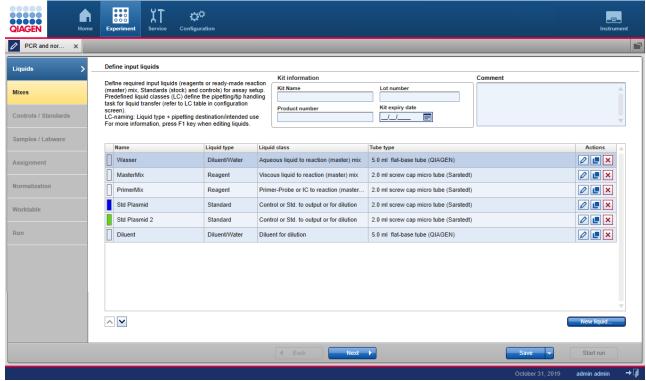
7.2.7.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will, e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click to open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.



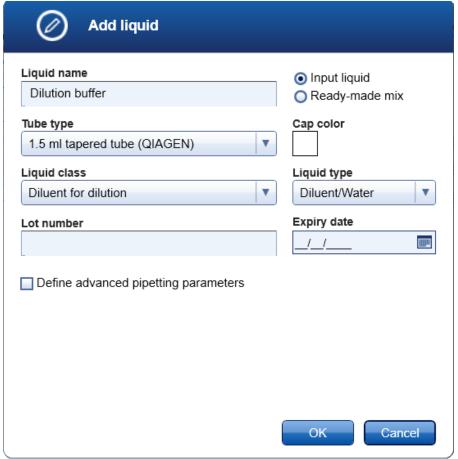
The buttons in the **Actions** column

of the table have the following functions:

Button	Action	Description
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
<u></u>	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.7.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.



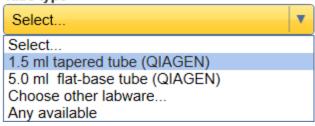
Add liquid dialog

- Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed. If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

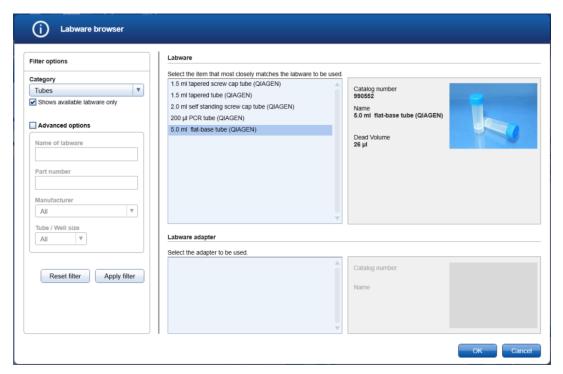
Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types
used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the
respective entry.

Tube type



Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the <u>Labware Manager</u>, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

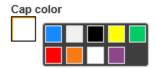
The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the **Labware browser**, refer to the section <u>Using the labware browser</u>.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- · the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

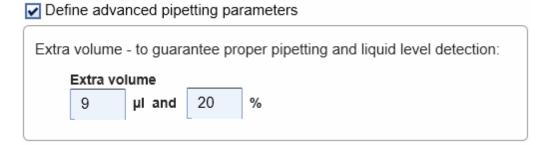
6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.



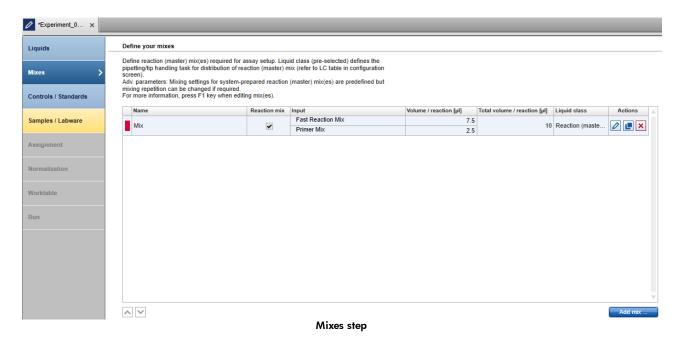
In the dialog, click **OK** to confirm your values and to close the dialog.
 Otherwise, click **Cancel** to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.7.2 Mixes step

The Mixes step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
- o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.



Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in Add new mix dialog input.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in <u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the **Mixes** screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click Next in the button bar to proceed to the Controls/Standards step, described in

Note: The **Next** button is disabled, if a row in the table is highlighted yellow. User prepared reaction (master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit() the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

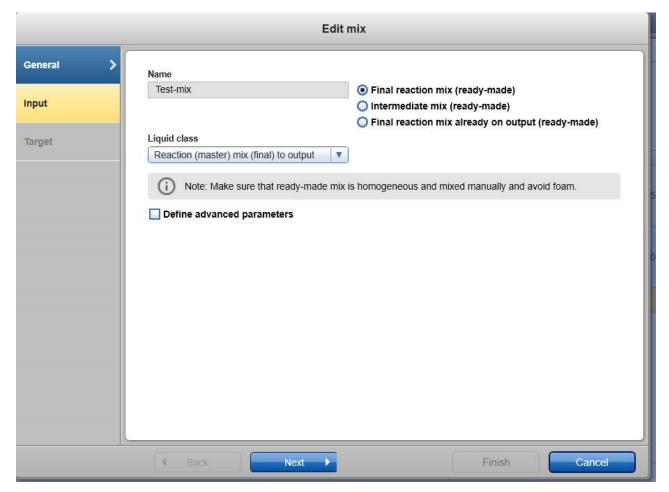
The buttons in the **Actions** column of the table have the following functions:

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.
	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

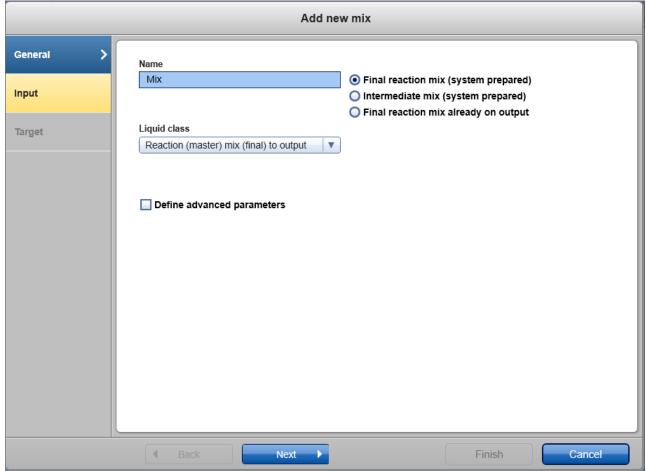
7.2.7.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.



Edit mix dialog



Add new mix dialog - General

 Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option

Description

Final reaction mix (ready-made)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QlAgility. In the later Assignment step, the mix will be available for selection. Duringthe run, the QlAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QlAgility as QlAgility will not perform any mixing steps.

Intermediate mix (readymade)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QlAgility. During the run, the QlAgility will pipette the mix into the tube, where the more specific mix is created.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QlAgility as QlAgility will not perform any mixing steps.

Final reaction mix already on output (ready-made) Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.

If the option is selected, the Input screen of the dialog will be skipped.

Final reaction mix (system prepared)

Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.

Intermediate mix (system prepared)

Use this option if this mix will be created by the QlAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QlAgility. Then the QlAgility will pipette the mix into the tube where the more specific mix is created.

Final reaction mix already on output

Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

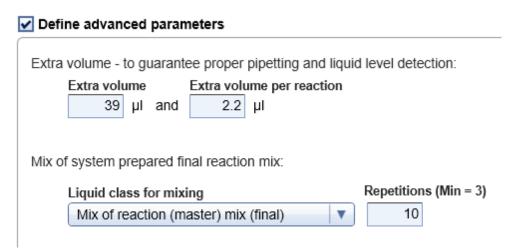
Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QlAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.



For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

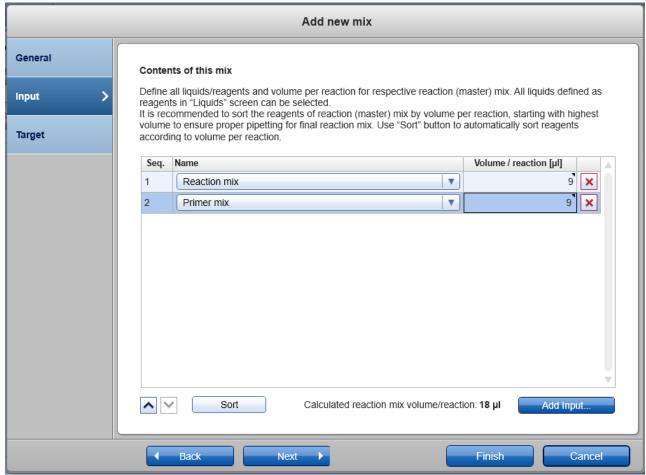
During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in Mixes step.

7.2.7.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.



Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

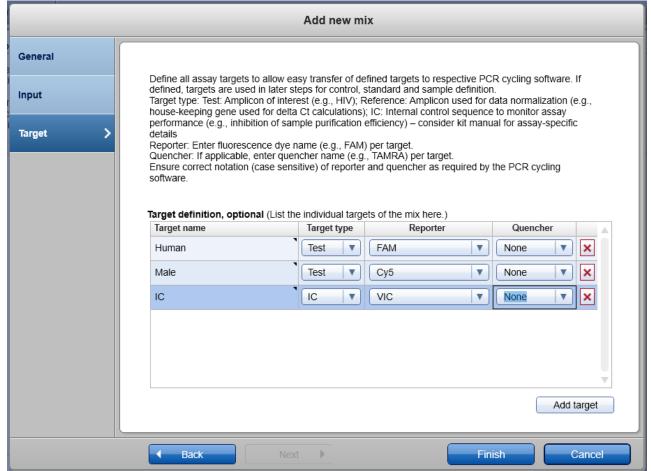
- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.
 Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.7.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.



Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type Description

Test Amplicon of interest (e.g., HIV)

Reference Amplicon used for data normalization (e.g., house-keeping gene used for delta

CT calculations)

IC Internal control sequence to monitor assay performance (e.g., inhibition of

sample purification efficiency) – consider kit manual for assay specific details)

Optional step: Click on the menu in the Reporter column and select an appropriate reporter dye for the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.



Green Yellow Orange ROX

FAM JOE

VIC SYBRGREEN1 CALFLUORRED610

SYBRGREENI HEX CY35

FLUORESCEIN TET **TEXASRED**

ALEXAFLUOR488 MAX ALEXAFLUOR568

CALFLUOR

GOLD540

YAKIMAYELLOW

Red Blue Crimson

CY5 **MARINABLUE** QUASAR705

QUASAR670 **EDANS** ALEXAFLUOR680

ALEXAFLUOR633 LIGHTCYCLERRED705 **BOTHELLBLUE**

LIGHTCYCLERRED640 ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in Mixes step.

7.2.7.3 Controls/Standards step

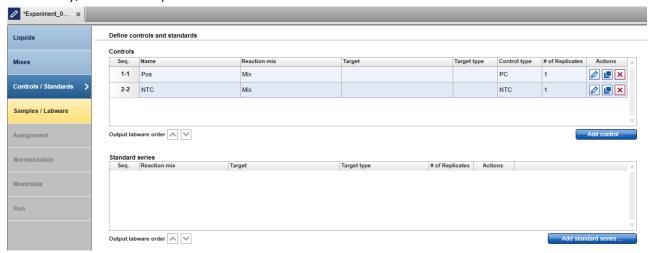
This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add standard series</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.



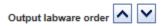
Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.7.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.



Add control dialog

1. Enter a control name in the **Control name** field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- 2. Select the input liquid from the **Liquid** menu. The menu lists all liquids that have been defined with liquid type **Pos./neg. control** or **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix** (ready-made), **Final reaction mix** (system prepared), **Final reaction mix in output** (ready-made) or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type



Option	Description
PC	Is a positive control for the intended assay.
NTC	Is a non-template control for the intended assay.
NC	Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

6. If the PC control type has been chosen, you can specify a custom volume for the control. Tick the **Use** specific volume box, type in a value in the **Volume** field, and select a diluent from the **Diluent** (used to fill up to sample volume) drop-down menu.

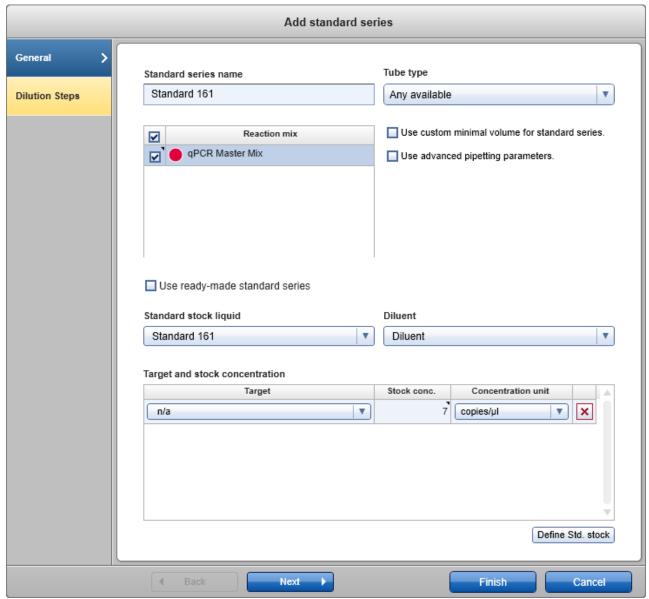


Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the Controls table.

7.2.7.3.2 Add standard series

In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.



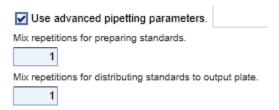
Add standard series dialog - General

- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.

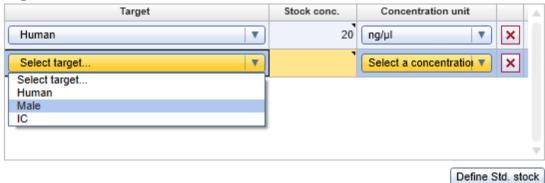


5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



- 6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.
 - Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.
- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 8. If the option Use ready-made standard series has not been checked, select the appropriate diluent from the Diluent menu. The menu lists all liquids that have been defined with liquid type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. Optional step. Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only. If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock concentration, enter concentration value 0.

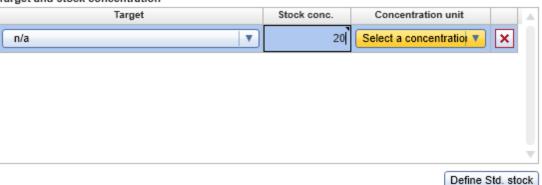
Target and stock concentration



Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration



Defining common stock, if no targets have been defined for the mix.

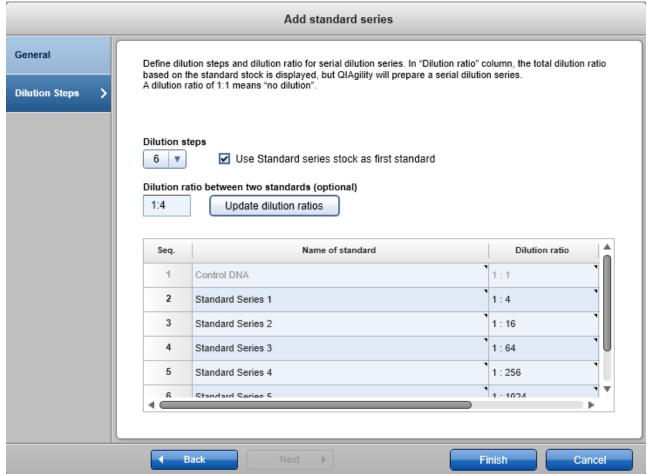
Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column	Description
Target	Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration.
	This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

7.2.7.3.3 Add standard series dilutions

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.



Add standard series dialog - Dilution steps

1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section Entering data.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.





Option "Use standard series stock as first standard" selected.

For each dilution step, if the option Use ready-made standard series has been checked in the
previous General screen, select the appropriate liquid from the corresponding menu in the Name of
standard cell.



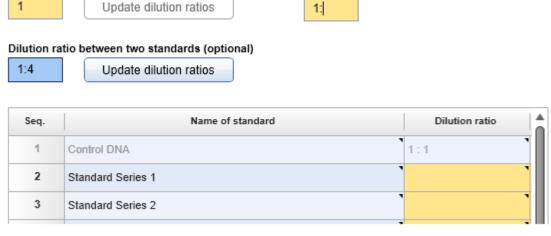
Selecting the appropriate liquid for each standard for a user prepared standard series.

If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

Dilution ratio

4. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the **Dilution ratio** field above the dilution steps and then click **Update dilution ratios** to apply it to the series.

Dilution ratio between two standards (optional)



Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

1:4 Update dilution ratios

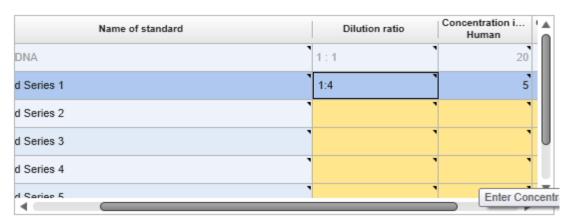
Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1:16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

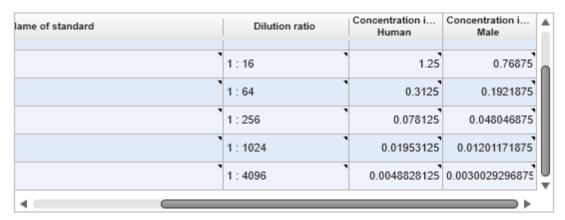
Update dilution ratios



Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.



Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

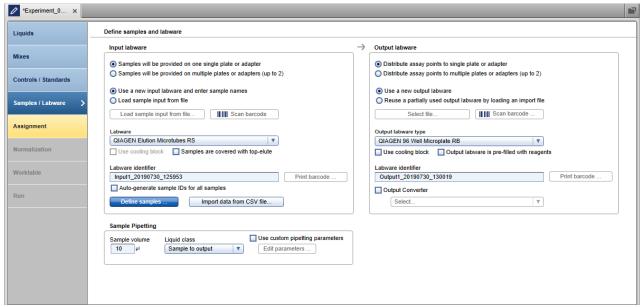
Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in Controls & standards steps with normalization.

7.2.7.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.



Samples / Labware step

Proceed as follows:

Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to two input labware</u>.

2. Select the output labware.

If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in Define output labware.

If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.

3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.



4. The liquid class used for sample transfer is predefined by default.

If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** drop-down menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.

5. Optional: If required, use custom pipetting parameters for samples. If you tick the box, the Edit parameters button becomes active. If you click the button, the Define advanced pipetting parameters dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to Advanced sample pipetting.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the Assignment step.

7.2.7.4.1 Define an input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

- If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.
 - Use a new input labware and enter sample names
 - O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the **Labware** drop-down menu on the left side of the screen.
- All previously selected labware types will be listed. The Choose other labware option will be listed last.

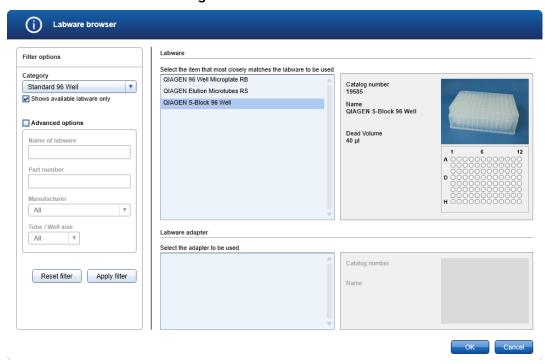
Labware

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapere

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware...

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the **Labware** browser, refer to the section <u>Using the labware browser</u>.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use** cooling block next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

6. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. Optional step: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample	Ds for all samples
Define samples	Import data from CSV file

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in Samples/Labware step.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option Load sample input from file has been checked, the Load sample input from file and Scan barcode buttons will be enabled.



Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to File dialoa.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode</u> dialog.

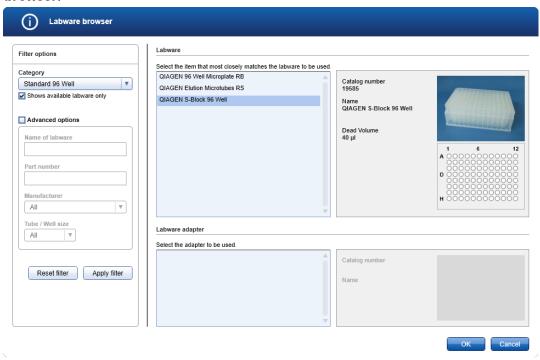
The software will load the sample information from the sample file.

13. If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the Labware drop-down menu. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

16. Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

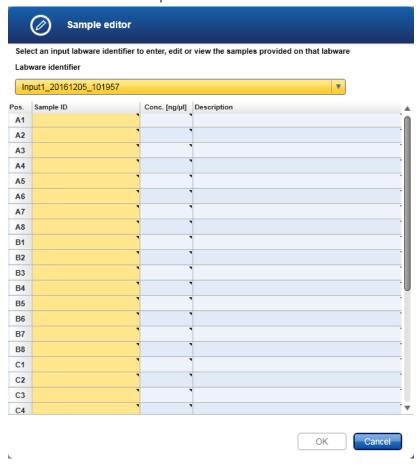
For details about the sample editor, refer to Edit samples.

- 17. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 - Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.
 - **Note:** Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.
- 19. Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in Samples/Labware step.

7.2.7.4.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



Note: For more details how to enter information into a table refer to the section **Entering data**.

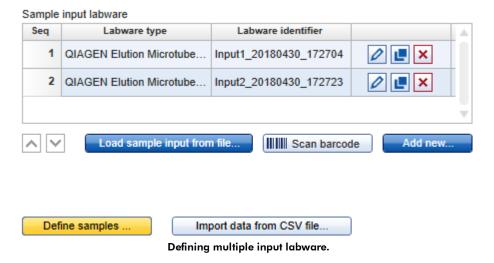
Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.7.4.3 Defining up to two input labware

If the option Samples will be provided on multiple plates or adapters (up to 2) has been selected, you can define up to two input labware types.

All defined input labware will be listed in the Sample input labware table. Initially, the table is empty.



 If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

Click the Load sample input from file button and select the desired sample file. For details, refer to
 <u>File dialog</u>. Click Open to close the file dialog. The software will load the sample information from the
 selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to Scan labware barcode dialog.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1-4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to **Editing sample names**.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in <u>Samples/Labware step</u>.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
<u>. </u>	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.7.4.4 Define output labware

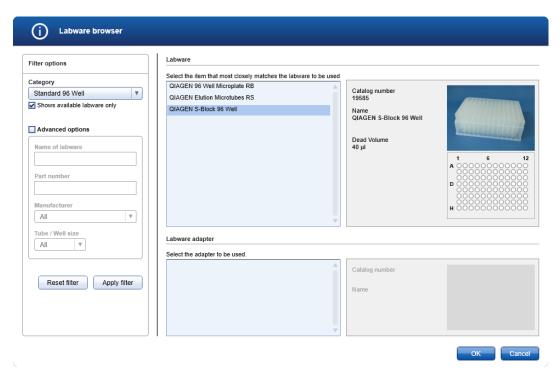
If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- You can either Use a new output labware or Reuse a partially used output labware by loading an import file. The option Use a new output labware will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

Select the output labware from the Output labware type menu on the right. Click Choose other labware to open the Labware browser.





In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QlAgility will consider this information and, e.g., not reuse tips.
- Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

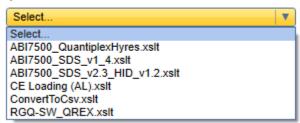
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.





Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in Samples/Labware step.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.



10. Click the Select file button and select the desired labware file. For details, refer to <u>File dialog</u>. Alternatively, click the Scan barcode button. In the Scan labware barcode dialog, scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

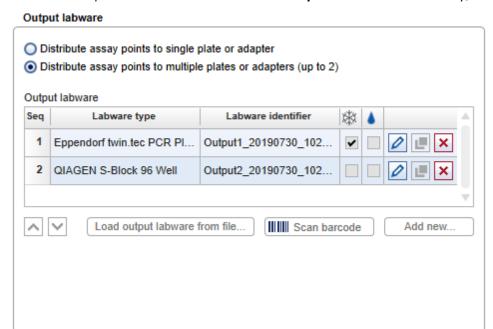
Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.7.4.5 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.



Defining multiple output labware

- If you would like to add output labware manually, click the **Add new** button and proceed to step 4.
 If you would like to load an output labware file, proceed to step 2.
 If you would like to provide output labware information by scanning a barcode, proceed to step 3.
- 2. Click the **Load output labware from file** button and select the desired file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the output information from the selected file.
 - If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.
- Click the Scan barcode button. The Scan labware barcode dialog opens. Scan the labware barcode and click OK to confirm. For details, refer to Scan labware barcode dialog.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.
 - If the labware is to be used with the cooling block, the box in the solumn will be ticked. If the labware is pre-filled with reagents, the box in the column will be ticked. To change these settings, edit the labware.

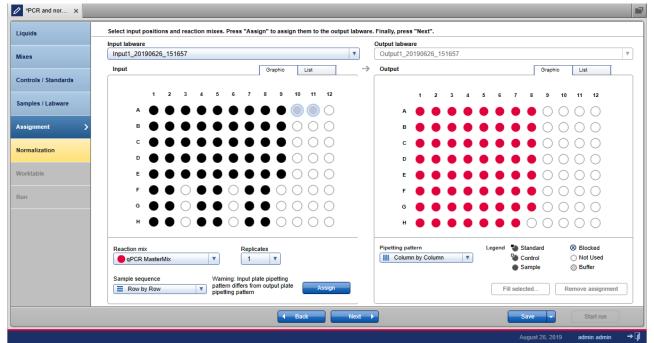
- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the Samples/Labware step.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.

7.2.7.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option Samples will be provided on multiple plates or adapters has been selected, you can switch between the different input labwares using the box above the Graphic and List tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

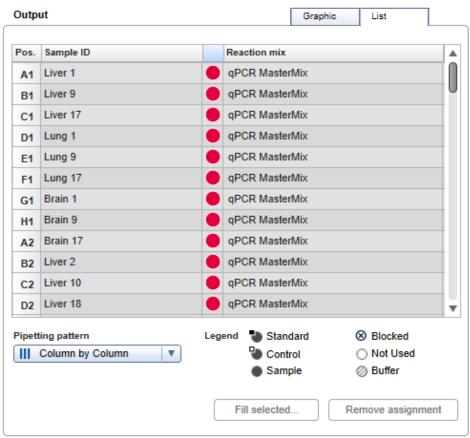
Assay points can be removed. For details, refer to Removing assay assignments.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the assay points that have been created.



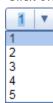
The List tab of the Output panel

7.2.7.5.1 Assigning samples with reaction mixes to the output labware

Proceed as follows:

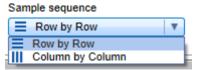
 From the Input labware menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous Labware selection step. The software will display its defined samples below in the Input panel.

- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.



Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click **Assign**.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

8. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.7.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the Output side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or CTRL and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

Click Remove assignment. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.

Note: The software will remove all replicates of the desired sample(s).

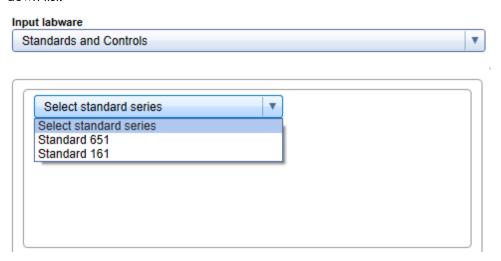
7.2.7.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

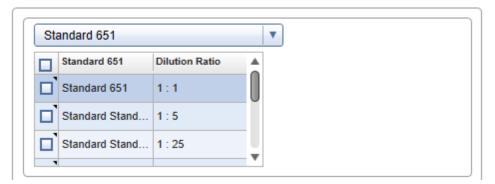
Proceed as follows:

- 1. Select **Standards and Controls** from the **Input labware** drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** drop-down list.



A table with dilution steps and dilution ratios will appear.



- Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.



5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 6. Click Assign.
- Tick the box next to one or more controls in the following table. To select all controls, tick the box in the table header.



8. Select a mix from the Reaction mix drop-down list.



9. Select the number of replicates from the Replicates drop-down list.



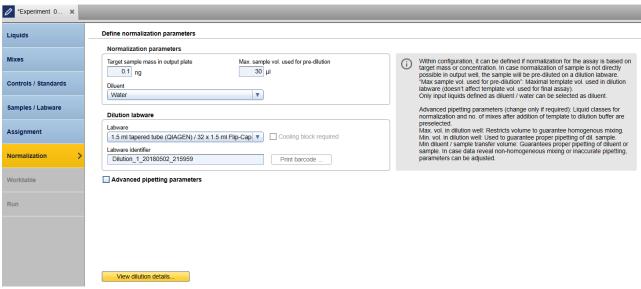
Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 10. Click Assign.
- 11. Repeat steps 2 10 as needed, until you have defined all the positions for the planned run.

7.2.7.6 Normalization step

In the **Normalization** step define the parameters for normalization. The QIAgility Setup Manager Software provides the option to normalize samples based on sample mass or sample concentration. When normalizing to a specific concentration, the sample will be diluted to the chosen target value and afterwards transferred to the output labware. In case normalization of a sample is not directly possible in the output position, the sample will be pre-diluted on a dilution labware. The software pre-calculates for each sample, if the target can be reached. Based on this calculation you can decide, if you want to include or exclude samples.

To configure if normalization is based on target sample mass or sample concentration, click **Configuration** in the main tool bar, click the **Settings** tab and then select the **Generic PCR user settings** step. For details refer to <u>settings</u>.



Normalization step

To define sample normalization:

1. Enter the Target sample mass or concentration that shall be reached in the output labware.

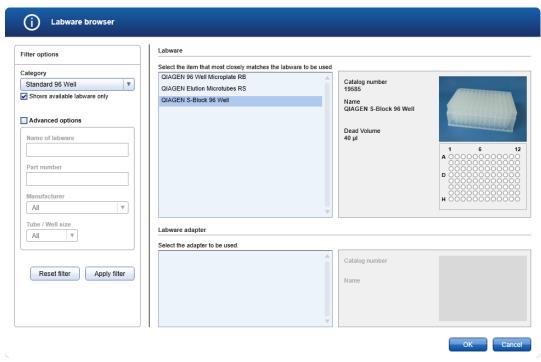
Note: When normalizing to target sample mass, the unit is predefined as "ng".

2. Enter into the field **Max. sample vol. used for pre-dilution** the maximal sample volume that shall be used in maximum for pre-dilution, e.i. the maximal template volume that will be used in the dilution labware to pre-dilute a sample.

Note: The higher the maximal sample volume for pre-dilution is set, the more flexible the software can calculate possible normalization strategies.

- 3. Click **Diluent** to select the diluent that shall be used for sample normalization. The menu lists all liquids that have been defined with liquid type **Diluent/Water** in the **Liquids** step. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 4. Click **Labware** to select the labware that shall be used to pre-dilute samples. All previously selected labware types will be listed. The **Choose other labware** option will be listed last. Select your dilution labware from the drop-down list. If it is not listed, click **Choose other labware** to open the Labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

- 5. **Optional:** If you would like to use the cooling block for the dilution labware, check the option **Cooling** block required next to **Labware**.
- 6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 8. **Optional:** Define advanced pipetting parameters. If you tick the box, parameters become visible. In case the initial sample concentration is too high or too near to target, parameters can be adjusted.

Advanced pipetting parameters Liquid class for pipetting diluent onto dilution labware Diluent to dilution . Liquid class for pipetting sample onto dilution labware No. of mix repetitions on dilution labware Sample for dilution ٧ 7 Max. volume in dilution well Min. volume in dilution well Min. diluent transfer volume Min. sample transfer volume 1400 µl 2 µI 20 ul 5 µI

Option **Description** Liquid class for pipetting diluent onto Preselected. dilution labware Defines the pipetting and tip handling for pipetting the diluent to the dilution labware. Liquid class for pipetting sample onto Preselected. dilution labware Defines the pipetting and tip handling for pipetting the sample to the dilution labware. Sample (template) will be pipetted after the diluent has been distributed. The value is predefined by the selected liquid class. No. of mix repetitions on dilution labware Defines the mix repetitions after the sample (template) has been added to the diluent on the dilution labware. Mix repetitions have been developed to guarantee proper mixing of water based diluents and samples for the default value of maximal volume in dilution well. In case maximal volume in dilution well is manually increased, it is recommended to increase mix repetitions. Max. volume in dilution well Predefined.

Restricts the volume in the dilution positions in order to

guarantee homogeneous mixing.

In case the maximal volume in dilution well is manually iincreased, it is recommende to increase mix repetitions. If the total volume of the selected dilution labware is smaller than the setting of this parameter, the total volume of the dilution

labware will be used.

Min. volume in dilution well Predefined.

Ensures proper creation of pre-dilution and pipetting of the

diluted sample to the output labware.

Min. diluent transfer volume Predefined.

Guarantees presize pipetting of diluent for pre-dilution. A

setting $< 2\mu l$ is not recommended.

Min. sample transfer volume Predefined.

Guarantees presize pipetting of sample (template) for pre-

dilution. A setting $< 1 \mu l$ is not recommended.

Click View dilution details to open a list with the results of pre-calculation for the samples. The button is colored yellow, if the normalization target will not be reached for all samples. Follow the steps described in Dilution details.

- 10. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 11. Close the lid.
- 12. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.7.6.1 Dilution details

The dialog lists the normalization pre-calculation for all samples. It is possible to enter individual normalization targets for output positions.



Dilution details

- 1. Check the calculation. Find a description of the table columns, below.
- Optional: You can enter an individual normalization target for positions on the output labware. Click into the Target mass / concentration field of the corresponding output position row and enter the new target value. The calculation for this position will automatically be updated.
- 3. For each sample that has yellow colored columns Include/Exclude, decide whether you want to include the sample into the run or not. Check the corresponding option. The sample position will no longer be colored yellow. To include all samples, click the Include all button below the table. For details refer to the description of the Include and Exclude columns, below.
- Optional: Samples that have no yellow color indication in Include/Exclude column do not have any
 restrictions in regards to normalization, but can also be excluded, if needed.

- 5. If no sample has yellow colored **Include/Exclude** column, the **OK** button will be enabled.
- 6. Click **OK** to confirm your selections and to close the dialog.

Proceed with step 10 in Normalization step.

The table contains all samples from the input labware. Samples that have not been previously assigned in the Assignemt step are listed last.

Column	Description
Include	If checked, the sample will be included in the run.
	All samples, previously assigned in the Assignment step, which have no restriction to reach the normalization target, are automatically included. Otherwise, the columns Include/Exclude are highlighted in yellow to indicate that a decision by the user is required.
	Check the option to include the sample.
Exclude	If checked, the sample will not be used in the run and will be automatically unassigned.
	All samples, previously assigned in the Assignment step, which have a restriction to reach the normalization target, are marked in yellow to indicate that a decision (include or exclude) by the user is required.
	After confirming the dialog with OK, the sample gets automatically unassigned. The following positions on the output labware will be filled up according to the selected Pipetting pattern and Pipetting order from the Assignment step.
Pos.	The sample position on the input labware.
Sample ID	The sample ID.
	If the sample ID is too long, it is cut off. To view the whole sample ID, hover the mouse over the field.
Target mass/ Target conc.	The normalization target. Initially, the value that has been entered into the Normalization step is shown.
	However, you can edit the value if you need an individual normalization target for the corresponding output position. If no target value has been entered, or if the target value is too high or too low, the field is highlighted in yellow to indicate that a valid target needs to be entered.
Output pos.	The position on the output labware.
Orig. conc.	The original sample concentration as imported from the sample input file or entered in the sample editor.
Dilution strategy	The dilution strategy is automatically selected by the software and cannot be changed by the user.
	If the normalization target cannot be reached, the reason is listed. Decide whether to include or exclude the sample. For details, refer to the description below.

Note: It is possible to influence the dilution strategies by changing normalization parameters. The software always used the dilution strategy for which the lowest reagent volumes are required.

Used sample volume The sample volume used during normalization.

The dilution strategy depends on

- the value for the normalization target
- · the original concentration of the sample
- the setting for maximum volume to be used for pre-dilution of a sample in the dilution labware
- the setting for minimum volume in the dilution well
- the sample volume for the run as specified in Samples/Labware
- the selected dilution labware (total volume and among of dilution positions)
- the maximum volume allowed in the dilution labware (advanced pipetting parameters for normalization)
- the minimum sample transfer volume (advanced pipetting parameters for normalization)
- the minimum diluent transfer volume (advanced pipetting parameters for normalization)

Dilution strategy	Description
Dilution in output position.	It is sufficient to dilute the sample directly in the output position within the defined sample volume for the assay setup.
Dilution uses 1 dilution position.	The sample will be diluted in one position of the dilution labware. Then the defined sample volume for the run will be transferred from the dilution labware to the output labware position.
Dilution uses 2 dilution positions.	The sample will be diluted using two positions of the dilution labware. Then the defined sample volume for the run will be transferred from the second dilution position on the dilution labware to the output labware position.
Dilution uses 1 dilution position and output position.	The sample will be pre-diluted in one position of the dilution labware. The last dilution step will be performed in the output position within the defined sample volume for the assay setup.
Original concentration too high.	The sample cannot be normalized to the given normalization target, because the original sample concentration is too high. The required dilution ratio cannot be created using the defined normalization parameters. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be diluted to this value.
Original concentration too low.	The original sample concentration is lower than the normalization target. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will

be used undiluted.

Original concentration too close to target.

The sample cannot be normalized to the given normalization target, because the defined normalization parameters do not allow such a small sample dilution ratio. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be used undiluted. Otherwise, the normalization parameters have to be changed (e.g., increase the Max. sample volume used for pre-dilution value).

7.2.7.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the Worktable

While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status.

The **Worktable** step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the Next and Back buttons, or just click on a position in the Worktable selector.

The Worktable selector displays the status of each worktable position:



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click Confirm all below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - Mix block

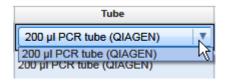
B Reaction mix (Mix) 1.5 ml tapered tube (QIAGEN) 0 μl C Reaction mix 1.5 ml tapered tube (QIAGEN) 295 μl D Primer mix 1.5 ml tapered tube (QIAGEN) 246 μl	
D Primer mix 1.5 ml tapered tube (QIAGEN) 246 μl	1.5 ml tapered tube (QIAGEN) 295 μl ☑
	1.5 ml tapered tube (QIAGEN) 246 μl ☑
E Dilution buffer 1.5 ml tapered tube (QIAGEN) 157 μl	1.5 ml tapered tube (QIAGEN) 157 μl ☑

Print loading instruction

Confirm all

Table of tubes to be loaded to the worktable position M1.

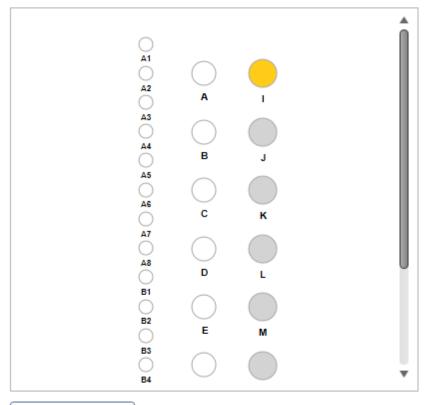
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the Labware tab on the right to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

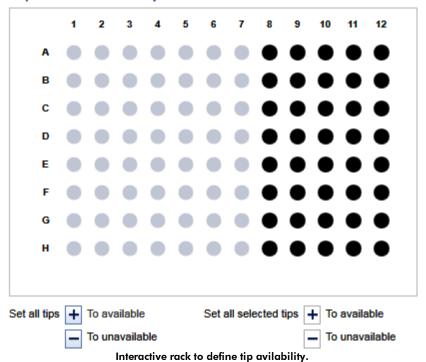
Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- 19. Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for Generic PCR experiments.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.7.8 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

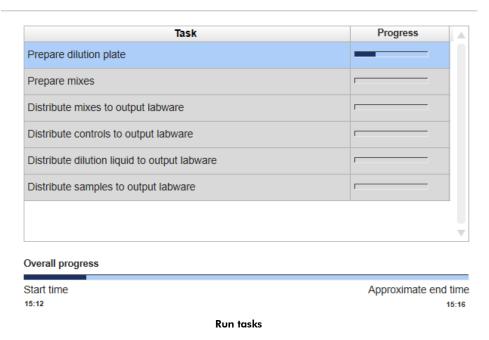
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.7.8.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.7.8.2 Pausing or canceling the run

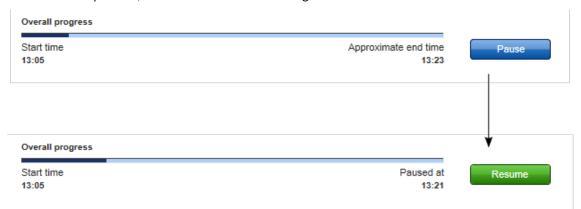
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- Click the Pause button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.7.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.qiagen.com/goto/QIAgility . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A $\frac{1}{1}$ can be created for documentation purposes. Refer to the $\frac{1}{1}$ user settings to configure the report options.
Save dilution plate	A file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the dilution labware and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

1. Remove the labware from the worktable.

- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.7.9 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information (incl. dilution labware)

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (incl. dilution strategy)
- Worktable information
- Detailed run information (incl. dilution labware)
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. Additionally, the report contains information about normalization parameters and dilution labware (both report types). Information about the dilution strategies used for the samples is available in the **Advanced** report, section **Sample information**.

Additional information is described below.

Detailed run information

In addition to the information described in <u>Report</u>, this section contains further information for both report types. It lists the normalization parameters as defined in the **Normalization** step, and contains a table that describes the dilution labware:

Column Description

Dilution position The position on the dilution labware.

Identifier Specifies the sample ID of the originating sample.

Type Sample.

Input The corresponding position on the input labware. If multiple input labwares were

used for the run, the position is enhanced by the input labware identifier.

Actual content Lists the volume and the components.

Concentration The sample concentration in the dilution labware position.

Flag Shows the status of the position: either ok, warning or error.

Sample information

The normalization strategy for each sample is provided, in addition.

7.2.8 PCR setup with serial dilution experiments

The Generic PCR Plug-in enables easy and convenient qPCR setup including serial dilution.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections Working with experiments for pathogen detection and Working with gene expression experiments, respectively. For generic PCR setup experiments without sample normalization, refer to section Generic PCR experiments. For generic PCR setup experiments with sample normalization, refer to the Generic PCR setup with normalization experiments. It is also possible to set up a run for sample normalization only. In this case, refer to section Normalization only experiments.

New experiments for generic PCR setup with serial dilution, are created by clicking **PCR Setup with Serial Dilution** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and **Mixes** step will be enabled, initially. Subsequent steps will become available, once the required information has been entered into the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has eight steps that will guide you through the setup process:

- 1. Liquids
- 2. Mixes
- 3. Controls/Standards
- 4. Samples/Labware
- 5. Assignment

- 6. Dilution
- 7. Worktable
- 8. Run

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

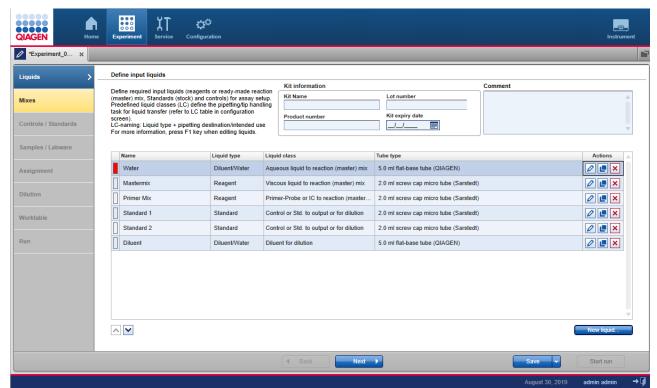
7.2.8.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will, e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.



The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The **Add liquid** dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the buttons below the table:

1. Click the row of the liquid to be moved.

2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.



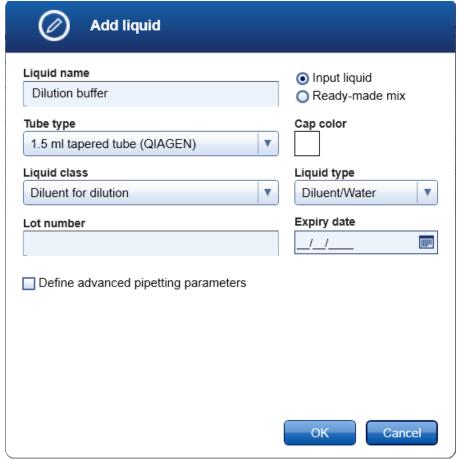
The buttons in the **Actions** column

of the table have the following functions:

Button	Action	Description
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
<u>e</u>	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.8.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.



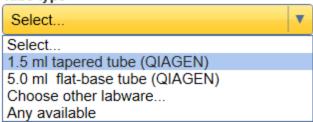
Add liquid dialog

- Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed. If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

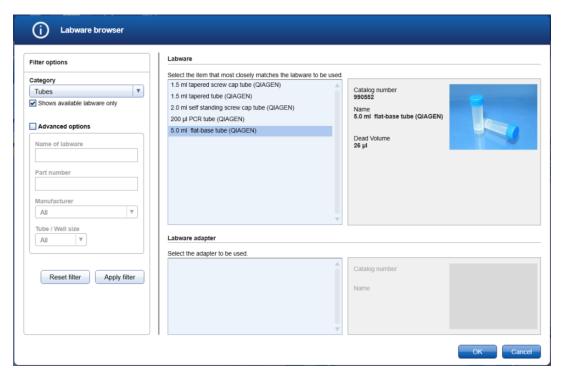
Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types
used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the
respective entry.

Tube type



Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the <u>Labware Manager</u>, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

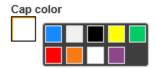
The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- · the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

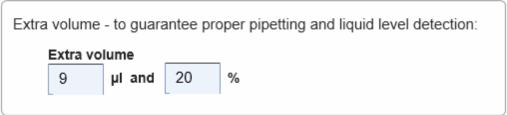
Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

☑ Define advanced pipetting parameters



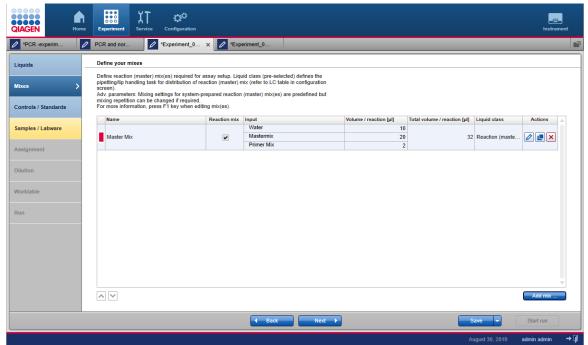
In the dialog, click OK to confirm your values and to close the dialog.
 Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.8.2 Mixes step

The Mixes step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.



Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described inAdd new mix dialog input.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in <u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the **Mixes** screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click Next in the button bar to proceed to the Controls/Standards step, described in

Note: The **Next** button is disabled, if a row in the table is highlighted yellow. User prepared reaction (master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit() the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

he buttons in the **Actions** column

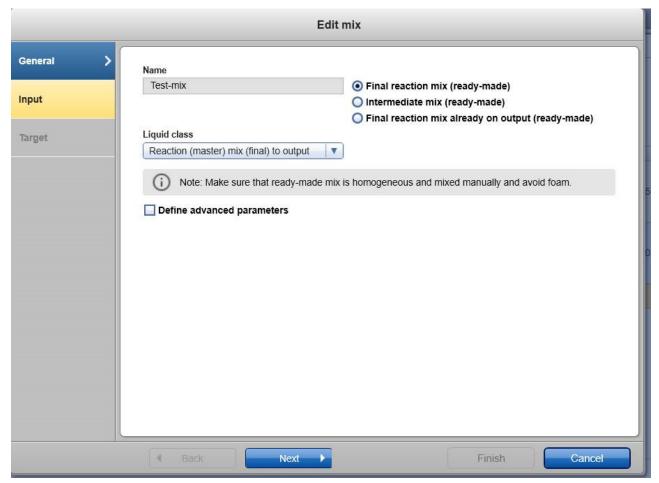
The buttons in the **Actions** column of the table have the following functions:

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.
	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

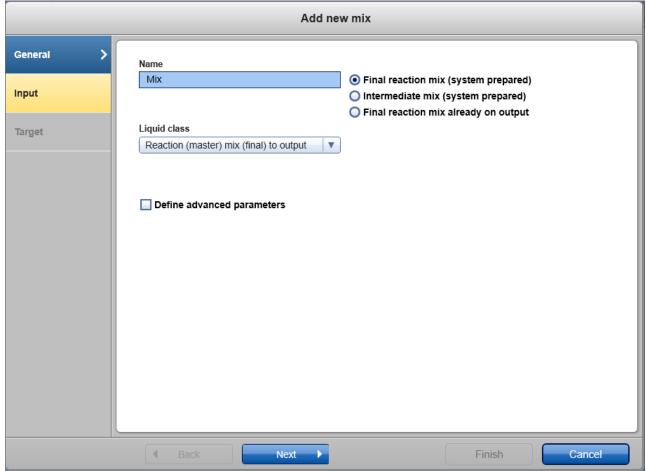
7.2.8.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.



Edit mix dialog



Add new mix dialog - General

 Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option

Description

Final reaction mix (ready-made)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In the later Assignment step, the mix will be available for selection. Duringthe run, the QIAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QlAgility as QlAgility will not perform any mixing steps.

Intermediate mix (readymade)

Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QlAgility. During the run, the QlAgility will pipette the mix into the tube, where the more specific mix is created.

Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QlAgility as QlAgility will not perform any mixing steps.

Final reaction mix already on output (ready-made) Only available for a mix that has been defined as a ready-made mix within the Liquid step.

Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.

If the option is selected, the Input screen of the dialog will be skipped.

Final reaction mix (system prepared)

Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.

Intermediate mix (system prepared)

Use this option if this mix will be created by the QlAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QlAgility. Then the QlAgility will pipette the mix into the tube where the more specific mix is created.

Final reaction mix already on output

Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.

If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

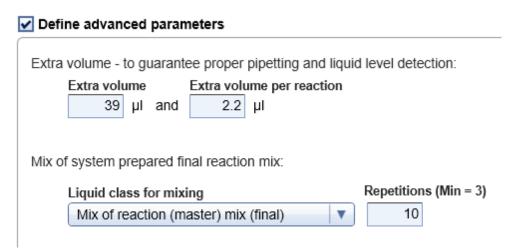
Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QlAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.



For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

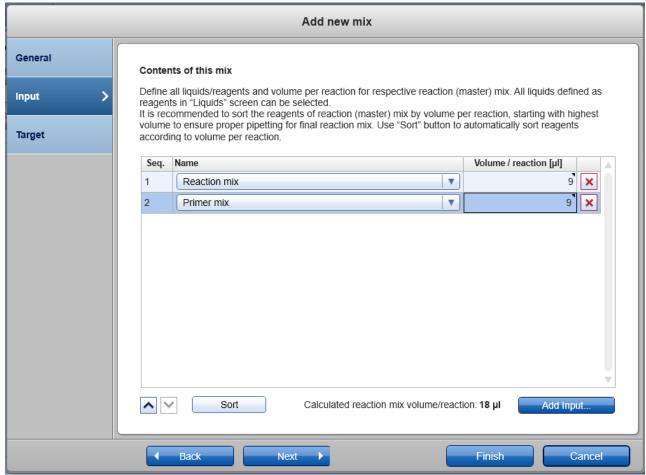
During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in Mixes step.

7.2.8.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.



Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

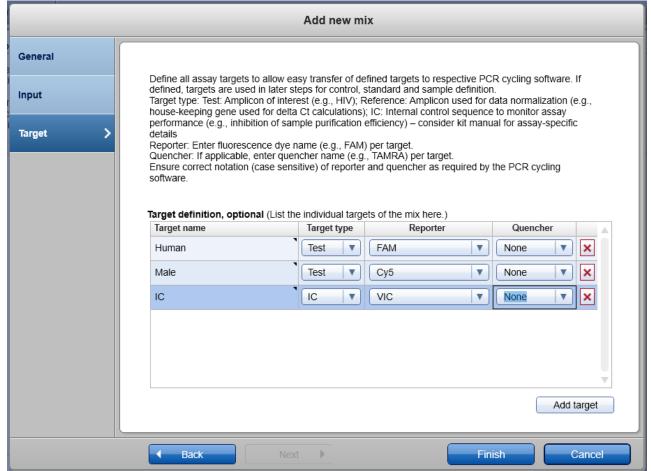
- 3. Define the volume per reaction for the input liquid. Left-click the Volume/reaction field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.
 Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.8.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.



Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type Description

Test Amplicon of interest (e.g., HIV)

Reference Amplicon used for data normalization (e.g., house-keeping gene used for delta

CT calculations)

IC Internal control sequence to monitor assay performance (e.g., inhibition of

sample purification efficiency) – consider kit manual for assay specific details)

 Optional step: Click on the menu in the Reporter column and select an appropriate reporter dye for the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.



Green Yellow Orange FAM JOE ROX

SYBRGREEN1 VIC CALFLUORRED610

SYBRGREENI HEX CY35

FLUORESCEIN TET TEXASRED

ALEXAFLUOR488 MAX ALEXAFLUOR568

CALFLUOR

GOLD540

YAKIMAYELLOW

Red Blue Crimson

CY5 MARINABLUE QUASAR705

QUASAR670 EDANS ALEXAFLUOR680

ALEXAFLUOR633 BOTHELLBLUE LIGHTCYCLERRED705

LIGHTCYCLERRED640 ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, save the output file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section Entering data.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in Mixes step.

7.2.8.3 Controls/Standards step

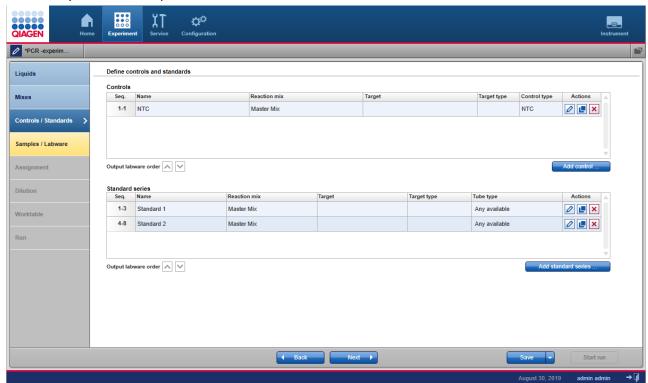
This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add standard series</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.



Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

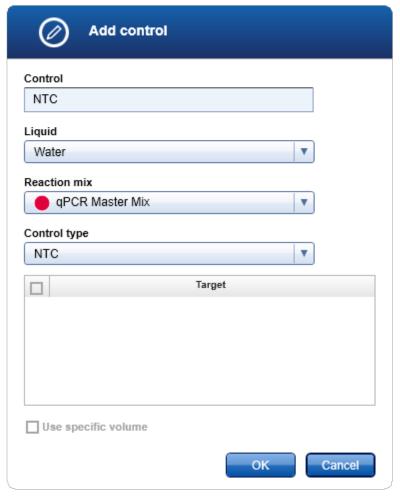
You can change the order of the controls and standard series using the up and down arrows below each table.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.8.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.



Add control dialog

1. Enter a control name in the **Control name** field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- 2. Select the input liquid from the **Liquid** menu. The menu lists all liquids that have been defined with liquid type **Pos./neg. control** or **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix** (ready-made), **Final reaction mix** (system prepared), **Final reaction mix in output** (ready-made) or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type



Option	Description
PC	Is a positive control for the intended assay.
NTC	Is a non-template control for the intended assay.
NC	Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

6. If the PC control type has been chosen, you can specify a custom volume for the control. Tick the **Use** specific volume box, type in a value in the **Volume** field, and select a diluent from the **Diluent** (used to fill up to sample volume) drop-down menu.

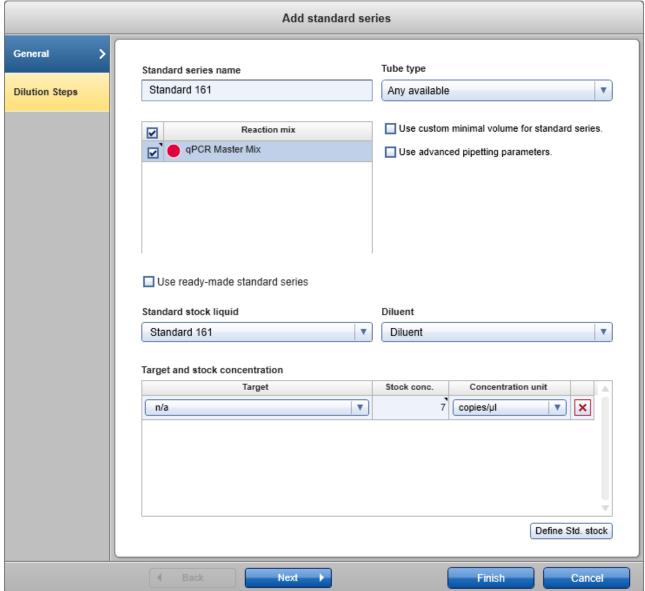


Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the Controls table.

7.2.8.3.2 Add standard series

In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.



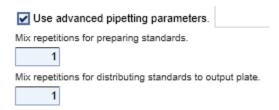
Add standard series dialog - General

- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.

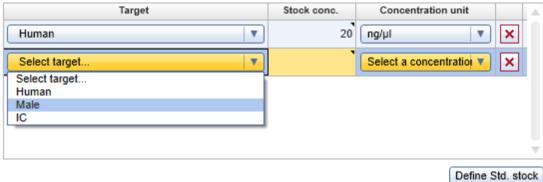


5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



- 6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.
 - Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.
- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 8. If the option **Use ready-made standard series** has not been checked, select the appropriate diluent from the **Diluent** menu. The menu lists all liquids that have been defined with liquid type **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only. If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock concentration, enter concentration value 0.

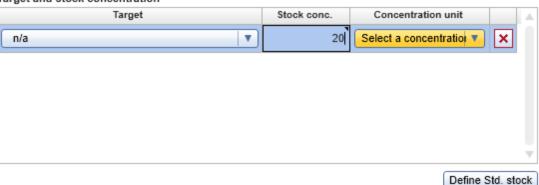
Target and stock concentration



Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration



Defining common stock, if no targets have been defined for the mix.

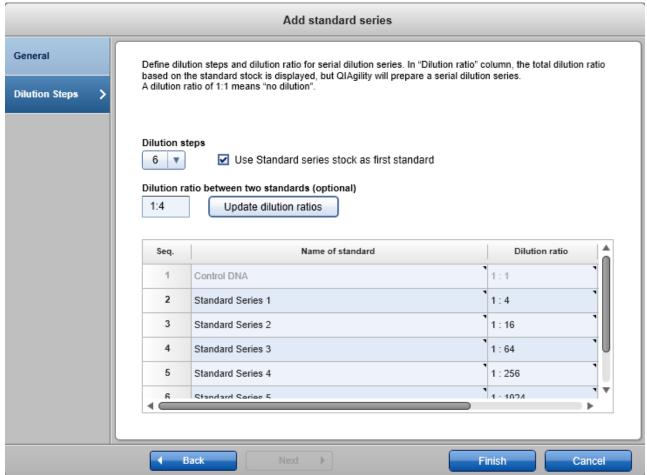
Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column	Description
Target	Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration.
	This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

7.2.8.3.3 Add standard series - dilutions

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.



Add standard series dialog - Dilution steps

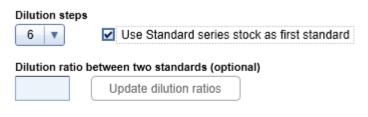
1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section Entering data.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.





Option "Use standard series stock as first standard" selected.

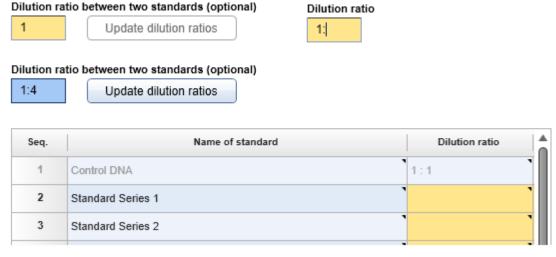
For each dilution step, if the option Use ready-made standard series has been checked in the
previous General screen, select the appropriate liquid from the corresponding menu in the Name of
standard cell.



Selecting the appropriate liquid for each standard for a user prepared standard series.

If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

 If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the Dilution ratio field above the dilution steps and then click Update dilution ratios to apply it to the series.



Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

1:4 Update dilution ratios

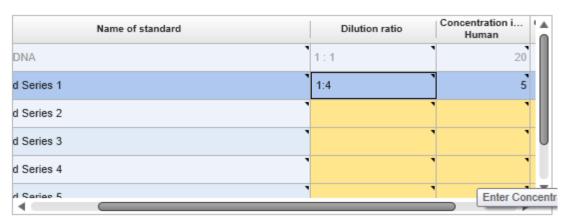
Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1:16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

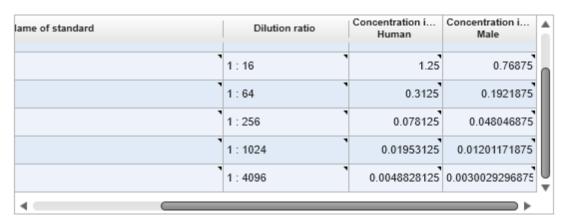
Update dilution ratios



Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.



Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

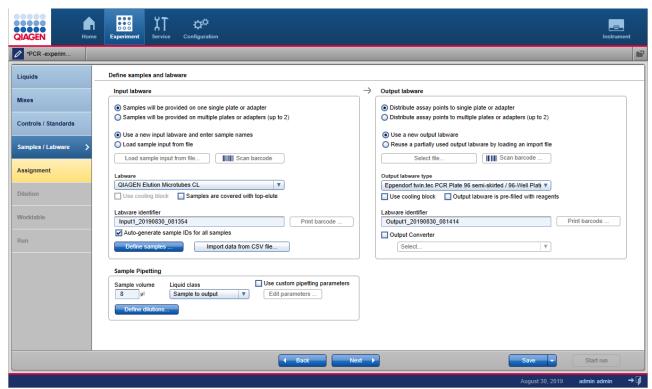
Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in Controls/Standards step.

7.2.8.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.



Samples / Labware step

Proceed as follows:

- 1. Select the input labware and define the samples.
 - If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define input</u> labware.
 - If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to two input labware</u>.
- 2. Select the output labware.
 - If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define output labware</u>.
 - If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.
- 3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.

Sample Pipetting Sample volume Liquid class Use custom pipetting parameters 8 µl Sample to output ▼ Define dilutions...

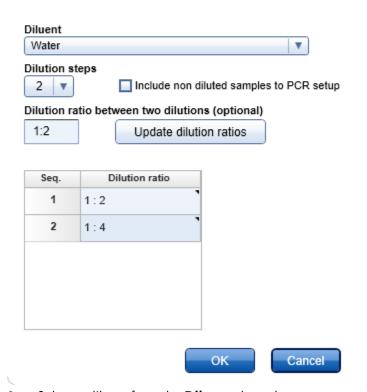
- 4. The liquid class used for sample transfer is predefined by default. If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** drop-down menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. To define sample dilutions, click the **Define dilutions** button. The **Define/Edit sample dilution series** dialog will open. For details, refer to <u>Define sample dilution series</u>.
- 6. Optional: If required, use custom pipetting parameters for samples. If you tick the box, the Edit parameters button becomes active. If you click the button, the Define advanced pipetting parameters dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to Advanced sample pipetting.
- 7. **Note:** Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.
- 8. Click **Next** to proceed.

7.2.8.4.1 Define/Edit sample dilution series dialog

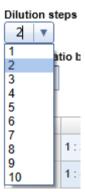
The **Define/Edit sample dilution series** dialog is used for defining and editing dilution steps and the dilution ratio for serial dilution series.



Define dilution steps and dilution ratio for serial dilution series. In "Dilution ratio" column, the total dilution ratio based on the sample is displayed, but QIAgility will prepare a serial dilution series. A dilution ratio of 1:1 means "no dilution".



- 1. Select a diluent from the **Diluent** drop-down menu.
- 2. Select the number of dilution steps from the **Dilution steps** drop-down list.



Note: The **Dilution steps** menu is an editable menu. If you want to work with more than 10 steps, just type the number into the field. For details about editable menus, refer to **Entering data**.

- 3. To include non-diluted samples in the setup, tick the box next to **Include non diluted samples to PCR** setup.
- 4. To specify the dilution ratio, type in a ratio in the **Dilution ratio between two dilutions (optional)** field and click the **Update dilution ratios** button, or type in a ratio for each sequence in the **Dilution ratio** column.
- 5. Click **OK** and proceed with step 6 in <u>Samples/Labware step</u>.

7.2.8.4.2 Define input labware

If the option Samples will be provided on one single plate or adapter has been selected, define the input labware according to the following steps:

- If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.
 - Use a new input labware and enter sample names
 - O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the **Labware** drop-down menu on the left side of the screen.
- All previously selected labware types will be listed. The Choose other labware option will be listed last.

Labware

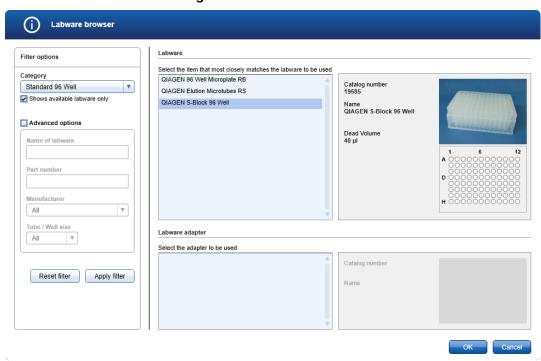
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes

Choose other labware...

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section <u>Using the labware browser</u>.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use** cooling block next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

6. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. Optional step: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples				
Define samples	Import data from CSV file			

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in Samples/Labware step.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option Load sample input from file has been checked, the Load sample input from file and Scan barcode buttons will be enabled.



Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to File dialoa.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to Scan labware barcode dialog.

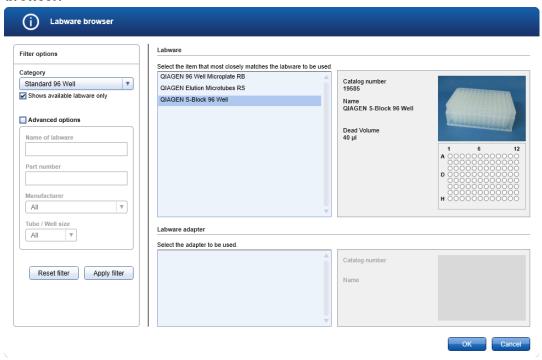
The software will load the sample information from the sample file.

13. If the sample information file also contains the labware type, it will automatically be selected in the **Labware** drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the Labware drop-down menu. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

16. Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

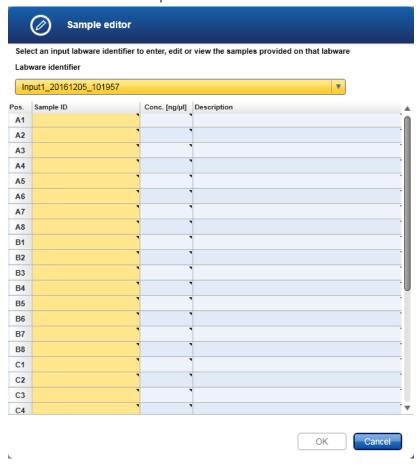
For details about the sample editor, refer to Edit samples.

- 17. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 - Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.
 - **Note:** Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.
- 19. Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in Samples/Labware step.

7.2.8.4.3 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



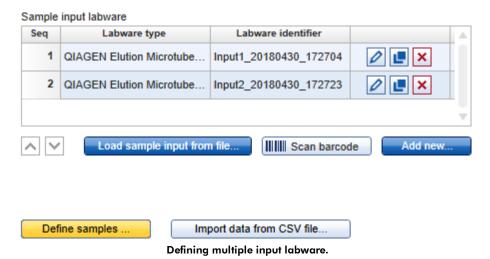
Note: For more details how to enter information into a table refer to the section **Entering data**.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.
- 7.2.8.4.4 Define up to two input labware

If the option Samples will be provided on multiple plates or adapters (up to 2) has been selected, you can define up to two input labware types.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.



1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

Click the Load sample input from file button and select the desired sample file. For details, refer to
 <u>File dialog</u>. Click Open to close the file dialog. The software will load the sample information from the
 selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to Scan labware barcode dialog.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1–4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in Samples/Labware step.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

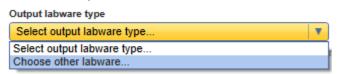
7.2.8.4.5 Define output labware

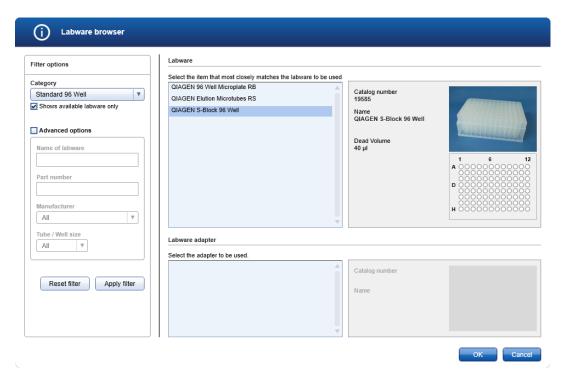
If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- You can either Use a new output labware or Reuse a partially used output labware by loading an import file. The option Use a new output labware will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

Select the output labware from the Output labware type menu on the right. Click Choose other labware to open the Labware browser.





In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

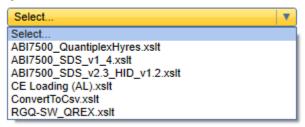
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.





Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with Step 3 of the <u>Samples/Labware step</u>.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.



10. Click the Select file button and select the desired labware file. For details, refer to <u>File dialog</u>. Alternatively, click the Scan barcode button. In the Scan labware barcode dialog, scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

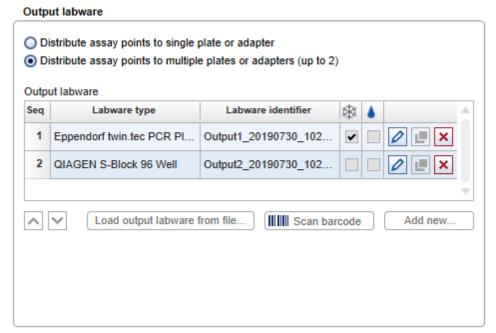
Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.8.4.6 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the **Output labware** table. Initially, the table is empty.



Defining multiple output labware

- If you would like to add output labware manually, click the Add new button and proceed to step 4.
 If you would like to load an output labware file, proceed to step 2.
 - If you would like to provide output labware information by scanning a barcode, proceed to step 3.
- 2. Click the **Load output labware from file** button and select the desired file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the output information from the selected file.
 - If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.
- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.
 - If the labware is to be used with the cooling block, the box in the solumn will be ticked. If the labware is pre-filled with reagents, the box in the column will be ticked. To change these settings, edit the labware.
- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the Samples/Labware step.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto Action

Description

The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.

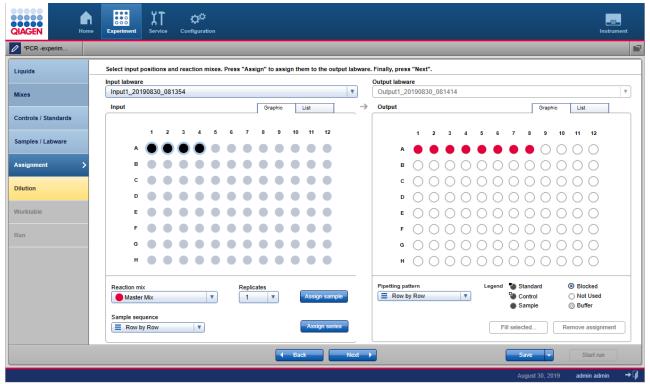
Duplicate the output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.

Delete the output labware

Deletes the row in the Sample output labware table.

7.2.8.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option Samples will be provided on multiple plates or adapters has been selected, you can switch between the different input labwares using the box above the Graphic and List tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

Assay points can be removed. For details, refer to Removing assay assignments.

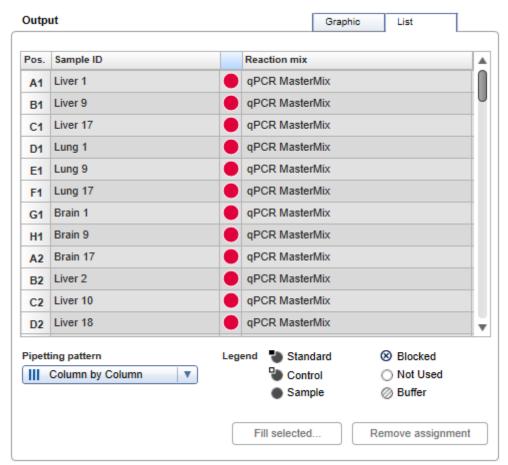
The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

It is possible to drag assigned samples, controls and standards within the output labware if a specific layout is required. Blocked positions cannot be used. If you drag a sample, control or standard to a position that is already in use, the respective sample/control/standard at that position and all following are automatically shifted to the next position, depending on the defined pipetting pattern.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-click on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the assay points that have been created.



The List tab of the Output panel

7.2.8.5.1 Assigning samples with reaction mixes to the output labware

Proceed as follows:

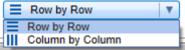
- From the Input labware menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous Labware selection step. The software will display its defined samples below in the Input panel.
- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.





Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click Assign.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate,

the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

8. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.8.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the **Output** side. Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

2. Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared. **Note:** The software will remove all replicates of the desired sample(s).

7.2.8.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

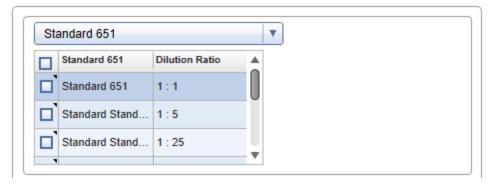
Proceed as follows:

- 1. Select **Standards and Controls** from the **Input labware** drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** drop-down list.



A table with dilution steps and dilution ratios will appear.



- 3. Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.



5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 6. Click Assign.
- 7. Tick the box next to one or more controls in the following table. To select all controls, tick the box in the

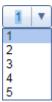
table header.



8. Select a mix from the Reaction mix drop-down list.



9. Select the number of replicates from the Replicates drop-down list.

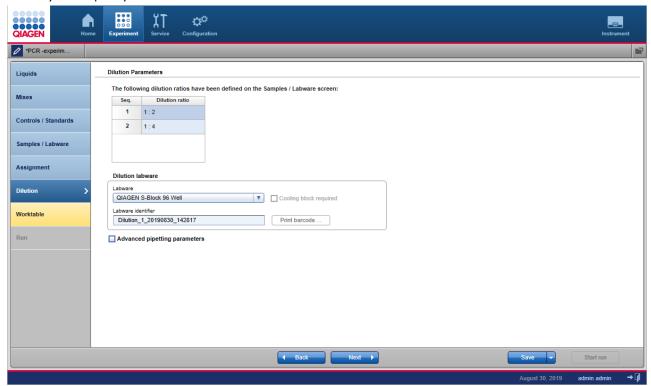


Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

- 10. Click Assign.
- 11. Repeat steps 2 10 as needed, until you have defined all the positions for the planned run.

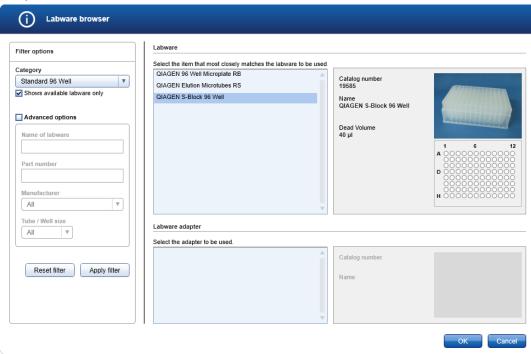
7.2.8.6 Dilution step

The **Dilution** step screen shows a summary of the dilution ratios defined in the **Samples/Labware** step and allows you to specify dilution labware.



To choose the labware, proceed with the steps below:

1. Select the labware from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the **Labware** browser, refer to the section <u>Using the labware browser</u>.

 Optional step: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.

To define advanced pipetting parameters, tick the box next to **Advanced pipetting parameters**.



You can specify the liquid class for pipetting the diluent or the sample onto dilution labware, the number of mix repetitions on the dilution labware, the maximal and minimal volumes in the dilution well, the minimal diluent transfer volume, and the minimal sample transfer volume.

If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).

Close the lid.

Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.8.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Worktable selector

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click Print loading instruction to generate a PDF document with the loading instructions for the run.

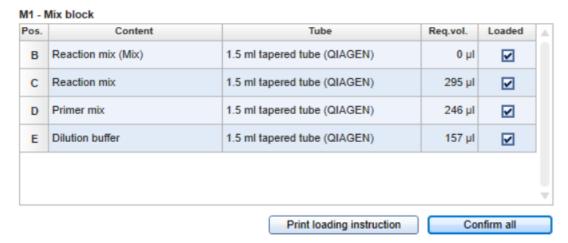
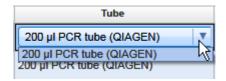


Table of tubes to be loaded to the worktable position M1.

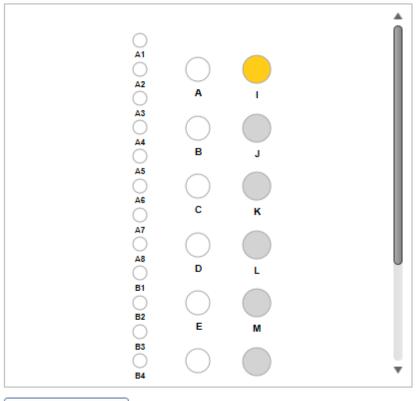
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the Labware tab on the right to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

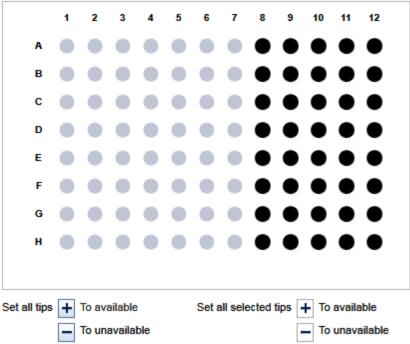
Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



Interactive rack to define tip avilability.

If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

 Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in Insert tips to tip rack

holder.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

 Optional step: A yellow Calibrate labware button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- 19. Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for Generic PCR experiments.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.8.8 Run step

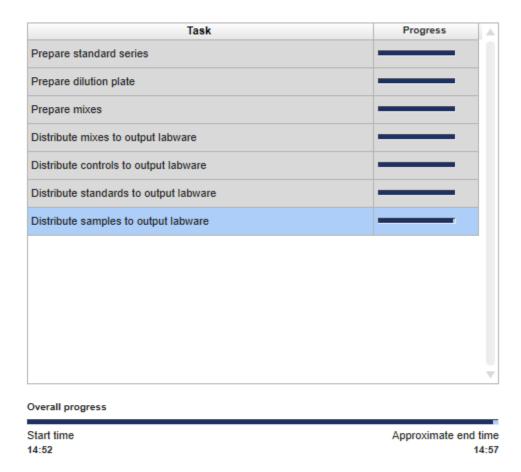
The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.8.8.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.



Run progress

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.8.8.2 Pausing or canceling the run

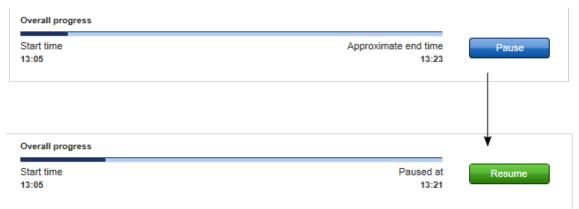
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

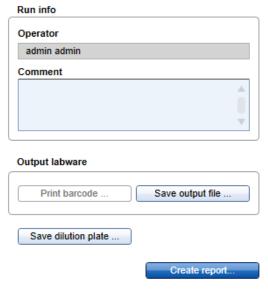
To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.8.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.

A file dialog opens to select directory and file name of the .xml file. Refer to User settings to define a standard directory.

The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.giagen.com/goto/QIAgility. You can find examples of output files in the "Examples" folder provided with the software:

C:\Users\Public\QIAGEN\QIAgility\Examples.

If an output converter has been selected in the **Samples/Labware** step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml, the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the User settings. Contact QIAGEN Technical Services if you need another file format.

Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second

run, the information from both runs can be saved in an output file. A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

Save dilution plate You can save an XML file with the contents of the dilution labware.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.

Create report

3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.9 Other workflow experiments

The Generic PCR Plug-in provides further workflows:

Normalization only

Use this workflow if samples will be normalized and transferred to the output labware without

transferring the reaction (master) mix.

<u>Prepare reaction (master) mix</u>
Use this workflow if only a reaction (master) mix

will be created for later reuse.

<u>Prepare and distribute reaction (master) mix</u>

Use this workflow if a reaction (master) mix will be

created and transferred to the output labware

without transferring the samples.

<u>Prepare standards</u>

Use this workflow if only standards will be created

for future use.

7.2.10 Normalization only experiments

The Generic PCR Plug-in enables easy and convenient setup of runs for sample normalization only.

For generic PCR setup experiments, including sample normalization, refer to section <u>Generic PCR with</u> <u>normalization experiments</u>.

Samples can be normalized based on mass or concentration. Ensure that the correct selection has been made in the <u>User settings for Generic PCR experiments</u> before the experiment is created. Changing the setting will take effect for new experiments, only.

New experiments for sample normalization only are created by clicking **Other workflows** from the **Home** screen and then selecting **Normalization only**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Samples/Labware** step will be enabled initially. Subsequent steps will become available once the required information has been entered into the **Liquids** and **Samples/Labware** step. The **Samples/Labware** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 6 steps that guide you through the run setup process:

- 1. Liquids
- 2. Samples/Labware
- 3. Assignment
- 4. Normalization
- 5. Worktable
- 6. Run

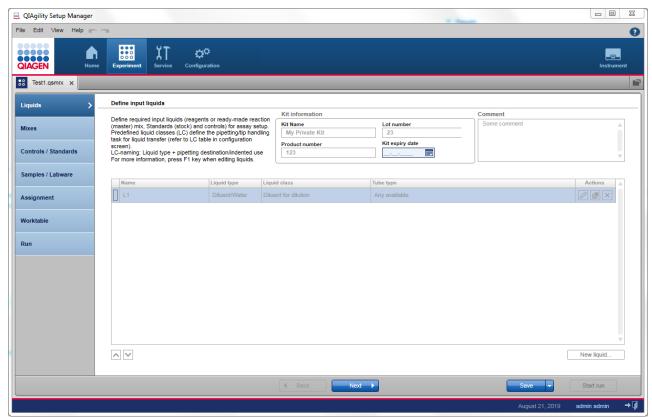
Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.10.1 Liquids step

The **Liquids** step is used to define the diluent that will be used for normalization. Samples will be defined later in the **Samples/Labware** step. Optionally, liquids of type diluent/water can be defined that will be used to fill empty positions in the output labware later in the **Assignment** step.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- Click the **New liquid** button below the table. 1.
- 2. The **Add liquid** dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

Finally, click **Next** in the button bar to proceed to the **Samples/Labware** step (Samples/Labware step).

Note: The Next button is disabled, if a row in the table is highlighted in yellow. Click do open the Edit liquid dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \(\textstyle \textstyle

- Click the row of the liquid to be moved. 1.
- 2. Click on the up and down arrows to move the currently selected row up or down.

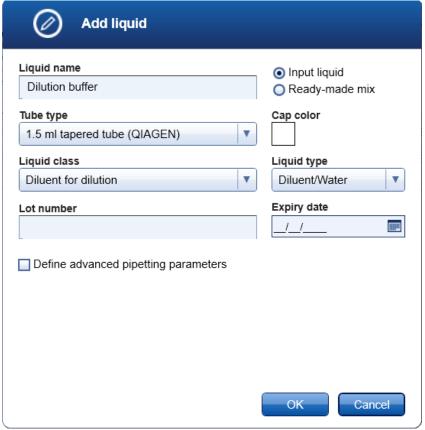
Use this function for a better overview. The report will list the liquids in this order.

of the table have the following functions: The buttons in the **Actions** column

Button	Action To edit a liquid	Description The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, some restrictions apply. Not all values can be changed. In this case, remove assignments to enable changes for defined input liquids.
<u>.</u>	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

Add liquid dialog 7.2.10.1.1

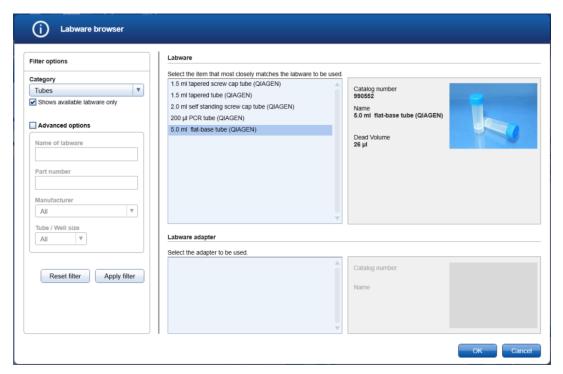
In the Add liquid dialog, a new input liquid of type diluent/water can be defined. If you would like to change the values for an existing liquid later, the Edit liquid dialog will open. Both dialogs show the same values of a liquid.



Add liquid dialog

- Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, when defining normalization, and in the loading instructions of the Worktable step.
- 2. Click **Tube type** to select the tube that will be used to place this liquid on the QlAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry. Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the <u>Labware Manager</u>, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the **Labware browser**, refer to the section <u>Using the labware browser</u>.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type, or make the tube available in the Labware Manager again.

Select the Liquid class that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid will be used for different purposes (e.g., a diluent used for normalization and to fill empty positions of the output labware) it is highly recommended to define multiple input liquids to allow precise definition of liquid class.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

- 4. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, this data will be present in the report.
- 5. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

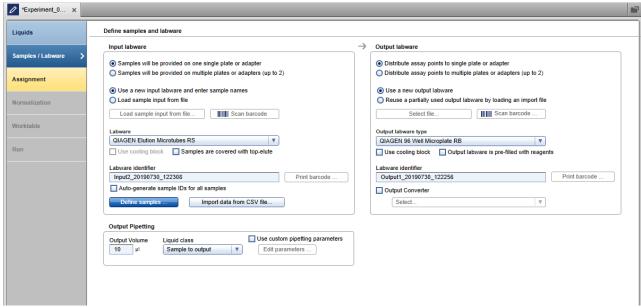
Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

6. In the dialog, click **OK** to confirm your values and to close the dialog.
Otherwise, click **Cancel** to close the dialog and discard all values. In this case, a new liquid is defined, or, in case of editing an existing liquid, the liquid is not changed.

7.2.10.2 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.



Samples / Labware step

Proceed as follows:

- Select the input labware and define the samples.
 - If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input labware</u>.
 - If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in Defining up to 3 input labware.
- 2. Select the output labware.
 - If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define the output labware</u>.
 - If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.
- 3. Enter the output volume for the run into the **Output volume** field in the **Output pipetting** panel. The value must be specified in μ l.



Entering output volume

Note: It is recommended to use sample volumes of at least 5 μ l, as it is technically not reliable to transfer less than 5 μ l volume into an empty labware.

- 4. The liquid class used for sample transfer is predefined by default.

 If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to Advanced sample pipetting.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the Assignment step.

7.2.10.2.1 Define an input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

- If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.
 - Use a new input labware and enter sample names
 - O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the **Labware** drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

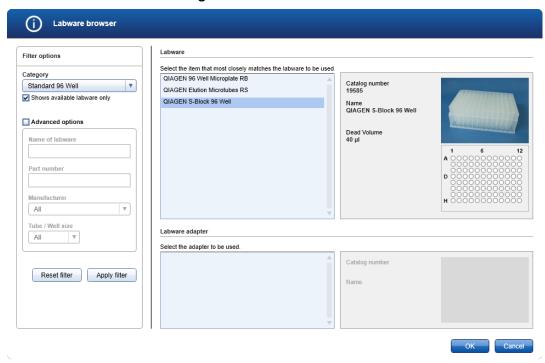
Labware

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapere

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware...

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section <u>Using the labware browser</u>.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

6. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. Optional step: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample	IDS I	for all samples
Define samples		Import data from CSV file

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in Samples/Labware step.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option Load sample input from file has been checked, the Load sample input from file and Scan barcode buttons will be enabled.



Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to File dialoa.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode</u> dialog.

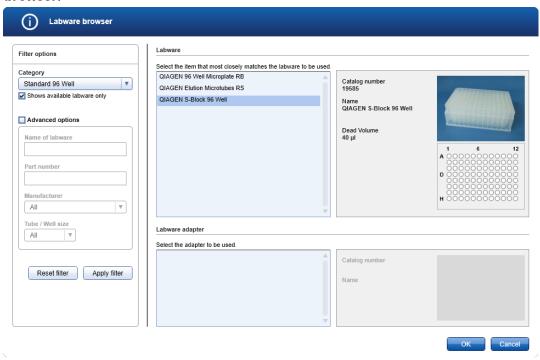
The software will load the sample information from the sample file.

13. If the sample information file also contains the labware type, it will automatically be selected in the **Labware** drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the Labware drop-down menu. If it is not listed, click Choose other labware to open the Labware browser.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section <u>Using the labware browser</u>.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

16. Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

For details about the sample editor, refer to Editing sample names.

17. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

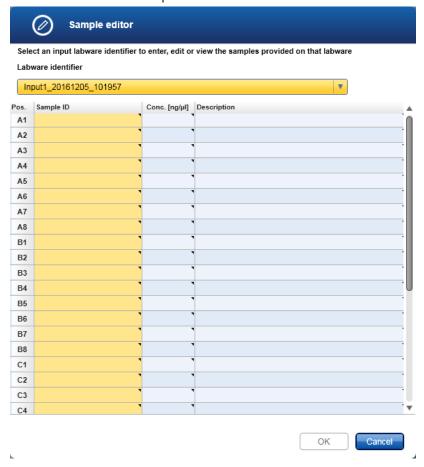
19. Optional step: Click the Print barcode button to print a barcode of the labware identifier.
Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.

20. Proceed with step 2 in Samples/Labware step.

7.2.10.2.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.



Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.
Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column Sample ID. Press and hold the SHIFT key and left-click to select multiple adjacent cells, or press and hold the CTRL key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on Auto-generate sample names. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on Auto-generate sample names.



Note: For more details how to enter information into a table refer to the section **Entering data**.

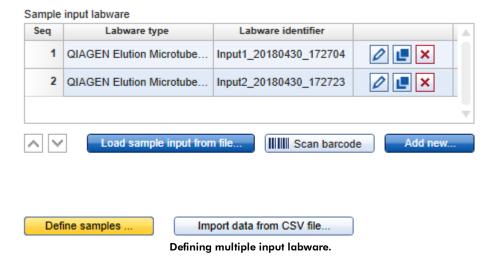
Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.10.2.3 Defining up to two input labware

If the option Samples will be provided on multiple plates or adapters (up to 2) has been selected, you can define up to two input labware types.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.



 If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

Click the Load sample input from file button and select the desired sample file. For details, refer to
 <u>File dialog</u>. Click Open to close the file dialog. The software will load the sample information from the
 selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to Scan labware barcode dialog.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1-4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to **Editing sample names**.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in Samples/Labware step.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
Ŀ	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

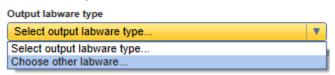
7.2.10.2.4 Define output labware

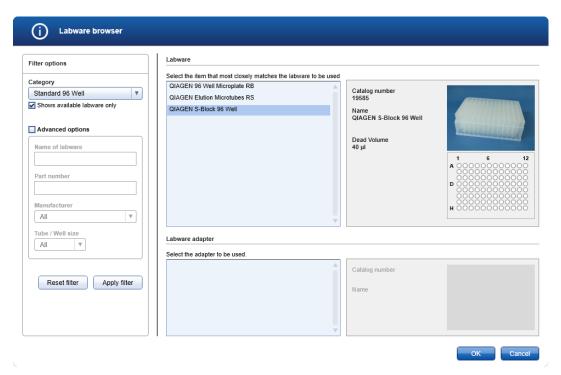
If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- You can either Use a new output labware or Reuse a partially used output labware by loading an import file. The option Use a new output labware will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.





In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

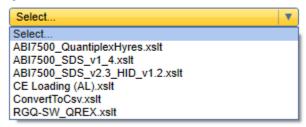
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.





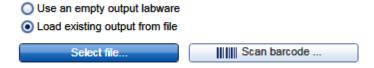
Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in Samples/Labware step.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.



10. Click the Select file button and select the desired labware file. For details, refer to <u>File dialog</u>. Alternatively, click the Scan barcode button. In the Scan labware barcode dialog, scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

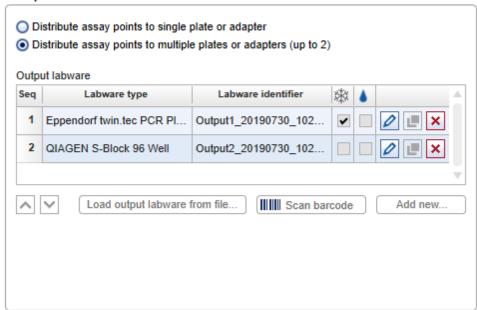
11. Proceed to step 3.

7.2.10.2.5 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the **Output labware** table. Initially, the table is empty.

Output labware



- 1. If you would like to add output labware manually, click the **Add new** button and proceed to step 4. If you would like to load an output labware file, proceed to step 2.
 - If you would like to provide output labware information by scanning a barcode, proceed to step 3.
- Click the Load output labware from file button and select the desired file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the output information from the selected file.
 - If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.
- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.
 - If the labware is to be used with the cooling block, the box in the solumn will be ticked. If the labware is pre-filled with reagents, the box in the column will be ticked. To change these settings, edit the labware.
- 6. Repeat steps 1-5 until all labware has been defined.

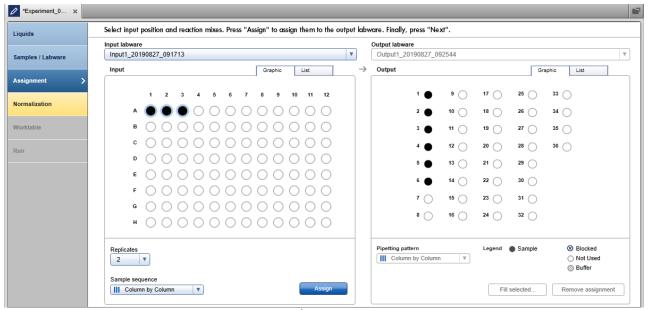
7. Proceed with step 3 of the <u>Samples/Labware step</u>.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.

7.2.10.3 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of samples to the output labware.



Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

•	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been assigned once to output labware.
	Selected sample	Currently selected sample.
		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
		Note: It is possible to use the same sample multiple times to create a dedicated layout on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option Samples will be provided on multiple plates or adapters has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a Replicates menu and a Sample sequence drop-down list.

In order to assign a sample to the output labware, set the number of replicates, choose a sample sequence and select the sample position(s). When you click the **Assign** button, the selected samples will be assigned to the output labware. Follow the procedure described in Assigning samples to the output labware.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. Positioning of samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. If you hover with the mouse cursor over an output position, the software displays the sample ID for that position.

Samples can be removed from the output labware. For details, refer to Removing assignments.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

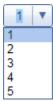
In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to Fill empty output positions.

Use the **List** tab of the output panel to view the samples on the output labware.

7.2.10.3.1 Assigning samples to the output labware

Proceed as follows:

- From the Input labware menu on the left side of the screen, select an input labware that was defined in the previous Labware selection step. The software will display its defined samples below in the Input panel.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to Entering data.

3. From the **Input** panel, select the sample position that will be assigned to the output labware. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.

- 4. Click Assign.
- 5. The software displays the assigned samples on the **Output** panel on the right side of the screen. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning on the output side follows the selection made for the **Sample sequence** in the **List** tab of the **Input** panel. You can also change the sequence of samples for the input labware by clicking the **Pos.** column header in the table in the **List** tab.
- 6. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.10.3.2 Removing assignments

To clear a position on the output side, follow these steps:

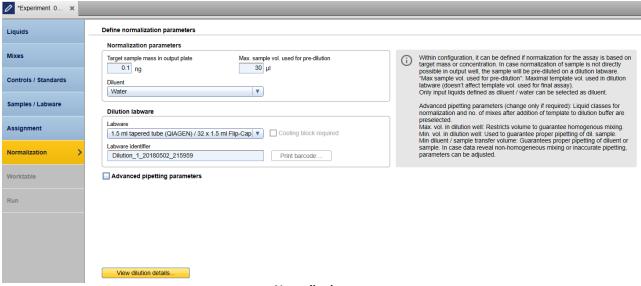
- Select the desired position on the Output side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or CTRL and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.

Note: The software will remove all replicates of the desired sample(s).

7.2.10.4 Normalization step

In the **Normalization** step define the parameters for normalization. The QIAgility Setup Manager Software provides the option to normalize samples based on sample mass or sample concentration. When normalizing to a specific concentration, the sample will be diluted to the chosen target value and afterwards transferred to the output labware. In case normalization of a sample is not directly possible in the output position, the sample will be pre-diluted on a dilution labware. The software pre-calculates for each sample, if the target can be reached. Based on this calculation you can decide, if you want to include or exclude samples.

To configure if normalization is based on target sample mass or sample concentration, click **Configuration** in the main tool bar, click the **Settings** tab and then select the **Generic PCR user settings** step. For details refer to <u>settings</u>.



Normalization step

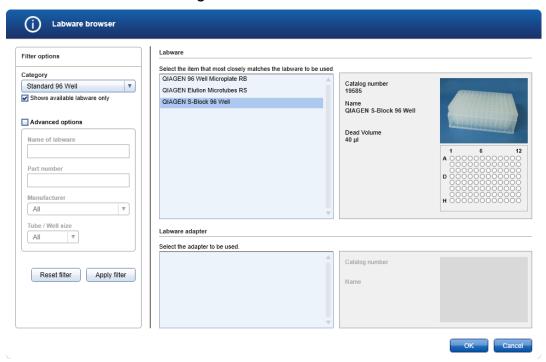
To define sample normalization:

- Enter the Target sample mass or concentration that shall be reached in the output labware.
 Note: When normalizing to target sample mass, the unit is predefined as "ng".
- 2. Enter into the field **Max. sample vol. used for pre-dilution** the maximal sample volume that shall be used in maximum for pre-dilution, e.i. the maximal template volume that will be used in the dilution labware to pre-dilute a sample.
 - **Note:** The higher the maximal sample volume for pre-dilution is set, the more flexible the software can calculate possible normalization strategies.
- 3. Click **Diluent** to select the diluent that shall be used for sample normalization. The menu lists all liquids that have been defined with liquid type **Diluent/Water** in the **Liquids** step. If a required liquid is not

listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

4. Click **Labware** to select the labware that shall be used to pre-dilute samples. All previously selected labware types will be listed. The **Choose other labware** option will be listed last. Select your dilution labware from the drop-down list. If it is not listed, click **Choose other labware** to open the Labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the **Labware** browser, refer to the section <u>Using the labware browser</u>.

- 5. **Optional:** If you would like to use the cooling block for the dilution labware, check the option **Cooling** block required next to **Labware**.
- 6. Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

7. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. **Optional:** Define advanced pipetting parameters. If you tick the box, parameters become visible. In case the initial sample concentration is too high or too near to target, parameters can be adjusted.

Advanced pipetting parameters

Liquid class for pipetting diluent onto dilution labware

Diluent to dilution ٧ Liquid class for pipetting sample onto dilution labware No. of mix repetitions on dilution labware Sample for dilution ٧ 7 Min. volume in dilution well Max. volume in dilution well Min. diluent transfer volume Min. sample transfer volume 1400 µl 20 µl 5 µl 2 µI **Description** Option Liquid class for pipetting diluent onto Preselected. dilution labware Defines the pipetting and tip handling for pipetting the diluent to the dilution labware. Preselected. Liquid class for pipetting sample onto dilution labware Defines the pipetting and tip handling for pipetting the sample to the dilution labware. Sample (template) will be pipetted after the diluent has been distributed. No. of mix repetitions on dilution The value is predefined by the selected liquid class. labware Defines the mix repetitions after the sample (template) has been added to the diluent on the dilution labware. Mix repetitions have been developed to guarantee proper mixing of water based diluents and samples for the default value of maximal volume in dilution well. In case maximal volume in dilution well is manually increased, it is recommended to increase mix repetitions. Max. volume in dilution well Predefined. Restricts the volume in the dilution positions in order to guarantee homogeneous mixing. In case the maximal volume in dilution well is manually iincreased, it is recommende to increase mix repetitions. If the total volume of the selected dilution labware is smaller than the setting of this parameter, the total volume of the dilution labware will be used. Min. volume in dilution well Predefined.

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Ensures proper creation of pre-dilution and pipetting of the

diluted sample to the output labware.

Min. diluent transfer volume Predefined.

Guarantees presize pipetting of diluent for pre-dilution. A

setting $< 2\mu l$ is not recommended.

Min. sample transfer volume Predefined.

Guarantees presize pipetting of sample (template) for pre-

dilution. A setting $< 1 \mu l$ is not recommended.

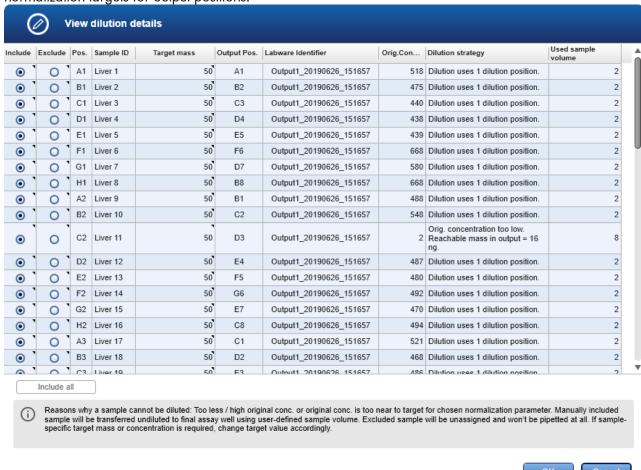
Click View dilution details to open a list with the results of pre-calculation for the samples. The button
is colored yellow, if the normalization target will not be reached for all samples.
 Follow the steps described in <u>Dilution details</u>.

- 10. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 11. Close the lid.
- 12. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.10.4.1 Dilution details

The dialog lists the normalization pre-calculation for all samples. It is possible to enter individual normalization targets for output positions.



Dilution details

- 1. Check the calculation. Find a description of the table columns, below.
- Optional: You can enter an individual normalization target for positions on the output labware. Click into the Target mass / concentration field of the corresponding output position row and enter the new target value. The calculation for this position will automatically be updated.
- 3. For each sample that has yellow colored columns Include/Exclude, decide whether you want to include the sample into the run or not. Check the corresponding option. The sample position will no longer be colored yellow. To include all samples, click the Include all button below the table. For details refer to the description of the Include and Exclude columns, below.
- Optional: Samples that have no yellow color indication in Include/Exclude column do not have any
 restrictions in regards to normalization, but can also be excluded, if needed.

- 5. If no sample has yellow colored **Include/Exclude** column, the **OK** button will be enabled.
- 6. Click **OK** to confirm your selections and to close the dialog.

Proceed with step 10 in Normalization step.

The table contains all samples from the input labware. Samples that have not been previously assigned in the Assignemt step are listed last.

Column	Description
Include	If checked, the sample will be included in the run.
	All samples, previously assigned in the Assignment step, which have no restriction to reach the normalization target, are automatically included. Otherwise, the columns Include/Exclude are highlighted in yellow to indicate that a decision by the user is required.
	Check the option to include the sample.
Exclude	If checked, the sample will not be used in the run and will be automatically unassigned.
	All samples, previously assigned in the Assignment step, which have a restriction to reach the normalization target, are marked in yellow to indicate that a decision (include or exclude) by the user is required.
	After confirming the dialog with OK, the sample gets automatically unassigned. The following positions on the output labware will be filled up according to the selected Pipetting pattern and Pipetting order from the Assignment step.
Pos.	The sample position on the input labware.
Sample ID	The sample ID.
	If the sample ID is too long, it is cut off. To view the whole sample ID, hover the mouse over the field.
Target mass/ Target conc.	The normalization target. Initially, the value that has been entered into the Normalization step is shown.
	However, you can edit the value if you need an individual normalization target for the corresponding output position. If no target value has been entered, or if the target value is too high or too low, the field is highlighted in yellow to indicate that a valid target needs to be entered.
Output pos.	The position on the output labware.
Orig. conc.	The original sample concentration as imported from the sample input file or entered in the sample editor.
Dilution strategy	The dilution strategy is automatically selected by the software and cannot be changed by the user.
	If the normalization target cannot be reached, the reason is listed. Decide whether to include or exclude the sample. For details, refer to the description below.

Note: It is possible to influence the dilution strategies by changing normalization parameters. The software always used the dilution strategy for which the lowest reagent volumes are required.

Used sample volume The sample volume used during normalization.

The dilution strategy depends on

- the value for the normalization target
- · the original concentration of the sample
- the setting for maximum volume to be used for pre-dilution of a sample in the dilution labware
- the setting for minimum volume in the dilution well
- the sample volume for the run as specified in Samples/Labware
- the selected dilution labware (total volume and among of dilution positions)
- the maximum volume allowed in the dilution labware (advanced pipetting parameters for normalization)
- the minimum sample transfer volume (advanced pipetting parameters for normalization)
- the minimum diluent transfer volume (advanced pipetting parameters for normalization)

Dilution strategy	Description
Dilution in output position.	It is sufficient to dilute the sample directly in the output position within the defined sample volume for the assay setup.
Dilution uses 1 dilution position.	The sample will be diluted in one position of the dilution labware. Then the defined sample volume for the run will be transferred from the dilution labware to the output labware position.
Dilution uses 2 dilution positions.	The sample will be diluted using two positions of the dilution labware. Then the defined sample volume for the run will be transferred from the second dilution position on the dilution labware to the output labware position.
Dilution uses 1 dilution position and output position.	The sample will be pre-diluted in one position of the dilution labware. The last dilution step will be performed in the output position within the defined sample volume for the assay setup.
Original concentration too high.	The sample cannot be normalized to the given normalization target, because the original sample concentration is too high. The required dilution ratio cannot be created using the defined normalization parameters. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be diluted to this value.
Original concentration too low.	The original sample concentration is lower than the normalization target. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will

be used undiluted.

Original concentration too close to target.

The sample cannot be normalized to the given normalization target, because the defined normalization parameters do not allow such a small sample dilution ratio. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be used undiluted. Otherwise, the normalization parameters have to be changed (e.g., increase the **Max. sample volume used for pre-dilution** value).

7.2.10.5 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- Worktable selector
- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

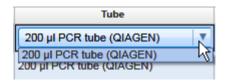
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - Mix block

B Diluent 1.5 ml tapered tube (QIAGEN) 1052 μl C Diluent 1.5 ml tapered tube (QIAGEN) 1052 μl	Pos.	Content	Tube	Req.vol.	Loaded
c Diluent 1.5 ml tapered tube (QIAGEN) 1052 ul	В	Diluent	1.5 ml tapered tube (QIAGEN)	1052 µl	✓
in the table (divident)	С	Diluent	1.5 ml tapered tube (QIAGEN)	1052 µl	✓

Table of tubes to be loaded to the worktable position M1.

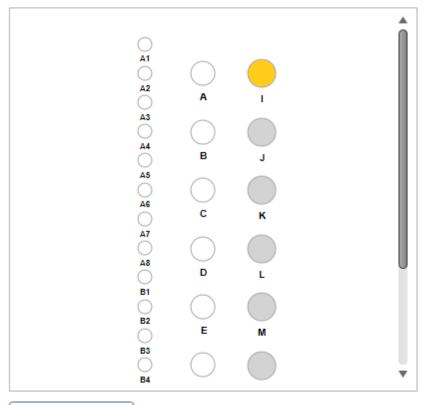
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes



Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the Labware tab on the right to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter

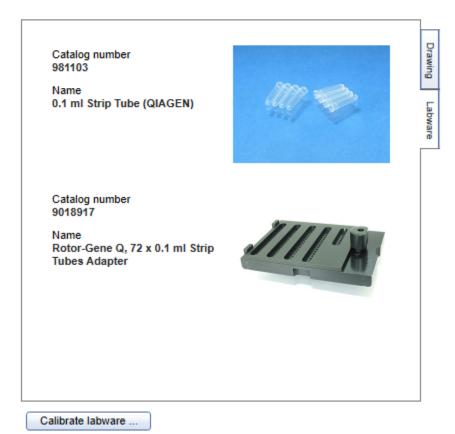
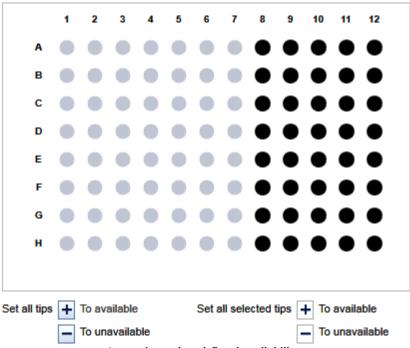


Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



Interactive rack to define tip avilability.

If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- 19. Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for Generic PCR experiments.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.10.6 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the

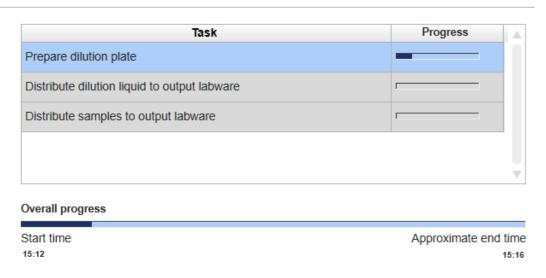
current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.10.6.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Run progress

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.10.6.2 Pausing or canceling the run

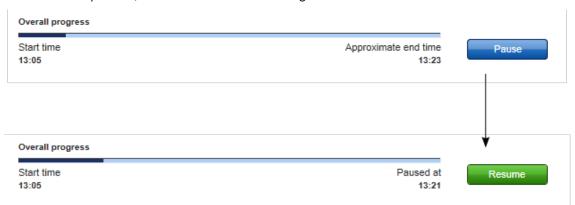
A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- Click the Pause button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.10.6.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.qiagen.com/goto/QIAgility . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Save dilution plate	A file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the dilution labware and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global

After the run has finished, follow these steps:

1. Remove the labware from the worktable.

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Settings tab of the **Configuration** environment.

- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.10.7 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information (incl. dilution labware)

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (incl. dilution strategy)
- Worktable information
- Detailed run information (incl. dilution labware)
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. In addition, the report contains information about normalization parameters and dilution labware (both report types). Information about the dilution strategies used for the samples is available in the **Advanced** report, section **Sample information**. As the experiment setup does not contain mixes, standard series and controls, the corresponding information is not present in the report.

Additional information is described below.

Detailed run information

In addition to the information described in <u>Report</u>, this section contains further information for both report types. It lists the normalization parameters as defined in the **Normalization** step, and contains a table that describes the dilution labware:

Column Description

Dilution position The position on the dilution labware.

Identifier Specifies the sample ID of the originating sample.

Type Sample.

Input The corresponding position on the input labware. If multiple input labwares were

used for the run, the position is enhanced by the input labware identifier.

Actual content Lists the volume and the components.

Concentration The sample concentration in the dilution labware position.

Flag Shows the status of the position: either ok, warning or error.

Sample information

The normalization strategy for each sample is provided, in addition.

7.2.11 Prepare reaction mix experiments

The QlAgility allows you to setup a run to create a reaction (master) mix for later reuse.

In case the reaction (master) mix will also be distributed to output labware, refer to <u>Prepare and distribute</u> reaction mix experiments, instead.

New experiments for creating a reaction (master) mix only are created by clicking **Other workflows** from the **Home** screen and then selecting **Prepare reaction mix**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will be enabled initially. Subsequent steps will become available once the required information has been entered into the **Liquids** and the **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 4 steps that guide you through the run setup process:

- 1. Liquids
- 2. Mixes
- 3. Worktable
- 4. Run

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

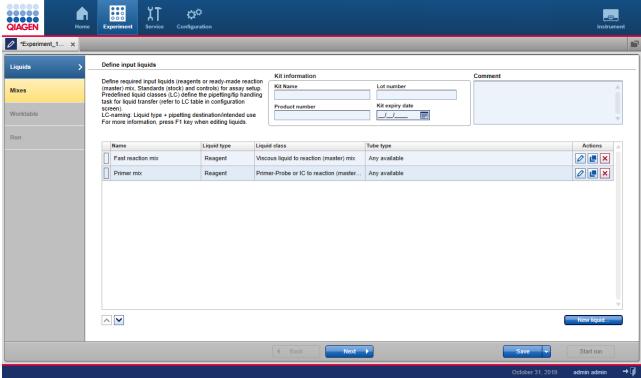
An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt

(Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The Start run button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.11.1 Liquids step

The **Liquids** step is used to define all input liquids required to be loaded onto the instrument's worktable. These are all kind of reagents that will be part of the reaction (master) mix(es). In this step, you can also provide information about the kit that is used during the run.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The **Add liquid** dialog opens.

- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.

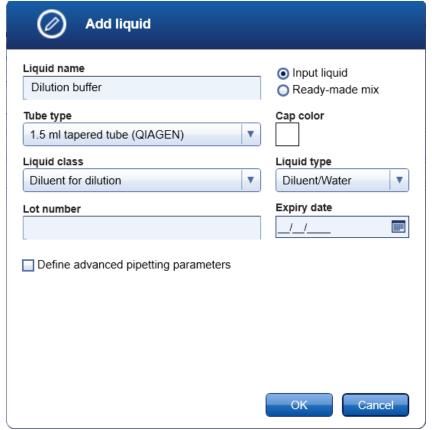
The buttons in the **Actions** column

The buttons in the **Actions** column of the table have the following functions:

Button	Action	Description
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, some restrictions apply. Not all values can be changed. In this case, remove the liquid from the reaction mixes to enable changes for defined input liquids.
	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.11.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you would like to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values of a liquid.

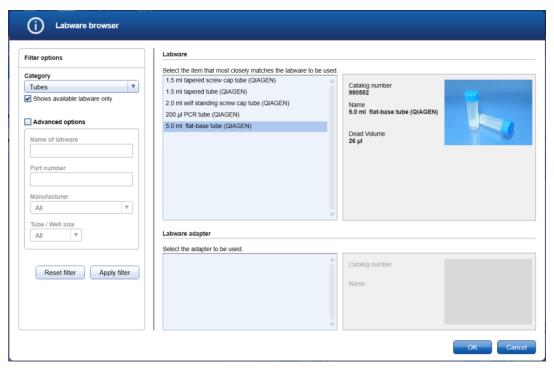


Add liquid dialog

- 1. Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g., when defining reaction mixes, and in the loading instructions of the **Worktable** step.
- The option Input liquid is automatically selected and cannot be changed.
 All liquids are input liquids, also if the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix.
- 3. Click **Tube type** to select the tube that will be used to place this liquid on the QIAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the needed tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section <u>Using the labware browser</u>.

Note: If you edit the liquid later and the **Tube type** is marked in yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type, or make the tube available in the Labware Manager again.

4. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

5. Select the **Liquid type**. The liquid type defines how the liquid will be used.

Liquid type	Description
Reagent	Use this type, if the liquid is a reagent.
Diluent/Water	Use this type, if the liquid is a diluent or water.

- 6. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, this data will be present in the report.
- 7. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

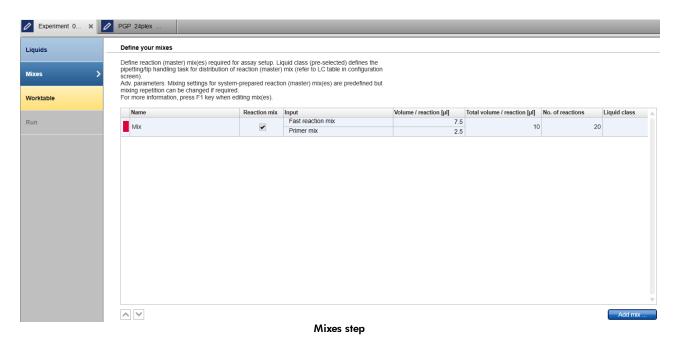
In the dialog, click OK to confirm your values and to close the dialog.
 Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or, in case of editing an existing liquid, the liquid is not changed.

7.2.11.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for the experiment. These are, for example:

- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.
- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that will be generated by the QIAgility during the run.

The screen lists all mixes that have already been defined in a table.



Repeat the steps 1–7 until all final reaction (master) mixes have been defined. At least one final reaction (master) mix needs to be defined.

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix as described in Add new mix input.
- 5. Click **Finish** to finish the definition of the mix and to close the dialog.
- 6. The defined mix will be listed in the table in the Mixes screen.
- 7. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 8. Close the lid.
- 9. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

Note: The Next button is disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix, of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Click the row of the mix to be moved to select it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Actions

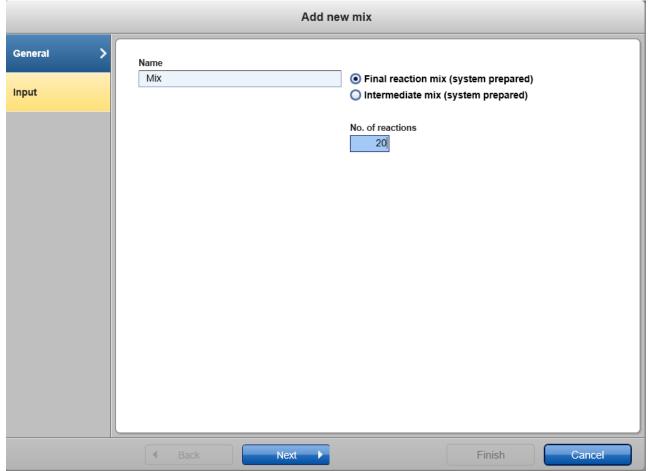
The buttons in the **Actions** column of the table have the following functions:

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already used as input for another mix, some restrictions apply. Not all values can be changed anymore.
Ŀ	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mixes name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

7.2.11.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.



Add mix dialog - General

- 1. Define the mix's name. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g., when defining more specific mixes, and in the loading instructions of the **Worktable** step.
- 2. Select, which kind of mix you define. Different options are available depending on the selection:

	Option	Description
Final reaction mix (system prepared)	Use this option for the final mix that will be created by the QIAgility.	
	(system prepared)	If selected, the field No.of reactions will be visible. Proceed to step 3.
	Intermediate mix (system prepared)	Use this option, if this mix shall be created by the QIAgility during the run, but will be part of a more specific mix. During the run, this mix will be created by the QIAgility first. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.
		Proceed to step 4.

- 3. For final reaction (master) mixes, only: enter the number of reactions for which the reaction mix shall be prepared into the field **No. of reactions**. Proceed to step 6.
- 4. For intermediate mixes, only: the liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only, if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

5. For intermediate mixes, only: optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as a reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

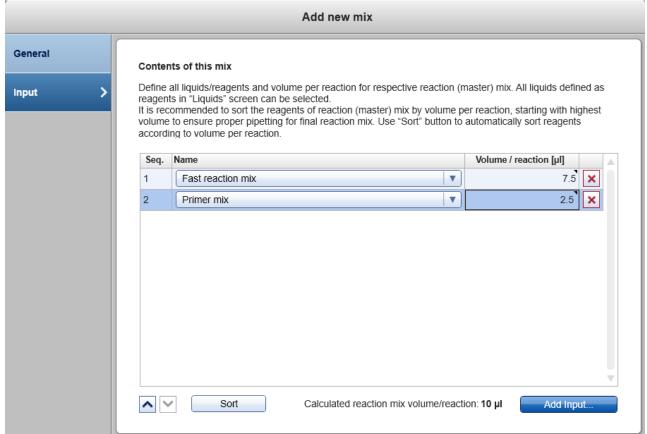
During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

6. Proceed with step 3 in Mixes step.

7.2.11.2.2 Add new mix input

The **Input** screen of the Add/Edit mix dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.



Add mix dialog - Input

To define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**.
- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For non-integer volumes use a point as a decimal delimiter.
- 4. Repeat the steps 1–3 above, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QlAgility will pipette the reagents into the tube where the mix is created in the order as listed in this table.
 Click the Sort button below the table, to automatically sort the list by volume per reaction, starting with the highest volume first.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.11.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Worktable selector

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

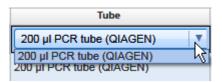
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - Mix block

Pos.	Content	Tube	Req.vol.	Loaded
В	Reaction mix (Mix)	1.5 ml tapered tube (QIAGEN)	0 μΙ	✓
С	Fast reaction mix	1.5 ml tapered tube (QIAGEN)	223 µl	✓
D	Primer mix	1.5 ml tapered tube (QIAGEN)	67 µl	✓

Table of tubes to be loaded to the worktable position M1.

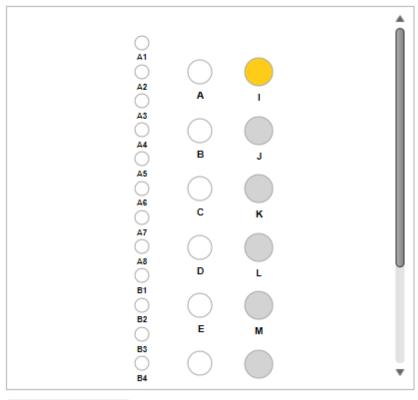
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware**Manager. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

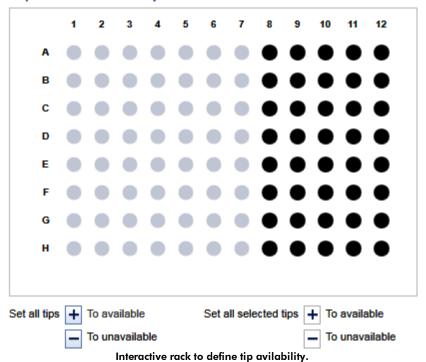


Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to Loading tips.

50 µl Conductive Filtered Tips



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- Optional step: Generate the loading instructions by clicking on Print loading instruction. A PDF will
 be generated containing the loading instructions for the run. Use this option, if, for example, liquids
 need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.11.4 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.11.4.1 Monitoring the run

Run progress

11:28

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Task Progress Prepare mixes Overall progress Start time Approximate end time

Run progress.

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

11:29

7.2.11.4.2 Pausing or canceling the run

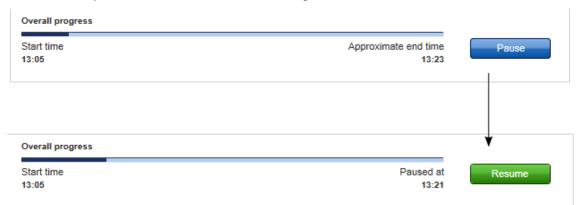
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.11.4.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, the option to create a report is available on the right side of the **Run** step screen. This option will still be available if you reopen the experiment later.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.11.5 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results (This section is empty.)
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Reaction results (This section is empty.)
- Exceptions/Pauses
- Sample information (This section is empty.)
- Worktable information
- Detailed run information
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. As the experiment setup does not contain samples, standard series and controls, the corresponding information is not present in the report.

7.2.12 Prepare and distribute reaction mix experiments

The QlAgility allows to setup a run to create a reaction (master) mix and distribute it to up to three output labware, or to distribute a reaction (master) mix to the output labware only.

In case the reaction (master) mix shall only be created for later reuse, but not distributed to an output labware, refer to <u>Prepare reaction mix experiments</u>, instead.

New experiments for creating and distributing a reaction (master) mix are created by clicking **Other workflows** from the **Home** screen and then selecting **Prepare and distribute reaction mix**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will initially be enabled. Subsequent steps will become available once the required information has been entered in the **Liquids** and the **Mixes** step. The **Mixes** step is colored yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 6 steps that guide you through the run setup process:

- 1. Liquids
- 2. Mixes
- 3. Output labware
- 4. Assignment
- 5. Worktable
- 6. Run

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

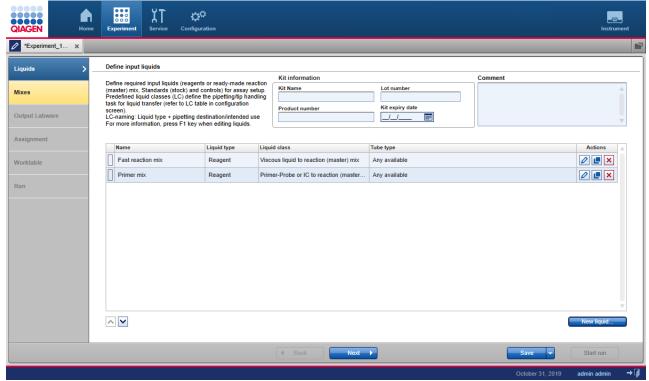
For more information about templates and experiments refer to the Experiments and templates concept.

7.2.12.1 Liquids step

The **Liquids** step is used to define all input liquids required to be loaded onto the instrument's worktable required for your setup. These are, for example:

- all kinds of reagents or diluents/water, that will be part of reaction (master) mixes
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the Mixes step.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (Mixes step).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click to open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.



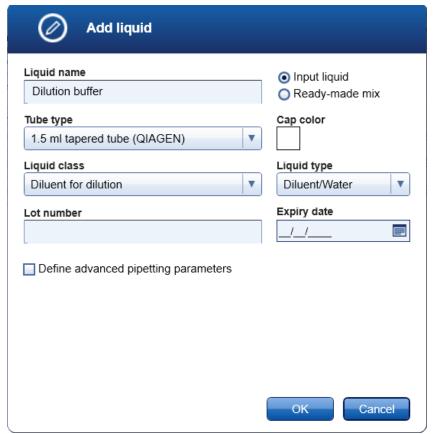
The buttons in the **Actions** column

of the table have the following functions:

Button	Action	Description
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, if, for example the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to positions on the output labware in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.12.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you later want to change the values for an existing liquid, the **Edit liquid** dialog will open. Both dialogs show the same values of a liquid.

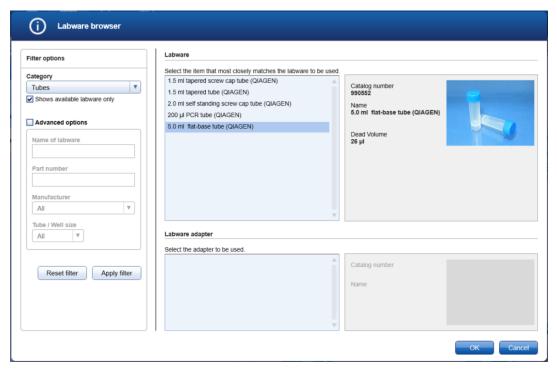


Add liquid dialog

- 1. Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, when defining reaction mixes, and in the loading instructions of the Worktable step.
- 2. Select, if the liquid is an input liquid or a ready-made reaction/master mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.
 - Select the option **Ready-made reaction/master mix** only, if the liquid is a final reaction/master mix that has already been prepared by user and is ready to be used and distributed to the output labware by the QlAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed. If the liquid is a mix that has already been prepared by user, but will be part of a more complex mix that shall be created during run by the QlAgility, select the option **Input liquid**.

3. Click **Tube type** to select the tube that will be used to place this liquid on the QIAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry. Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the <u>Labware Manager</u>, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the needed tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked in yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Select the **Liquid class** that will be used to pipette the liquid during he run. Select the liquid class that fits best

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- · the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid is intended to be used for different purposes it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

5. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g., when defining reaction (master) mixes.

Liquid type Reagent	Description Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
Diluent/Water	Use this type, if the liquid is a diluent or water. Later in the experiment setup, a liquid of this type is available for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid. Later in the Mixes step, when you define the content for a mix, a
	liquid of this type is available for selection.

- Optionally, enter the Lot number and/or the Expiry date of the liquid for documentation purposes. If entered, this data will be present in the report.
- 7. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

8. In the dialog, click **OK** to confirm your values and to close the dialog.

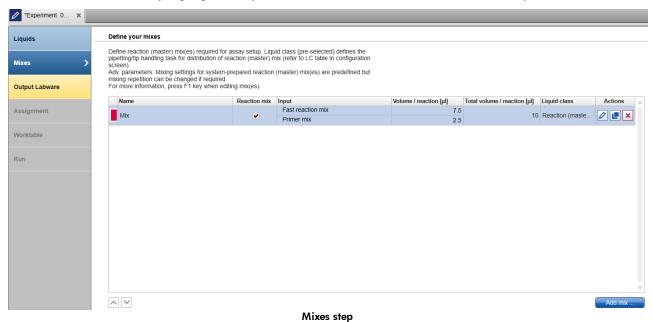
Otherwise, click **Cancel** to close the dialog and discard all values. In this case, no new liquid is defined, or --in case of editing an existing liquid-- the liquid is not changed.

7.2.12.2 Mixes step

The Mixes step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.



Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the Add mix button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- In the dialog Input screen define the volume per reaction and the content of the mix; as described in 4. Add new mix input.
- 5. Click **Finish** to finish the definition of the mix and to close the dialog.
- The defined mix will be listed in the table in the Mixes screen. 6.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click Next in the button bar to proceed to the Output Labware step, described in Output labware step.

Note: The Next button is disabled, if a row in the table is highlighted yellow. User prepared reaction (master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit(the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Click the row of the mix to be moved to select it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

The buttons in the **Actions** column



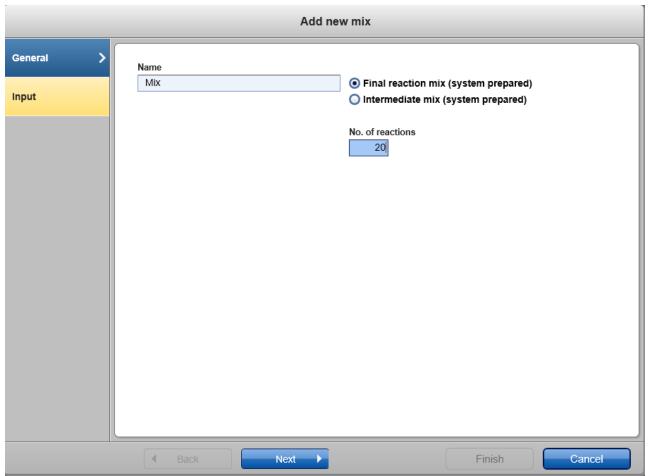
of the table have the following functions:

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already used as input for another mix, or if it is a final reaction mix and has already been assigned to positions on the output labware in the Assignment step, some restrictions apply. Not all values can be changed anymore.
	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mixes name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

7.2.12.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you later want to change the values for an existing mix, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.



Add mix dialog - General

- 1. Define the mix's name. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g., when defining more specific mixes, and in the loading instructions of the Worktable step.
 - For a ready-made mix, the name is already defined from the liquid dialog definition.
- 2. Select, which kind of mix you define. For ready-made mixes and other mixes different options are available:

Option	Description
Final reaction mix (ready-made)	Only available for a mix which has been defined as ready-made mix within the Liquid step.
	Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QlAgility. In the later Assignment step, the mix will be available for selection. During the run, the QlAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.
	Ensure that the reaction (master) mix is homogenously mixed before it is loaded to the QIAgility as the QIAgility will not perform any mixing steps.
Intermediate mix (ready- made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	Use this option if this mix has already been prepared by the user and will be part of a more specific mix that will be created during the run by the QlAgility. During the run, the QlAgility will pipette the mix into the tube where the more specific mix is created.
	Ensure that the reaction (master) mix is homogenously mixed before it is loaded to the QIAgility as the QIAgility will not perform any mixing steps.
Final reaction mix (system prepared)	Use this option, if during the run the mix will be created by the QIAgility first, and then distributed to the output labware.
Intermediate mix (system prepared)	Use this option, if this mix will be created by the QIAgility during the run, but will be part of a more specific mix. During the run, this mix will be created by the QIAgility first. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** which fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QlAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix is transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – that are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents it is recommended to increase the mixing repetition to 15 or more.

For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in Mixes step.

7.2.12.2.2 Add new mix input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.



Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

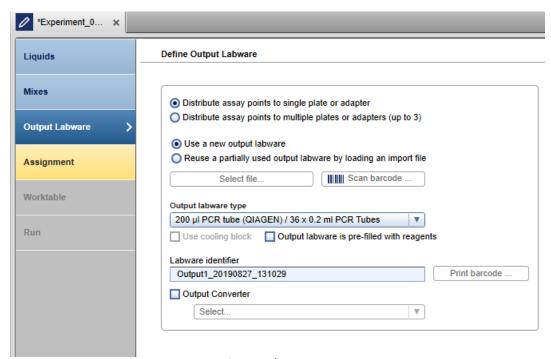
- Define the volume per reaction for the input liquid. Left-click the Volume/reaction field and then enter
 the value. The total volume per reaction of the mix is displayed below the table. It summarizes the
 volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal
 delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.
 Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.12.3 Output labware step

Use the **Output Labware** screen to select the output labware for the run. You can distribute assay points to one plate or adapter or up to three plates or adapters. To define output labware on a single plate or adapter, follow the steps in <u>Define one output labware</u>. To define multiple labware, follow the steps in <u>Define up to three output labware</u>.



Output Labware step

7.2.12.3.1 Define one output labware

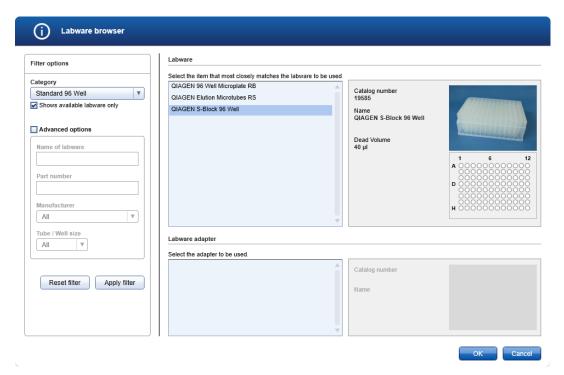
If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- You can either Use a new output labware or Reuse a partially used output labware by loading an import file. The option Use a new output labware will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.





In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the **Labware** browser, refer to the section <u>Using the labware browser</u>.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. Optional: If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the Labware identifier field. This identifier will be used in the report and in the sample output file.

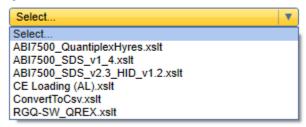
Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. Optional: If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to Output converter to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, None is the only entry in the list.





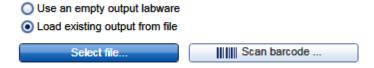
Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed to step 12, below.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

The Select file and Scan barcode buttons will be enabled if the Load existing output from file option has been selected.



10. Click the Select file button and select the desired labware file. For details, refer to <u>File dialog</u>. Alternatively, click the Scan barcode button. In the Scan labware barcode dialog, scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

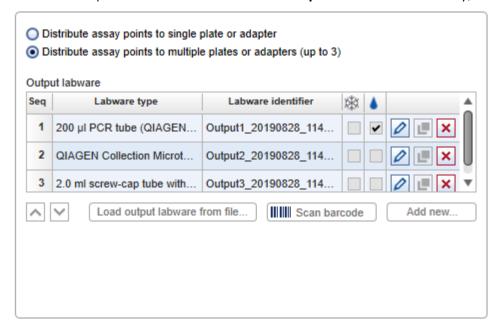
- 11. Proceed to step 3.
- 12. Click **Next** to proceed.

The next step is the Assignment step.

7.2.12.3.2 Define up to three output labware

If the option **Distribute assay points to multiple plates or adapters (up to 3)** has been selected, you can define up to three output labware types by following the steps below.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.



- 1. If you would like to add output labware manually, click the **Add new** button and proceed to step 4. If you would like to load an output labware file, proceed to step 2.
 - If you would like to provide output labware information by scanning a barcode, proceed to step 3.
- Click the Load output labware from file button and select the desired file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the output information from the selected file.
 - If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.
- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.
 - If the labware is to be used with the cooling block, the box in the solumn will be ticked. If the labware is pre-filled with reagents, the box in the column will be ticked. To change these settings, edit the labware.
- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Click **Next** to proceed.

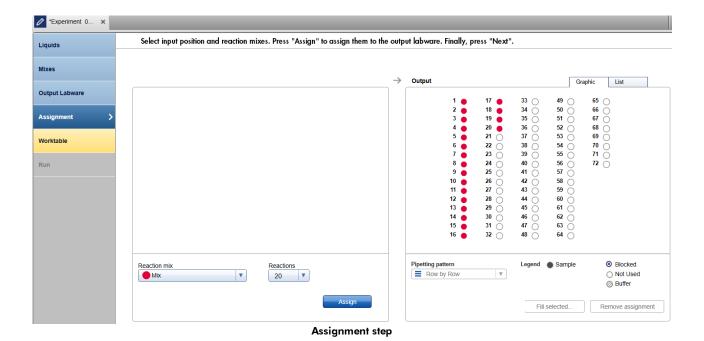
The next step is the **Assignment** step.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.

7.2.12.4 Assignment step

The **Assignment step** screen shows a graphic visualization of the output labware on the right. It is designed to support flexible and intuitive assignment of final reaction (master) mix(es) to the output labware.



At the bottom on the left is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Reactions** menu.

In order to assign a final reaction (master) mix to the output labware, select the reaction (master) mix. Then set the number of reactions. When you click the **Assign** button, the defined number of reactions for the selected mix will be created on the output labware. Follow the procedure described in <u>Assigning reaction mixes to the output labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The graphic will be updated when you confirm an assignment. The reactions are colored with the reaction mixes. If you hover with the mouse cursor over an output position, the software displays the reaction mix for that position.

Reactions can be removed. For details, refer to Removing assignments.

The menu at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column by column).

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the reaction (master) mixes for the output positions.

7.2.12.4.1 Assigning reaction mixes to the output labware

Proceed as follows:

- From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- Click on the down arrow of the **Reactions** drop-down menu to define the number of reactions for the mix:



Note: The reactions menu is an editable menu. If you want to work with more than 5 reactions, just type the number into the field. For details about editable menus, refer to Entering data.

- 3. Click Assign.
- 4. The software creates the defined number of reactions for the selected reaction mix on the **Output** panel on the right side of the screen. The positioning follows the selection made for the **Pipetting pattern**.

Note: To create a desired output layout you can use the **Assign** button multiple times with different selections for reaction mix and number of reaction.

- 5. Repeat the steps above until all positions of the output side have been defined for the planned run.
- 6. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 7. Close the lid.
- 8. Click **Next** to enter the worktable step. If the instrument has not been used before, it will initialize. **Note:** While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.12.4.2 Removing assignments

To clear a position on the output side, follow these steps:

- Select the desired position on the Output panel. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or CTRL and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click **Remove assignment**. The assignment will be removed, and the position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared. The following positions will be shifted to fill the gap.

7.2.12.5 Worktable step

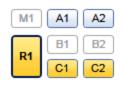
Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Worktable selector

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for

each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

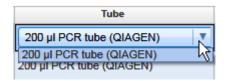
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - Mix block

Pos.	Content	Tube	Req.vol.	Loaded	
В	Reaction mix (Mix)	1.5 ml tapered tube (QIAGEN)	0 μΙ	✓	
С	Fast reaction mix	1.5 ml tapered tube (QIAGEN)	223 µl	✓	
D	Primer mix	1.5 ml tapered tube (QIAGEN)	67 µI	~	
		'			-

Table of tubes to be loaded to the worktable position M1.

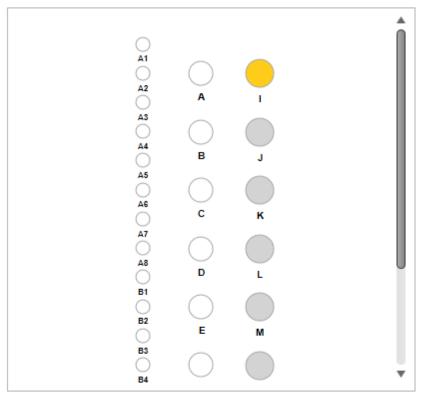
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

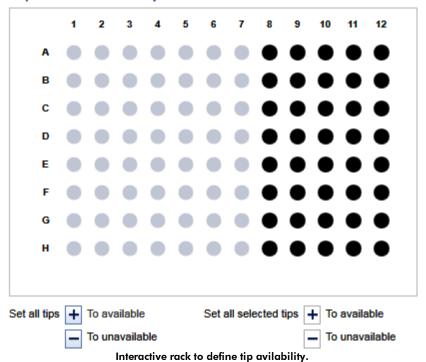


Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack holder</u>.

- 4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.
 - After the calibration steps have been completed, the worktable screen will be displayed again.
- 5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.
 - **Note:** For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.
- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.12.6 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

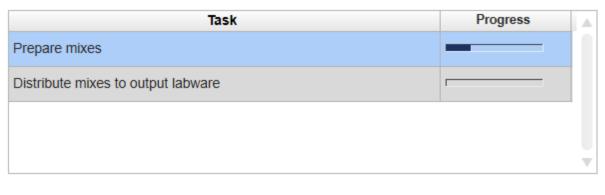
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.12.6.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Overall progress

Start time Approximate end time 15:12 15:16

Run progress.

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.12.6.2 Pausing and canceling the run

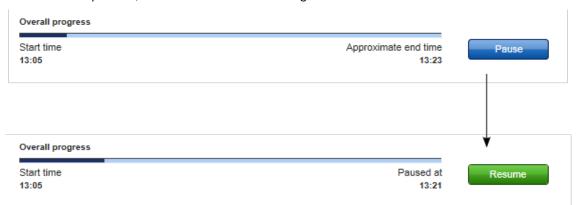
A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

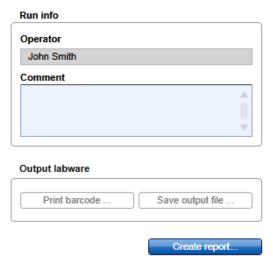
To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.12.6.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to print a barcode.



Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.12.7 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

• General information

- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (This section is empty.)
- Worktable information
- Detailed run information
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. As the experiment setup does not contain samples, standard series and controls, the corresponding information is not present in the report. The section **Reaction results** lists the actual content of the output positions, only.

7.2.13 Prepare standards experiments

The QIAgility allows you to prepare standards without PCR, so that they can be used in your future PCR experiments.

New experiments for preparing standards are created by clicking **Other worfkflows** from the **Home** screen and then selecting **Prepare standards**. The experiment will then be defined in the Experiment environment of the software. When a new experiment is created, only the **Liquids** and **Controls/Standards** steps will be initially enabled. Subsequent steps will become available once the required information has been entered into the **Liquids** and **Controls/Standards** step. The **Controls/Standards** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has four steps that guide you through the run setup process:

- 1. <u>Liquids</u>
- 2. Controls/Standards
- 3. Worktable
- 4. <u>Run</u>

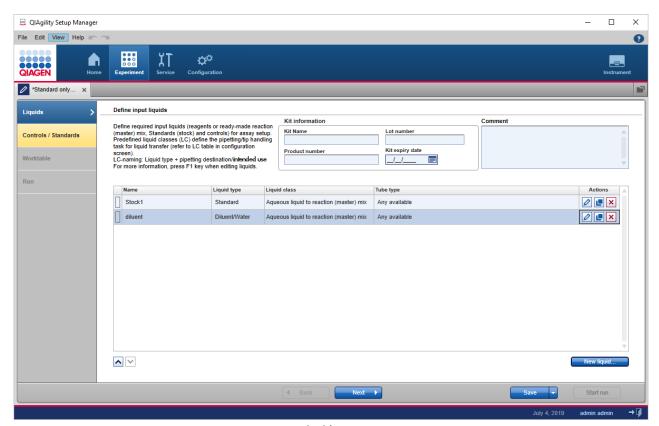
Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save** as buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is *.qsmrx, and for templates *.qsmrt (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.qsmrqt (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.13.1 Liquids step

The Liquids step is used to define standards and diluents.



Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

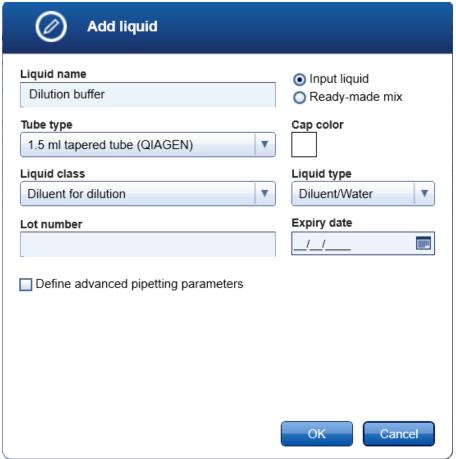
To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The **Add liquid** dialog opens.
- 3. Define the liquid as described in Add liquid dialog.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

7.2.13.1.1 Add liquid dialog

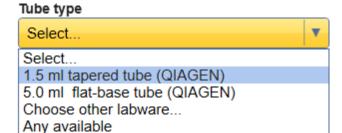
In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.



Add liquid dialog

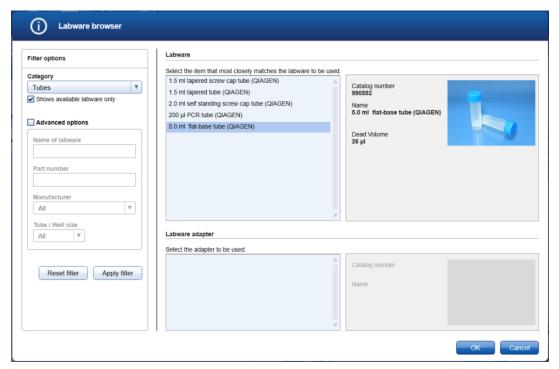
 Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup.

2. Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the respective entry.



Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.



In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the **Labware browser**, refer to the section <u>Using the labware browser</u>.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

3. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

4. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

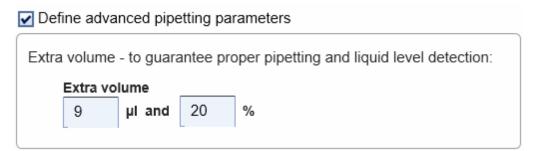
Liquid type	Description
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QlAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.

- 5. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each

liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.



In the dialog, click **OK** to confirm your values and to close the dialog.
 Otherwise, click **Cancel** to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.13.2 Controls/Standards step

This step is used to define standard series.

The screen contains one table: **Standard series**. The table has an **Actions** column with interactive buttons to manage the standard series.

Adding a standard series

- 1. Click on Add standard series.
- 2. The Add standard series dialog will open. For details, refer to Add standard series.
- 3. The standard will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all standard series have been defined.
- 5. Click **Next** to proceed.

The buttons in the Actions column of the Standard series table have the following functions:

Button	Action	Description
0	To edit a standard series	The Edit standard series dialog will open, displaying the values that have been entered in the Add standard series dialog.
	To duplicate a standard series	The Duplicate standard series dialog will open, displaying the values that have been entered for the original standard series. The standard series names will initially display as a copy of the standard series.
×	To delete a standard series	Deletes the row in the Standard series table.

Changing the order of the output labware

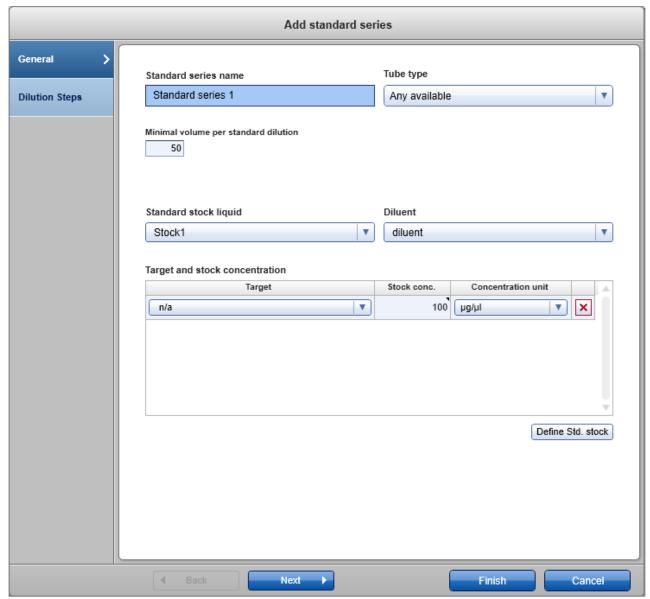
You can change the order of the standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output labware will be based on the order indicated in these tables.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.13.2.1 Add standard series

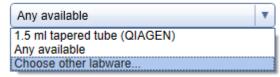
In the **Add standard series** dialog, a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.



Add standard series dialog - General

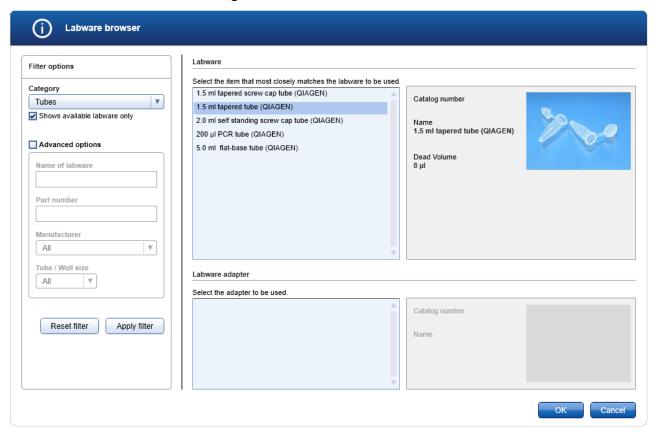
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Click on the **Tube type** drop-down menu, which is on the right side of the **Standard series name** field.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Tube type



4. Select the tube type from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.



In the filter options, select the labware **Category** from the drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the labware from the list at the top. If the labware requires an adapter, select the adapter from the **Labware adapter** list. Click **OK** to close the **Labware browser**.

The selected labware will be displayed and added to the **Tube type** drop-down menu.

- 5. Specify a minimal volume per standard dilution in the **Minimal volume per standard dilution** field.
- Select the appropriate liquid from the Standard stock liquid drop-down menu. The menu lists all liquids that have been defined with liquid type Standard. If a required liquid is not listed as expected,

- check if the liquid has already been defined in the **Liquids** step and if the correct liquid type has been selected.
- 7. Select the appropriate diluent from the **Diluent** drop-down menu. The menu lists all liquids that have been defined with liquid type **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the **Liquids** step and if the correct liquid type has been selected.
- 8. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration.



Defining common stock

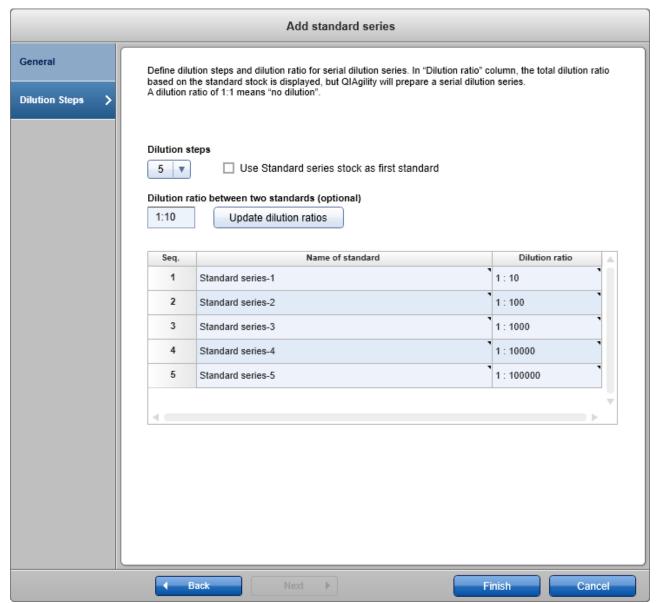
Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column	Description
Target	N/A is selected.
Stock conc.	Stock concentration.
	This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration.

9. In the dialog, click Next to proceed to the **Dilution steps** screen.

7.2.13.2.2 Add standard series dilutions

For general information about the Controls/Standards step, refer to <u>Control/Standards step</u>. For information about the **General** screen of this dialog, refer to <u>Add standard series</u>. Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.



Add standard series dialog - Dilution steps

1. Select the number of dilution steps from the Dilution steps menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> data.

- 2. Enter a meaningful name into the **Name of standard** cell of each row. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the loading instructions of the Worktable step.
- If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the Dilution ratio field above the dilution steps and then click **Update dilution ratios** to apply it to the series

Dilution ratio between two standards (optional)



Seq.	Name of standard	Dilution ratio	
1	Standard series-1		7
2	Standard series-2		7

Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)



Seq.	Name of standard	Dilution ratio
1	Standard series-1	1:4
2	Standard series-2	1:16
	•	•

After the "Update dilution ratios" button has been clicked

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard.

Dilution ratio between two standards (optional) Update dilution ratios Seq. Name of standard Dilution ratio 1 Standard series-1 1:4 2 Standard series-2

Entering dilution ratio for dilution step

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed.

- 4. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 5. Proceed with step 4 in Controls/Standards step.

7.2.13.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Worktable selector

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click Print loading instruction to generate a PDF document with the loading instructions for the run.

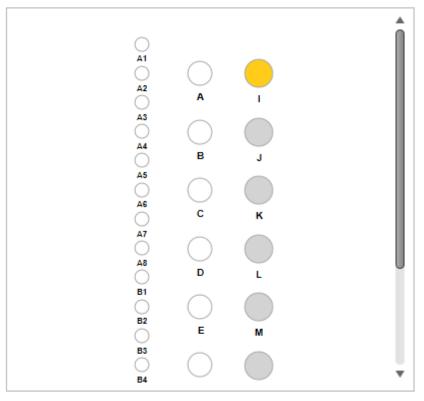
R1 - Reagent block

Pos.	Content	Tube	Req.vol.	Loaded	Α
1	Stock1	1.5 ml tapered tube (QIAGEN)	25 µl	V	
J	Standard series 1-2	1.5 ml tapered tube (QIAGEN)	0 μΙ	✓	
K	Standard series 1-3	1.5 ml tapered tube (QIAGEN)	0 μΙ	✓	
L	Standard series 1-4	1.5 ml tapered tube (QIAGEN)	0 μΙ	V	
M	Standard series 1-5	1.5 ml tapered tube (QIAGEN)	0 μΙ	✓	
N	Standard series 1-6	1.5 ml tapered tube (QIAGEN)	0 μΙ	V	
					Ų
		Print loading instruction	Co	nfirm all	

Table of tubes to be loaded to the R1 worktable position

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

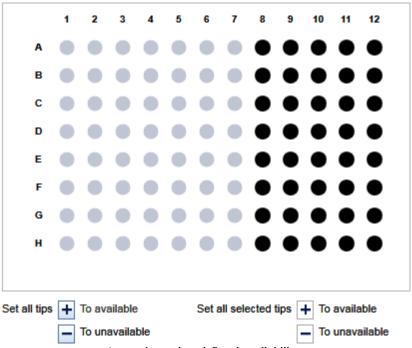


Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.

50 µl Conductive Filtered Tips



Interactive rack to define tip avilability.

If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> holder.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has

not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the **Calibrate labware** button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again.

 Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 10. Optional step: If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the Loaded column, or click

Confirm all to confirm all positions.

- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.13.4 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

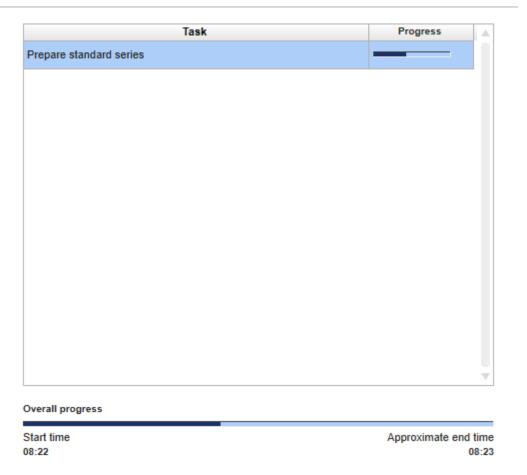
During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.13.4.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress



Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.13.4.2 Pausing or canceling a run

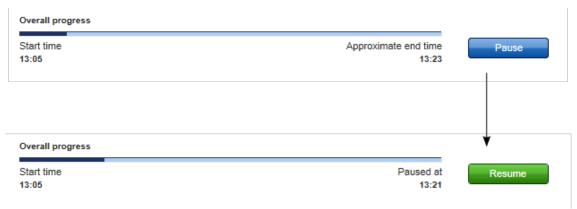
A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.



To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

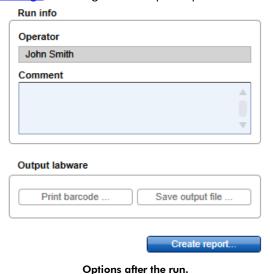
- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.13.4.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report.

After a run is completed, it is possible to create a <u>report</u> for documentation purposes. Refer to the <u>user settings</u> to configure the report options.



After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.3 Administrative tasks

This section describes various administrative tasks for the QIAgility.

7.3.1 Configuring QG

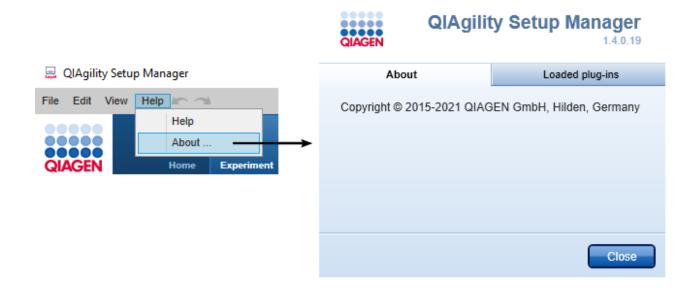
To set up the QIAgility, follow the instructions in the following sections, in the order listed below:

- 1. Unpacking and installation
- 2. Software installation

- 3. Managing users
- 4. Customizing settings
- 5. <u>Labware manager</u>
- 6. <u>Instrument calibration</u>

7.3.2 Viewing software version

When you contact QIAGEN Technical Services for support purposes, you may be asked to provide the software version number of your installation. To see the version number, click on **Help** from the main menu and select the **About** command. The **About** dialog will be displayed, and the current version number will be displayed in the top right of the dialog.



Click on **Loaded plug-ins** for information about the installed plug-ins.

7.3.3 View system audit trail

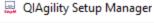
The audit trail is a versatile tool to keep track of events that occur in the QIAgility Setup Manager Software. You can filter for specific events using the date or date ranges and user criteria. Results matching the criteria are displayed and can be exported to a PDF file.

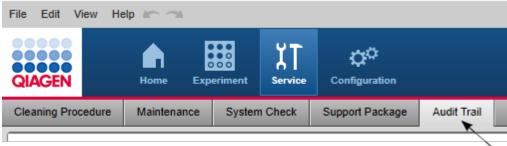
Note: This function is only available for users with administrator user role.

Relevant events are:

- Starting a run
- Completion of a run
- Stopping a run

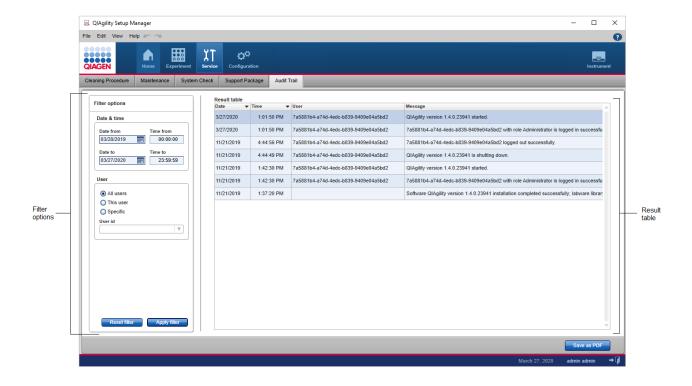
- Creating a report
- Saving calibration data





The audit trail is placed as separate tab in the "Service" environment.

You can apply filters from the **Filter options** group box on the left to find audit trail entries that meet specific criteria. Criteria you can use are specific dates, date ranges or specific users. All audit trail entries that meet the criteria are listed in the **Result table** on the right of the screen. You can also generate a PDF file of the results.



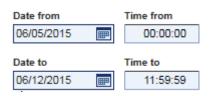
To filter for specific audit trail entries

- 1. Click on the Audit Trail tab in the Service environment.
- 2. The **Audit Trail** is displayed.
- 3. From the **Filter options** group box on the left, select the filter criteria.

Note: You can combine time/date filter and user filter for more advanced queries:

Time/date filter

Enter the desired date/time to the **Date from/Time from** and **Date to/Time to** fields:



Note: You can use the date picker (**)** to open an interactive calendar for entering the dates

User filter

Select one of the three options to filter for all users, the current user or a specific user. In the latter case, select a user ID from the **User id** drop-down menu below:



- 4. Click on **Apply filter** to apply the selected filter.
- 5. All entries in the audit trail that meet the selected filter will be displayed in the **Result table** on the right of the screen.

Exporting filter results

To export the results of a specific filter, click on the **Print to PDF** button at the bottom right. A PDF will created and opened, displaying the current result table.

Note: To view reports generated in PDF format, a PDF reader must be installed on the computer, see <u>Computer</u>.

Maintenance procedures

8 Maintenance procedures

There are two procedures required to carry out the maintenance. Following these procedures ensures optimum performance of your QIAgility instrument.

- Regular maintenance
- Periodic maintenance

Before undertaking any maintenance procedures, we recommend that you familiarize yourself with the safety information by referring to section <u>Maintenance safety</u>.

Important: Switch the instrument off and disconnect the line power cord from the power outlet before servicing.

WARNING/ CAUTION



Risk of electric shock [W15]

Do not open any panels on the QIAgility.

Risk of personal injury and material damage

Only perform maintenance that is specifically described in this user manual.

8.1 Servicing

The QlAgility is supplied with a warranty that lasts for 1 year, beginning on the date of shipment. The warranty includes all repairs due to mechanical breakdown. Application development, software upgrades, accessories and disposable items are not included in the warranty.

QIAGEN offers comprehensive Service Support Agreements, including warranty extensions, full cover support agreements and instrument/application training, including on-site installation. Service Support Agreements maximize productivity and ensure high performance from your instrument. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Field Service Specialist or your local distributor for more information about flexible Service Support Agreements from QIAGEN .

8.2 Regular maintenance

CAUTION

Damage to the instrument [C4]



Do not use solvents or reagents containing acids, alkalis or abrasives to clean the QlAgility.

WARNING/ CAUTION

Risk of electric shock [W15]



Do not open any panels on the QIAgility.

Risk of personal injury and material damage

Only perform maintenance that is specifically described in this user manual.

CAUTION

Damage to the QIAgility lid [C5]



Do not use alcohol, solvents, reagents containing acids, alkalis or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use distilled water only.

CAUTION

Damage to the QIAgility side panels [C6]



Never clean the instrument side panels with alcohol or alcohol-based solutions.

Cleaning agents

The following disinfectants and detergents are recommended for cleaning the QIAgility.

Note: If using disinfectants different from those recommended, ensure that their compositions are similar to those described below.

General cleaning of the QIAgility:

- Mild detergents
- 70% ethanol (except for the QIAgility lid)
- Mikrozid[®] Liquid (Schülke & Mayr GmbH)

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Disinfection

- Ethanol-based disinfectants can be used for disinfection of surfaces, such as the worktable: e.g., Mikrozid Liquid, Mikrozid Wipes. Do not use ethanol-based disinfectants to clean the lid.
- Disinfectants based on glyoxal and quaternary ammonium salt can be used for submerging worktable items, such as the adapter and tip ejector: e.g., 10 g glyoxal, 12 g lauryldimethylbenzylammonium chloride, 12 g myristyldimethylbenzylammonium chloride, and 5–15% nonionic detergent per 100 g liquid, Lysetol[®] AF (Gigasept[®] Instru AF in Europe, Schülke & Mayr GmbH, cat. no. 107410; or DECON-QUAT[®] 100, Veltek Associates, Inc., USA, cat. no. DQ100-06-167-01)

Removal of RNase contamination

5 PRIME RNaseKiller (5 PRIME, cat. no. 2500080) for cleaning surfaces and submerging worktable items

Removal of nucleic acid contamination

- DNA-ExitusPlus[™] (AppliChem, cat. no. A7089,0100) for cleaning surfaces and to submerge worktable items
- ANIGENE (formerly TriGene, Medichem International) for submerging worktable items

8.2.1 Cleaning the QIAgility

- Do not use spray bottles to spray cleaning or disinfectant liquids onto surfaces of the QIAgility workstation. Spray bottles should be used only for items that have been removed from the instrument.
- Follow manufacturer's safety instructions for handling cleaning agents.
- Follow manufacturer's instructions for soaking time and concentration of the cleaning agents. Immersing for longer than the recommended soak time can harm the instrument.

Cleaning the QIAgility:

- Remove all adapters and the tip ejector from the worktable.
 To clean the adapters, refer to <u>Cleaning accessories</u>. For instructions for removing and cleaning the tip ejector, refer to <u>Maintenance of the tip ejector</u>.
- 2. Clean the worktable inside the instrument using a moist, soft cloth.
- 3. Wipe the instrument with a damp cloth only.
- 4. Discard waste according to local safety regulations.

Note: Do not wipe the rails. These rails support the pipettor head and allow it to slide backwards and forwards easily. Wiping the rails will remove the grease and make them more susceptible to rust.

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8.2.2 Cleaning the lid

The instrument lid is manufactured from a special UV-absorbent acrylic glass.

- Do not use alcohol, alcohol solvents or reagents containing acids, alkalis or abrasives to clean the instrument lid as this could damage the lid and its UV absorbtion.
- If solvents or saline, acidic or alkaline solutions are spilled on the QIAgility, wipe the spilled liquid away immediately.

Clean the instrument lid only with distilled water and a clean, soft cloth.

Alternatively, a cleaning product specifically designed to clean acrylic glass can be used. Any cleaning product should first be tested on a small, inconspicuous section of the instrument lid.

8.2.3 Cleaning accessories

Cleaning adapters

- Do not use a dishwasher to clean adapters as this will damage the anodization of the parts.
- Do not allow household bleach to come in contact with anodized parts.
- 1. Wash briefly in a quaternary ammonium-based solution.
- 2. Rinse with room-temperature, deionized water and allow to drain and dry.

Cleaning the guide rails

The X, Y and Z guide rails are the coated hardened steel rails supporting the linear bearings that allow the robotic arm to easily slide back and forth. Do not wipe these guide rails with a cloth as doing so removes the grease.

- 1. Check for splashed reagents daily.
- 2. Wipe only the areas which have splashed reagent on them and then move the robotic arm back and forth over the axis to redistribute the grease.

The linear bearings incorporate seals to keep the bearings free of dust or grit.

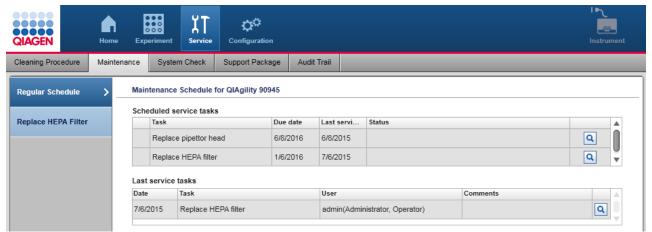
8.3 Periodic maintenance

In addition to the regular maintenance procedures which should be performed very frequently, there are also periodic maintenance tasks which need to be performed less often. These periodic tasks mainly focus on the exchange of hardware parts such as the HEPA filter, among others.

Periodic maintenance tasks are listed in the software in the <u>Maintenance schedule</u> in the **Service** environment.

8.3.1 Maintenance schedule

QlAgility Setup Manager Software provides a maintenance tracking tool to give you an overview of scheduled upcoming service tasks and recent tasks. You can find this tool in the **Regular Schedule** step of the **Maintenance** tab in the **Service** environment:



Scheduled tasks (upper table) and recent tasks (lower table)

The maintenance scheduler lists the periodic tasks for maintenance of the <u>pipettor head</u> and the <u>HEPA</u> filters, and consists of two tables which are described below:

- Scheduled service tasks
- Last service tasks

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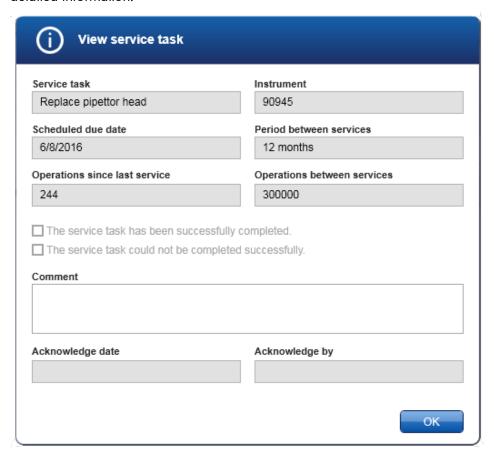
Scheduled service tasks table

Column First column	Description If the task is due, a maintenance symbol is displayed. If the task is due because the service period has been reached, a screwdriver will be displayed. If the task is due because the number of operations between services has been exceeded, a part of a large screwdriver will be displayed.	
	Note : The number of operations is counted for the P-axis movements of the pipettor head only.	
Task	Description of the task	
Due date	Date the task is due	
Last service date	Date the task was last performed	
Status	Current status of the task. The cell is empty if the task is not due. If the task is due, the reason why the task is due will be displayed: because the service period has been exceeded, or if the number of operations between services has been exceeded, or both.	
View icon (🔍)	Opens the View service task dialog with detailed information; see below	

Note: If one task is due, the software indicates this also with a screwdriver symbol on the instrument icon. Additionally, a message is shown if a task becomes due (the message is shown only once).

Last service tasks table

The **View** icon () has the identical functionality in both tables: it opens the **View service task** dialog with detailed information.



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Field Description Service task Description of the task Schedule due date Date the task is due Operations since last service Number of operations (e.g., number of P-Axis movements of the pipettor head) that have been performed since the last service Note: This field is only displayed for the pipettor head maintenance task. **Note**: This field is not available from the **Last service tasks** table. Instrument Serial number of the instrument Period between services Proposed period between two service dates Operations between services Recommended number of operations between two services Note: This field is only displayed for the pipettor head maintenance task. Note: This field is not available from the Last service tasks table. The service task has been successfully completed. The service task could not be completed successfully. Result of the service task: whether or not the task has been completed successfully. Comment Comment noted by the QIAGEN Field Service Specialist Acknowledge date Date the task has been performed Acknowledge by User ID who has acknowledged the task

8.3.2 Maintenance of the QlAgility pipettor

QIAGEN recommends that a regular preventive maintenance service is carried out on the QIAgility. The service should be performed by a trained QIAGEN Field Service Specialist. Contact your local QIAGEN Field Service Specialist or your local distributor for more information.

We recommend regular servicing of the pipettor. The recommendation will be displayed by the maintenance schedule. As P-axis movements and the date of pipettor installation are tracked by the software, the software will remind you with a message. In addition, the instrument icon will display a screwdriver as long as the maintenance is due.

The pipettor head maintenance task can only be acknowledged by QIAGEN Field Service Specialist.

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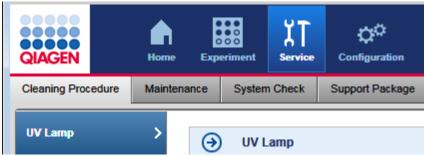
8.3.3 Maintenance of the QIAgility instrument

To remove DNA or RNA from the worktable and to eliminate cross-contamination, the QIAgility must be routinely decontaminated. Proceed as follows:

- Remove all adapters and the tip ejector from the worktable. Wash the adapters in detergent and rinse
 with clean water.
 - For information how to remove the tip ejector, refer to Cleaning the tip ejector.
- 2. Soak the components in ANIGENE (formerly TriGene, Medichem International; for more information, see **www.medi-mark.co.uk**) or one of the tested cleaning agents listed in the section Regular maintenance, for 15–30 min.
- 3. Thoroughly rinse the components in nucleic acid-free water.
- 4. Briefly rinse with ethanol and dry with a soft paper towel.
- 5. Dampen the instrument worktable with ANIGENE or one of the tested worktable cleaning agents listed above, and allow to stand for 15–30 min. Wipe dry with clean paper towels.
- 6. Dampen the worktable with nucleic acid-free water and wipe dry with clean paper towels. Repeat this 3 times.
- 7. Dampen the worktable with ANIGENE or one of the tested worktable cleaning agents listed above, and allow to air dry.
- 8. Replace the tip ejector and close the lid.
- 9. Switch on UV lamp.

8.3.4 Using UV lamp

The UV lamp function is located in the Service environment under the Cleaning Procedure tab.



UV lamp function in "Service" environment

WARNING



UV irradiation hazard [W9]

An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp. Under no circumstances should this interlock be disabled.

To use the UV lamp, follow the instructions below.

- 1. Click on the **Service** environment button. If not yet selected, click on the **Cleaning Procedure** tab header. There is only the UV lamp function.
- Empty the worktable!
 During UV irradiation, the robotic arm moves to ensure the worktable is fully exposed.
- Ensure that the tip ejector is in place!
 The tip ejector must be in place, to prevent exposure to UV irradiation outside the instrument.
- 4. Close the lid.
- 5. There are three options to start the UV lamp operation:
 - a) Click **Start** to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically.
 - b) Click **Start and shutdown application**, to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically and the software will automatically close. The instrument itself does not automatically switch off.
 - c) Click **Start and shutdown computer**, to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically and the computer will automatically shut down. The instrument itself does not automatically switch off.

Note: During UV irradiation, the pipettor head moves. All items must be removed from the worktable to ensure the worktable is fully exposed. The UV lamp does not irradiate certain areas, including the pipettor head, due to construction constraints.

Note: The operating time of the UV lamp can be configured by the administrator in the <u>Settings</u>.

8.3.5 Cleaning the tip ejector

1. Remove the retention by pulling the chain connected to the tip ejector.



2. Remove the tip ejector by carefully pulling the tip chute upwards.



Note: If it is stuck, slightly vibrate while pulling.

- 3. Soak it in ANIGENE (formerly TriGene, Medichem International; for more information, see **www.medi-mark.co.uk**) or one of the tested cleaning agents listed in section Regular maintenance above, for 15–30 min.
- 4. Thoroughly rinse the components in nucleic acid-free water.
- 5. Briefly rinse with ethanol and dry with a soft paper towel.
- 6. Replace the tip ejector by lowering it onto the keeper bracket on the worktable.
- 7. Press the sliding retention pin into the side slot opening.



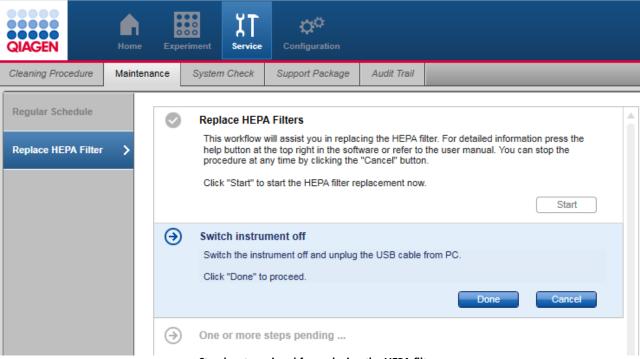
8.3.6 Replacing the HEPA filter

HEPA filters should be replaced on a regular basis. The QlAgility Setup Manager Software will remind you when it is time to replace the HEPA filters. Additionally, the instrument icon will display a screwdriver as long as the maintenance is due.

The software provides a step-by-step wizard to guide you through all steps to replace the HEPA filter. The wizard can be accessed via the **Replace HEPA filter** step in the **Maintenance** tab of the **Service** environment.

Follow the instructions displayed in every step. After you confirm that you have completed the instructions in a step, click **Done** to continue to the next step. The next step will only be displayed once you have successfully completed the instruction and no errors have occurred. While the wizard is active, all other parts of the software become unavailable.

To leave the wizard without completion, click **Cancel**.



Step-by-step wizard for replacing the HEPA filters

To exchange the HEPA filters:

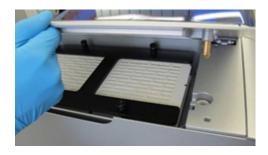
- Click the Service button in the main toolbar to switch to the Service environment. Click on the Maintenance tab and then click on the Replace HEPA filter step.
- 2. Click **Start** to start the procedure now. The next step will be displayed.

3. If the instrument is still switched on, you will be prompted to switch it off. Empty the worktable. If the pipettor head is holding a tip, manually remove it. Then switch off the instrument and disconnect the line power cord from the power outlet. If the instrument is connected via USB cable, unplug the USB cable from the computer. Finally, click **Done** to proceed.

Note: When the instrument is switched off, the pipettor head will drop approximately 70 mm.

4. Lift the HEPA filter cover and remove it.





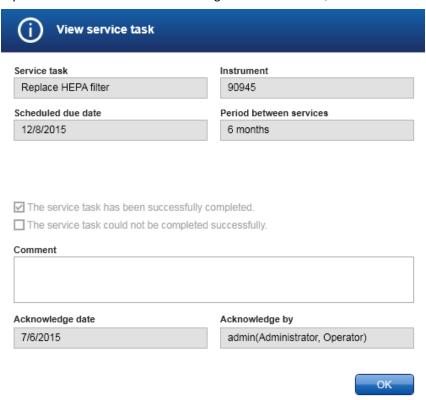
- 5. Unscrew the four thumb screws. See schematic in the step-by-step wizard.
- 6. Remove the H frame.
- 7. Remove the old HEPA filters.
- 8. Insert new HEPA filters with the arrows down.
- 9. Reassemble the H frame.
- 10. Screw the four thumb screws tight.
- 11. Re-assemble the cover onto the instrument.
- 12. Click **Done** to finish the task. The software automatically acknowledges the task as successfully completed. The task will be listed in the **Last service tasks** table of the **Regular Schedule**. In the task details the task will be marked as successfully completed.
- 13. Reconnect the instrument to the computer.
 - If the USB cable had been unplugged from the computer, plug it in again. Then switch on the instrument. It takes some time for the software to recognize the instrument again.

Note: If you leave the wizard by clicking **Cancel**, the task will still be listed in the **Last service tasks** table. However, in the task details, the task will be marked as not successfully completed. The service task remains due, indicated in the **Scheduled service tasks** table and on the instrument icon.



Performed HEPA filter exchange is listed in the "Last service tasks" table.

If you click the **View** button on the right side of the task, the detailed view opens:



Details of the performed HEPA filter task.

Troubleshooting

9 Troubleshooting

If you need to contact QIAGEN Technical Services for technical assistance, note down the steps that occurred leading up to the issue. Alternatively, save or choose the applicable support package from the run, and then send this to QIAGEN Technical Services. This will help the QIAGEN Field Service Specialist resolve the issue.

It is also possible to export support packages, even if the instrument is powered down.

A warning will be issued when an error, such as insufficient tips, is encountered during the run; the error will also be listed in the report.

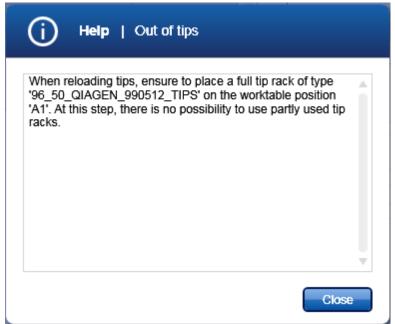
If you receive a software warning or error message that contains a **Help** button, click on it to get more information.

Example:



Run out of tip message during a run.

Click on the **Help** button in the message dialog.

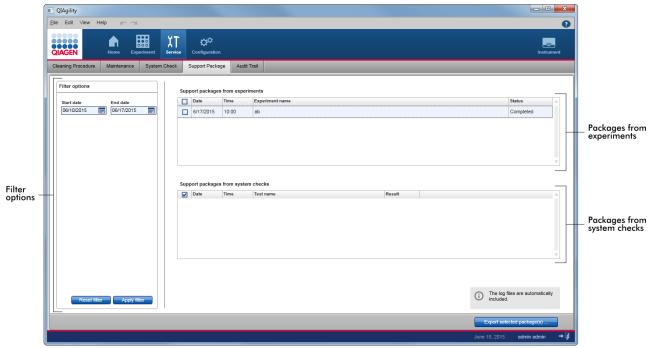


After clicking the messages' Help button.

Click the **Close** button to return to the message dialog.

9.1 Support packages

Support packages are folders containing multiple files that help QIAGEN Technical Services to analyze issues that might have been observed. The function to export support packages is located in the **Support Package** tab in the **Service** environment:



Screen to export support packages.

Use the filter options to search for support packages created between the start and the end date. The right part of the screen consists of two tables that list support packages created from experiment runs and support packages created from performed <u>system checks</u>. Click on **Export selected package(s)** to export the selected support packages to a definable directory.

Support packages are exported for reporting different scenarios, including:

- an issue during a run
- the results of performed system checks
- instrument issues

Note: Support packages older than 90 days will be automatically deleted.

Exporting a support package

- 1. Click on the Support Package tab in the Service environment.
- 2. The **Support Package** screen will be displayed.

- 3. To filter for support packages created within a specific date range:
 - a) Enter a start date and an end date, or use the 🗐 icon to interactively select a date. Ensure that the range covers the date the run or the system test was performed.
 - b) Click on Apply filter.
- 4. All available support package information created within the selected date range will be displayed in the **Support packages from experiments** table and the **Support packages from system checks** table, listing the run experiments and the test results, respectively.
- 5. Activate the check boxes for the support package you want to export.

Activate the check box of the experiment(s) if you want to report issues during run. Activate the check box of the performed system test to report the results.

Note: If you don't select any experiment run or system test result, the support package will contain the currently available log files only.

- 6. Click on Export selected packages.
- 7. The **Select folder** file dialog is opened.
- Select/create the target directory (where the files should be saved in) and click OK.
 Note: The target directory folder must have a read/write access.
- 9. The support package is exported to the selected directory.
- 10. Navigate to the newly created directory and send all files in it to QIAGEN Technical Services.

The directory contains the support packages of the selected experiments and/or system test results, including plug-in specific files, user information, settings, labware database and instrument specific data combined in a zip file. Additionally, current log files and the audit trail are copied to the destination directory (unzipped). Even if the software stops due to a program failure during a run, the support package will be completed when the software is restarted.

The file name of the support package file for an experiment run is generated from a time stamp, instrument and experiment data according to the following naming scheme:

Time stamp Instrument type Instrument serial number Experiment name Experiment status.zip

Log files are created for analysis by QIAGEN Technical Services only.

9.2 System setup

	Comments and suggestions		
Instrument does not run			
a) Power cord not connected	Check the power connection.		
b) Fuse blown	Replace the fuse according to the following procedure:		
	1. Switch off the QIAgility using the main power switch.		

- 2. Unplug the power cord from the power outlet and from the rear of the instrument.
- 3. Use a small flat-bladed screwdriver to remove the fuse-carrier assembly located directly to the right of the area where the cord connector enters into the inlet.
- 4. Replace the fuse. Replace only with type F5A250V.
- 5. Reinsert the fuse-carrier assembly and plug in the power cord.
- 6. Turn on the power and check the instrument for normal operation. If the instrument does not function normally, or continues to blow fuses, unplug the system and contact QIAGEN Technical Services.
- c) Instrument does not initialize on software start

The instrument does not initialize on software start. It initializes just before use, e.g., before calibration or during worktable planning before a run.

Computer software cannot communicate with system

a) USB cable not connected between the computer and the instrument

Check the connection of the USB cable. See section Connecting the computer to the QIAaility.

b) Instrument is not switched on

Switch the instrument on. If the instrument is connected to the computer via the USB cable, the software automatically connects to the instrument. This can take a few seconds

c) A communication problem occurred

If the instructions of a) and b) have already been followed, but the software does not connect to the instrument, restart the computer. If the problem persists, collect the support package and contact QIAGEN Technical Services.

QIAgility Setup Manager Software does not launch

a) QIAgility Setup Manager Software not installed

Install the QIAgility Setup Manager Software. See section Software installation.

b) Old version of Microsoft Windows

The QlAgility Setup Manager Software operates with Windows 7 Professional (32- or 64-bit) and Windows 10 (64-bit). See section Computer.

QIAgility Setup Manager Software cannot be installed

 a) Message during installation: Wrong SiLabs USB driver version has been detected on the computer. The wrong driver version may have been installed by another QIAGEN software, e.g the old QIAgility Software.

If the message shows up, allow the installer to update the driver to the version needed by QlAgility Setup Manager Software. Otherwise, QlAgility Setup Manager Software cannot be installed correctly. However, the other software may become

inoperable with this driver version. It is not possible to have both an old version of QIAgility software and the QIAgility Setup Manager Software installed on the same computer.

b) Message during installation: .NET Framework needs to be installed has been answered with No The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft .NET Framework. This may take some time. If you click **No**, the QIAgility Setup Manager Software will not be installed.

c) Message during installation - Windows
 7 only: Installation of .NET Framework failed.

On computers with Windows 7, ensure that the Windows Service Pack 1 has been installed. To check if the Service Pack 1 has been installed, click the **Windows Start** icon at the bottom left of the **Windows** screen. Then right-click on the entry **Computer** in the right bar and select **Properties** from the appearing context menu. Check that the Service Pack 1 is mentioned in the **Windows edition** section.

d) Message during installation: SQL Server needs to be installed has been answered with No The appropriate version of the Microsoft SQL Server will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft SQL Server. This may take some time. If you click **No**, the www.qiagen.com/ will not be installed.

e) Policy prevents software installation from an Unknown Publisher

Contact your local IT administrator.

f) On Windows 10 only. An update installation fails at step "Stopping services."

On Windows 10 only, when updating from QIAgility Setup Manager Software 1.0 or 1.1. Please note that QIAgility Setup Manager Software version 1.2 is the first version that can be used on Windows 10.

To get the new version installed:

- 1. Take a backup of your experiments, reports and output files.
- 2. Uninstall the previous version.
- 3. Install the new version.

Windows security message shown during the Device Driver Installation Wizard

 a) A Windows security confirmation message is shown during QIAgility Setup Manager Software driver installation: QIAGEN GmbH drivers attempt to be installed Click Install to install the QIAgility driver.

If you do not install the driver software, the QIAgility Setup Manager Software

- a) will not be able to operate the QIAgility if you install the QIAgility Setup Manager Software for the first time, or
- b) might not be able to operate the QIAgility correctly in case of an update installation because an older driver version remains active on the computer.

b) A security warning message is shown during driver installation of the QlAgility Setup Manager Software: Unknown publisher of driver software If the message is shown in the context of an installation of the QlAgility Setup Manager Software, allow to install the driver anyway.

If you do not install the driver software, the QIAgility Setup Manager Software

- a) will not be able to operate the QIAgility if you install the QlAgility Setup Manager Software for the first time, or
- b) might not be able to operate the QIAgility correctly in case of an update installation because an older driver version remains active on the computer.
- c) Policy prevents driver installation from an Unknown Publisher

Contact your local IT administrator.

Device Driver Installation Wizard fails

On Windows 10, the Secure Boot option is used together with UEFI Boot

If you use UEFI Boot, the Secure Boot option must be disabled.

9.3 Operation

Comments and suggestions

Experiment setup

a) The Next button in the Sample/Labware screen is not available Change the output converter to an available one.

An output converter that is not available has been selected.

An adapter for the output plate is not available.

b) Samples in the quick order are not assigned

Samples IDs used in a quick order must be unique.

yellow

c) The Calibrate labware button remains If a protocol uses the same plate/adapter combination with and without a cooling block, the calibration must be completed in the calibration wizard. For more information, refer to Calibrate labware.

possible to add a second output plate if output

d) In PCR Setup with Serial Dilution, it's not If the Rotor Disc 100 is used as input labware, and it should also be used as output labware, the Rotor Disc 100 must be selected the Rotor Disc 100 is used as the input and first, and then another output plate type can be added. If different output labware is added first, you cannot add the Rotor Disc 100 afterward.

e) Reporter is not mapped in Q-Rex v1.1

The reporte/fluorophore map is not aligned with Q-Rex v1.1. Refer to the mapping list below:

- Green: FAM, SYBRGREEN1, SYBRGREENI, FLUORESCEIN, ALEXAFLUOR488
- Yellow: JOE, VIC, HEX, TET, MAX, CALFLUOR, GOLD540, YAKIMAYELLOW
- Orange: ROX, CALFLUORRED610, CY35, TEXASRED, ALEXAFLUOR568
- Red: CY5, QUASAR670, ALEXAFLUOR633, LIGHTCYCLERRED640
- Blue: MARINABLUE, EDANS, BOTHELLBLUE, ALEXAFLUOR350, AMCAX
- Crimson: QUASAR705, ALEXAFLUOR680, LIGHTCYCLERRED705
- f) Labware on the worktable position has not yet been calibrated

The run can only be started if all labware is calibrated on the positions that have been planned for the run. The yellow Calibrate labware button indicates that the labware on the position has not yet been calibrated. Follow the instructions in Starting a run.

or B2 but has not yet been calibrated on that position

g) An additional tip rack is planned on B1 The software plans additional tip racks to support completely using previously started tip racks and to minimize the need of tip reloading during run. If a tip rack has not yet been calibrated on that additional position, the Calibrate labware button is yellow and the run cannot be started. The button will remain yellow even if you decide not to use this position for tips in this run (i.e., you define all tips on this position as unavailable and do not check the box next to Loaded). Click the button to calibrate the tip rack on this position. In general, we recommend that you also calibrate 50 μ l tip racks on worktable positions B1 and B2.

Running out of master mix

- a) Master mix remains in the tube after completion of the run, but insufficient volume of master mix was delivered to the last reactions aliquoted
- Ensure that the height calibration of the master mix tube is set correctly.
- b) Correct initial volume was supplied or made but all master mix is consumed before all reactions are made
- The pipettor may be aspirating larger volumes than expected. Ensure that the volume calibration data are correct and have been configured properly.
- c) Source reagent remains in the tube after completion of the run, but the master mix has run out before all reactions aliquoted

Ensure that the height calibration of all reagent wells used for construction of the master mix is set correctly.

Uneven levels in reaction tubes

a) Air bubbles trapped in sample tubes or master mix tubes

The liquid-level sensor functions equally well on air bubbles or the liquid surface. Centrifuge the sample plate to remove air bubbles.

b) Liquid droplets appear on reaction tubes above the liquid surface

The tip is colliding with the base of the tube causing liquid ejected under pressure to spray when the tip is retracted. Height calibration needs to be raised to prevent tip contact with the tube base during dispense.

c) None of the above

Pipettor is operating erratically and may be clogged. Organize a service for the pipettor.

Defined mix is not created by the QIAgility

a) The reaction/master mix has not been assigned with samples

For the run, the software will create only reaction (master) mixes (with their components like intermediate mixes and single reagents) that have been assigned to samples in the Assignment step.

Software locks or crashes

a) Screen savers and power save modes not disabled on the computer

Disable screen savers and power save modes on the computer.

Z-axis not rising after power has been switched on

a) Power cords not securely plugged in

Ensure that all power cords are securely plugged in, then try switching the instrument on again.

b) An internal fault on the instrument has occurred

Contact QIAGEN Technical Services.

Replicates do not provide high reproducibility

a) Incorrect reagent volumes entered

Insert the correct volumes for each reagent.

b) Less than 5 μ l DNA template used in reactions

It is recommended to use at least 5 μ l DNA template in reactions.

c) Air bubbles trapped in sample tubes

Centrifuge the sample plate to remove air bubbles.

Software is very slow

a) Virus scanners running on the computer

Refer to chapter <u>Computer</u>. Follow the instructions to minimize influence of other processes to the software.

Instrument pipets in mid-air or pipets no liquid

a) Level sensor malfunction

Static electricity on the plasticware may be interfering with operation of the liquid-level sensor. Generate a support package and contact QIAGEN Technical Services.

Liquid in source well, but received "Insufficient Liquid" error message

a) Incorrect tip configuration The instrument may have been configured to pick up tips that

were not present in the Tip Rack Holder. Ensure that tip

configuration is correct.

b) Incorrect Z-calibration If the Z-calibration is too high, the tip cannot reach low enough into the tube to aspirate all the fluid. Ensure that Z-calibration is

correct. Use automatic Z-calibration whenever possible.

c) Physical plate does not match model The software uses models of tubes and wells to translate the selected in software detected liquid surface height into a volume. When the wells or tubes are physically different than the model chosen, the volume

> calculated may be much lower than the actual volume present, causing aspiration steps to fail. Ensure the plate chosen in the

software matches the physical plate.

d) Master mix has been loaded with the The software uses models of tubes to translate the detected required volume, but insufficient liquid surface height into a volume. For highly viscous liquids,

this might result in an "Insufficient Liquid" error, if only the minimum required volume has been loaded. For master mix, load the tubes provided with the QIAGEN kit. They already contain some extra liquid. Or, add an extra volume of 10%.

Error messages recorded in post-run report, but the run was completed

a) Volumes of reagents or samples below the minimum volume which is required for liquid-level sensing.

volume of master mix was detected

If liquid was not detected, an error is recorded in the post-run report. Add an extra 20 μ l volume as required. Alternatively, dilute samples and reagents and pipet larger volumes rather than pipetting small volumes of concentrated solutions.

An error message is received during worktable planning

a) The run cannot be planned, because no suitable labware has been found. Check the labware availability in the Labware Manager (Configuration environment).

The software looks for tube and adapter combinations to place all liquids on the worktable, taking into account the required volumes for reagents, dilutions for standard series and intermediate mixes that need to be created along with the tubes' dead volumes. The software only considers the available labware. If one or more liquids or empty tubes cannot be placed, the software will try another tube/adapter combination. If no combination can be found for placing all required tubes, this error message will appear.

To overcome this issue, check and try the following:

a) Check that you have made tubes available so that all positions on the adapters for M1 and R1 can be used.

Comments and suggestions

- b) Check if different adapters for M1 and R1 are available to support either more small-sized tubes or fewer large-sized tubes.
- c) In the assignment step, reduce the number of assay points on the output labware. If the worktable can be planned, you can try to increase the assay points on the output labware stepwise.

A non-readable error message is received when the software is started

a) Correct plate definitions not installed

Uninstall and then reinstall the software. See section <u>Software</u> installation.

Important: Copy all user files to another location outside **C:** \Users\Public\QIAGEN\QIAgility before uninstalling the software, because they will be removed during the uninstallation process.

An error message is shown on software start that the disc is nearly full

a) Not enough free hard disc capacity available

Follow the instructions in the message and remove files from the hard disc. Otherwise, it will not be possible to start a run.

Output file in additional format is empty (for experiments created with Generic PCR Plug-in only)

a) Targets have not been defined for final reaction (master) mix

Output converters rely on the target definition made in the experiment. If it is missing, the file in the additional format cannot be created and is empty.

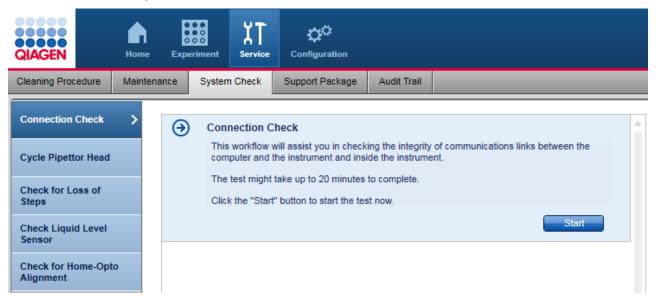
When defining final reaction (master) mix(es) ensure to define all targets. When defining controls ensure to select all targets. When defining standard series that shall be created by QlAgility ensure to define stock concentrations for all available targets; for targets with no stock concentration, enter concentration value 0.

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9.4 Self-diagnostic tools

QlAgility Setup Manager Software provides several different checks to verify whether the most important instrument parts are working correctly. The tests are designed to collect information to help with troubleshooting. They are bundled in the **System Check** tab of the **Service** environment:

Note: The tests are only available if the instrument is connected.



Each check is carried out in a separate step, and the steps are independent from each other, so you can start any test you choose. The checks are built in a step-by-step wizard format. The initial step shows a description of the check, its duration and a **Start** button. Click the **Start** button to begin the check and to display the instructions for the next step. Follow the instructions on the screen. After you have finished, click **Done** to proceed to the next step. To cancel a test, click **Cancel** or **Stop**.

Check name	Description	Duration
Connection Check	Checks the connection between computer and instrument and within the instrument itself	20 min
Cycle Pipettor Head	Checks the cycling of the pipettor head	10–15 min
Check for Loss of Steps	Checks whether the pipettor head movement is working correctly	40–45 min
Check Liquid-Level Sensor	Checks whether the liquid-level detection is working correctly	90 min
Check for Home-Opto Alignment	Checks if the instrument can perform the homing routine correctly	5 min

Important: Before starting a test, read the details first by clicking the link in the table.

Note: These tests should only be performed by experienced users.

To perform a test:

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- 1. Click the **Service** button in the main toolbar and then then click the **System Check** tab to switch to the **System Check** tab of the **Service** environment.
- 2. Click on the step marker of the test you would like to perform. The description of the test is shown.
- 3. Click **Start** to start the test. The next step is displayed.
 - a) Follow the instructions, if any, and then click **Done** to confirm and proceed.
 - b) Or, wait while the instrument performs an automatic step. The software will then proceed to the next step automatically.
 - Repeat this for all steps of the test.
- 4. If the test is finished, the software displays the test result as a last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Or, click to another part in the software for another task.

9.4.1 Connection check

This test checks the communications links between the computer and the instrument and inside the instrument.

- 1. In the step marker, click **Connection Check**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- 3. Wait while the instrument checks the connection links.
- 4. If the test finished, the software displays the test result as a last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**.

If the test failed, save the <u>Support package</u> subsequently. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Or, click to another part in the software for another task.

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9.4.2 Cycle pipettor head

If the QIAgility has been unused for a long period of time, it is recommended to cycle the pipettor head. This helps to distribute the grease evenly.

- 1. In the step marker, click on **Cycle Pipettor** head. The description is shown.
- 2. Click **Start**. The next step is displayed.
- 3. Empty the worktable. Remove the tip ejector, all plates, tubes, adapters, accessories and tips. **Important:** Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument cycles the pipettor head.
- 7. If the task finished, the software displays the last step. The task result is automatically saved.
 Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Click on the **Support Package** tab if you want to export the result, or click to another part in the software for another task.

To cancel the task, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

9.4.3 Check for loss of steps

This test establishes whether the instrument has reliable movement.

- 1. In the step marker, click on **Check for Loss of Steps**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- Empty the worktable. Remove the tip ejector, all plates, tubes, adapters, accessories and tips.
 Important: Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument moves the robotic arm with the pipettor head.
- 7. If the test is finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

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If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click to another part in the software for another task.

9.4.4 Check liquid-level sensor

This test checks that the liquid sensor circuitry is functioning correctly, so that it only triggers when the conductive tip touches the liquid.

- 1. In the step marker, click on Check Liquid-Level Sensor. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- 3. Prepare the worktable as displayed by the software.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument checks the sensor. This will be repeated several times.
- 7. If the test is finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click on another part in the software for another task.

9.4.5 Check for home-opto alignment

This test checks if the instrument can perform the homing routine correctly.

- 1. In the step marker, click on **Check for Home-Opto Alignment**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- Empty the worktable. Remove the tip chute, all plates, tubes, adapters, accessories and tips.
 Important: Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument moves the robotic arm.

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7. If the test finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and is checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click to another part in the software for another task.

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Glossary

10 Glossary

Glossary terms in alphabetical order

Α

Aspirate To draw liquid up into the pipette.

Audit Trail A record of actions. The audit trail tracks user actions and changes to the

instrument.

В

C

Calibration Before the instrument can be used, the locations of work items such as the

tip ejector or labware must be determined. This process is termed calibration. All labware that will be used needs to be calibrated on the

respective worktable positions.

Calibration report The calibration report is a printed document delivered with QIAgility. It

defines the P-axis movements to be performed for a given volume. The

values must be entered when configuring QIAgility.

Conductive Tip QlAgility uses conductive tips to detect the liquid level.

Control Controls are used to verify the PCR process. There are negative controls

(NTC, non-template control) and positive controls.

.csv file A file format, which can be used to import sample data. In general, a .csv

file represents comma separated values (CSV), like in a table, where each line in the file represents a row in the table and a comma (,) represents the

cell border to the next column.

Cycler The thermal cycler (also known as a Thermocycler, PCR Machine or DNA

Amplifier) is a laboratory apparatus used to amplify segments of DNA via the polymerase chain reaction (PCR) process. Rotor-Gene Q is the cycler

developed by QIAGEN.

D

Dispense To distribute the liquid out of the pipette.

Dilution labware For experiments created with the Generic PCR Plug-in, only.

Labware that is used during sample normalization to create predilutions.

Dilution position/well Position/well on the dilution labware/plate.

Е

Expiry Date

The date after which QIAGEN can no longer guarantee that a kit performs

according to its specification.

Extra Volume Additional volume that is needed for pipetting and LLD. The software

automatically calculates the extra volume needed for a run.

F

Final reaction (master) mix A liquid that contains all reagents for a PCR reaction, including target-

specific components, and will be distributed to the output labware.

G

GUI Abbreviation for Graphical User Interface.

Η

HEPA Filter Abbreviation for High Efficiency Particulate Airfilter. Air filter to prevent

airborne contamination underneath the instrument hood.

Home Position The position where the pipettor head moves to reset all axis.

Input liquid For experiments created with the Generic PCR Plug-in, only.

A liquid, e.g., a reagent or water, which will be loaded on the QIAgility for

the run or will be used by the QIAgility to create a mix.

In the **Add liquid** dialog the options **Input liquid** and **Ready-made mix** are available. If the option **Ready-made mix** is selected in the **Mixes** step, a mix with the same name as the liquid is listed automatically. You need to select the option **Ready-made mix**, if the liquid is the final reaction (master) mix. Select the option **Input liquid**, if the liquid should not be listed as a

mix in the Mixes step.

Intermediate mix For experiments created with the Generic PCR Plug-in, only.

A liquid that serves as a generic pre-mix that will be used to create more

specific reaction (master) mixes, e.g., final reaction (master) mixes.

J

Κ

Kit Ready-to-use composition of reagents and consumables for a given

protocol.

L

Labware Library The labware library is delivered with the software and contains all labware

to be used on the QIAgility instrument.

Labware Type Labware is grouped into types such as tubes, 96-well or 384-well plates, to

enhance customer convenience while looking for specific labware.

Lid The instrument lid isolates the worktable, maintaining sample integrity and

acting as a safety mechanism.

Liquid class Liquid classes define the pipetting and tip handling for respective liquid

transfers. Liquid classes are defined by QIAGEN.

Liquid-Level Detection

(LLD)

Ability to determine the filled in volume in the labware.

Liquid type For experiments created with the Generic PCR Plug-in, only.

> The liquid type defines how the liquid will be used. During experiment setup, the software uses the liquid type to present sub-sets of defined liquids

for selection.

Loading instruction For experiments created with the Generic PCR Plug-in, only.

> During worktable setup, printable loading instructions can be created. These list the worktable positions for the labware and the required

minimum volumes of liquids to be loaded.

Log file A log file contains information about (instrument) actions that were

performed.

M

QIAgility User Manual 02/2022 10-4 Master Mix A master mix is a provided liquid that contains all reagents for a PCR

reaction, except target-specific components.

Master Mix Block Special adapter that can be placed on position M1. The Master Mix Block is

intended to hold up to five tubes on the instrument worktable.

Ν

Normalization Based on the imported original sample concentration, samples will be

normalized to reach the chosen target concentration/mass value. Samples

can be normalized based on mass or concentration.

0

Output converter If the output file is going to be saved after the run for import to a

downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, an output converter is needed. The

output converter defines the required file format.

For more detailed information, refer to the section **Run completion** of the respective experiment type, within the section <u>Working with experiments</u>.

Ρ

P-Axis The direction of pipetting piston movement.

Passive reference A passive reference is a fluorescence dye added to the reaction mix to

provide the PCR cycler with the ability to normalize the fluorescence signals

between all wells.

Pipette Order Defines the order of samples, controls and standards on the output plate.

Pipettor Head Part of the instrument that is directly involved in the transfer of liquid.

Pipetting Piston Part of the pipettor head.

Plate Labware with wells that are connected physically and arranged in a two

dimensional way. Plates can be placed in an adapter or as standalone

onto the instrument worktable.

Power switch A button located at the back of the QIAgility. It allows the user to switch the

QlAgility on and off.

Premade Reaction Mix A pooled group of reagents that provides the chemicals necessary for a

PCR reaction. The mix is prepared by a user prior to a run, and is placed

ready to use on the instrument.

Same as Ready-made reaction mix.

Q

QDEF QIAGEN Data Exchange Format. XML files that describe plates as well as

adapters containing tubes. This file format enables the seamless transfer of plates (or adapters with tubes) together with a corresponding list of

samples from one instrument to the next, in a workflow.

An output file in QDEF format can be saved after a run, describing the

output labware. If sample normalization was part of the run, a file

describing the dilution labware can also be created.

A detailed description of the file format can be found at

www.qiagen.com/goto/QIAgility.

R

Reagent A reagent can be combined with other reagents to make a master mix or

added individually to a reaction. Reagents are always dispensed into

reaction wells before samples.

Reagent Block Special adapter that can be placed on position R1. The Reagent Block is

intended to hold up to three different tube types on the instrument

worktable.

Reformatting Reformatting means exclusive pipetting of a certain liquid from one input

container into one or more defined target container.

Replicate Number of identical reactions derived from the same control or standard

sample.

Report A report can be generated after each run and contains information on the

well contents and run settings.

Ready-made reaction mix A liquid that has already been prepared by the user, which contains all

reagents for a PCR reaction, including target-specific components, and is ready to be loaded onto the QIAgility for distribution to the output labware.

Same as Premade reaction mix.

S

Sample Samples are source liquids. Samples may have unique identifiers. Each

sample is used as an ingredient in a final PCR setup.

SBS format Dimensional standard for labware to be used on QIAgility. Abbreviation for

Society for Biomolecular Sciences.

Standard Series A standard series is a dilution series with known concentrations.

Support Packages A support package contains useful information about the last run which is

important when contacting QIAGEN Technical Services for technical

assistance.

Τ

Target The specific nucleic acid sequence to be amplified in a PCR reaction.

Tips are disposables that are used to pipette liquids. They are provided in

the Tip Rack Holder and must be set to available or unavailable.

Tip Disposal Box

The tip disposal box collects used tips at the side of the instrument.

Tip Ejector Used tips are ejected through the tip ejector into a tip disposal box at the

side of the instrument.

Tube Single container for liquids. A tube is usually placed in an adapter onto the

instrument worktable.

U

V

Volume Calibration Volume calibration defines the number of units that the P-axis motor must

move to accurately deliver a given volume of liquid. Refer to <u>Instrument</u>

calibration.

W

Well A hole in a plate that can contain liquids.

Worktable The instrument worktable supports tips racks, adapter plates and tubes.

Χ

XY Calibration The exact xy-position of each item on the worktable has to be calibrated

manually. Refer to <u>Instrument calibration</u>.

Υ

Ζ

Z Calibration The exact height of the tube/well bottoms above the worktable has to be

calibrated. Refer to <u>Instrument calibration</u>.

Appendices

11 Appendices

11.1 Appendix A

11.1.1 Technical data

QIAGEN reserves the right to change specifications at any time.

11.1.2 Environmental conditions

Operating conditions

Power 100–240 V AC, 50–60 Hz, 250 VA

Mains supply voltage fluctuations are not to exceed

±10% of the nominal supply voltages.

Fuse F5A 250 V fuse

Overvoltage categoryll

Air temperature 15–30°C (59–86°F)

Relative humidity* 40–70%

Altitude* Up to 2000 m (6500 ft.)

Place of operation For indoor use only

Pollution degree 2

Environmental class 3K2 (IEC 60721-3-3)

3M2 (IEC 60721-3-3)

Transportation conditions

Air temperature -25°C to 60°C (-13°F to 140°F) in manufacturer's package

Relative humidity Max. 75% (noncondensing)

Storage conditions

Air temperature 15°C to 30°C (59°F to 86°F) in manufacturer's package

Relative humidity Max. 75% (noncondensing)

^{*}Conditions under which equipment is electrically safe to operate

Mechanical data and hardware features

Dimensions Width: 54 cm (21.3 in.)

Depth: 63 cm (24.8 in.) without cables

72 cm (28.3 in.) with cables

Height: 45 cm (17.7 in.) lid closed

86 cm (33.9 in) lid open

Mass Instrument (unpacked): 51 kg (112.4 lb.) standard configuration

Accessories (unpacked): 8 kg (17.6 lb.)

Capacity Up to 96 samples per run

11.1.3 Waste Electrical and Electronical Equipment

This section provides information about disposal of waste electrical and electronic equipment by users.

The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.



Recycling can be provided by QIAGEN upon request at additional cost. In the European Union, in accordance with the specific WEEE recycling requirements and where a replacement product is being supplied by QIAGEN, free recycling of its WEEE-marked electronic equipment is provided.

To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

11.1.4 FCC declaration

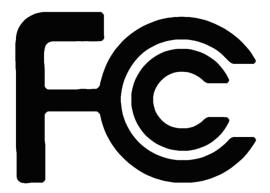
The "United States Federal Communications Commission" (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

This device complies with part 15 of the FCC. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This Class A digital apparatus complies with Canadian ICES-003.

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.



QIAGEN GmbH, Germany is not responsible for any radio television interference caused by unauthorized modifications of this equipment or the substitution or attachment of connection cables and equipment other than those specified by QIAGEN GmbH, Germany. The correction of interference caused by such unauthorized modification, substitution or attachment will be the responsibility of the user.

11.1.5 Declaration of conformity

Name and address of the legal manufacturer:

QIAGEN GmbH QIAGEN Strasse 1 40724 Hilden Germany

An up-to-date declaration of conformity can be requested from QIAGEN Technical Services.

11.2 Appendix B

11.2.1 Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications. All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

11.3 Appendix Kit Compatibility

The following QIAGEN kits are supported by the first release of the QIAgility Setup Manager Software Gene Expression Plug-in.

Product	Cat. no.
QuantiTect [®] Probe PCR Kit (1000 x 50 μl)	204345
QuantiTect Probe PCR Kit (200)	204343
QuantiTect Multiplex PCR NoRox Kit (1000)	204745
QuantiTect Multiplex PCR Kit (1000)	204545
QuantiTect SYBR $^{ ext{@}}$ Green PCR Kit (1000 x 50 μ l)	204145
QuantiTect SYBR Green PCR Kit (200)	204143
QuantiFast® SYBR Green PCR Kit (400)	204054
QuantiFast SYBR Green PCR Kit (4000)	204057
QuantiFast SYBR Green PCR Kit (2000)	204056
QuantiFast Multiplex PCR +R-Kit (400)	204754
QuantiFast Multiplex PCR +R-Kit (2000)	204756
QuantiFast Probe PCR Kit (400)	204254
QuantiFast Probe PCR Kit (2000)	204256
Rotor-Gene SYBR Green PCR Kit (2000)	204076
Rotor-Gene SYBR Green PCR Kit (400)	204074
Rotor-Gene Multiplex PCR Kit (400)	204774
Rotor-Gene Multiplex PCR Kit (80)	204772
Rotor-Gene Probe PCR Kit (400)	204374
QuantiNova® Probe PCR Kit (500)	208254
QuantiNova Probe PCR Kit (100)	208252
QuantiNova Probe PCR Kit (2500)	208256
QuantiNova SYBR Green PCR Kit (100)	208052
QuantiNova SYBR Green PCR Kit (500)	208054
QuantiNova SYBR Green PCR Kit (2500)	208056

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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The following QIAGEN kits are supported by the first release of the QIAgility Setup Manager Software Pathogen Detection Plug-in.

Cat. no.
211354
211352
211011
211031
208652
208654
211013
211015
211033
211035

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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11.4 Appendix C Consignes de sécurité

Avant d'utiliser le QlAgility il est impératif de lire attentivement ce manuel et de porter une attention particulière aux consignes de sécurité. Afin de garantir un fonctionnement de l'appareil en toute sécurité et de maintenir l'appareil en bon état de marche, il est impératif de suivre les instructions et consignes de sécurité fournies dans le présent manuel d'utilisation.

Les types d'informations de sécurité suivants sont fournis tout u long du manuel.

AVERTISSEMENT



Le terme **AVERTISSEMENT** signale des situations risquant d'entraîner des accidents corporels dont l'utilisateur, ou d'autres personnes, pourraient être victime.

Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

ATTENTION



Le terme **ATTENTION** signale des situations risquant d'entraîner des détériorations de l'appareil ou de tout autre matériel.

Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Les conseils donnés dans ce manuel ont pour but de venir compléter les exigences de sécurité habituelles en vigueur dans le pays de l'utilisateur, et non de s'y substituer.

11.4.1 Utilisation appropriée

AVERTISSEMENT/ ATTENTION



Risque d'accident corporel et de détérioration du matériel [W1]

L'utilisation inappropriée du QIAgility peut entraîner des accidents corporels ou une détérioration de l'appareil.

L'utilisation du QIAgility est exclusivement réservée au personnel qualifié ayant été convenablement formé.

La maintenance du QIAgility doit uniquement être effectuée par des spécialistes du Service Après-Vente QIAGEN.

AVERTISSEMENT/ ATTENTION



Risque de surchauffe [W2]

Afin de garantir une bonne ventilation, laisser un dégagement d'au moins 10 cm sur les côtés et à l'arrière du QlAgility. Les fentes et les ouvertures qui assurent la ventilation de l'appareil ne doivent pas être obstruées.

AVERTISSEMENT/ ATTENTION



Risque d'accident corporel et de détérioration du matériel [W3]

Le QlAgility est trop lourd pour être soulevé par une seule personne. Afin d'éviter tout accident corporel et toute détérioration du matériel, ne pas soulever l'appareil seul.

AVERTISSEMENT/ ATTENTION



Risque d'accident corporel et de détérioration du matériel [W3]

Le QlAgility est trop lourd pour être soulevé par une seule personne. Afin d'éviter tout accident corporel et toute détérioration du matériel, ne pas soulever l'appareil seul.

AVERTISSEMENT/ ATTENTION



Atmosphère explosive [W5]

Le QIAgility n'est pas conçu pour être utilisé dans une atmosphère explosive.

AVERTISSEMENT/ ATTENTION



Risque d'explosion [W6]

Le QIAgility a été conçu pour l'utilisation des réactifs et substances fournis par les kits QIAGEN.

L'utilisation de réactifs et de substances autres que celles indiquées peut entrainer un risque d'incendie ou d'explosion.

ATTENTION



Détérioration de l'appareil [C1]

La lumière directe du soleil peut décolorer les pièces de l'appareil et abîmer les éléments en plastique.

Tenir le QIAgility à l'abri de la lumière directe du soleil.

ATTENTION



Détérioration de l'appareil [C2]

Éviter de renverser de l'eau ou des produits chimiques sur le QIAgility. La détérioration de l'appareil dû au déversement de liquides annule la garantie.

AVERTISSEMENT



Risque d'accident corporel [W7]

Pendant les étapes de calibration des positions, le bras robotisé se déplace alors que le capot de l'appareil est relevé. Ne jamais actionner ou cliquer sur aucunes touches tant que des parties du corps se trouvent dans l'enceinte de l'appareil.

En cas d'urgence, éteindre le QIAgility à l'aide de l'interrupteur d'alimentation situé à l'arrière de l'appareil et débrancher le cordon d'alimentation de la prise de courant.

11.4.2 Sécurité électrique

Avant l'entretien, débrancher le cordon d'alimentation de la prise de courant.

AVERTISSEMENT/ ATTENTION



Danger électrique [W8]

Toute interruption du conducteur de protection (conducteur de terre/de masse) à l'intérieur ou à l'extérieur de l'appareil ou toute déconnexion de la borne du conducteur de protection est susceptible de rendre l'appareil dangereux. Toute interruption intentionnelle est interdite.

Tensions mortelles à l'intérieur de l'appareil.

Lorsque l'appareil est relié à l'alimentation, les bornes peuvent être sous tension et l'ouverture de capots de l'appareil ou le retrait de pièces risque d'exposer des éléments sous tension.

AVERTISSEMENT/ ATTENTION



Danger d'irradiation UV [W9]

Un dispositif électronique de verrouillage empêche la lampe UV de fonctionner tant que le capot de l'appareil est ouvert. Ce dispositif ne doit en aucun cas être désactivé.

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Afin que le QlAgility fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants:

- Le cordon d'alimentation de l'appareil doit être branché sur une prise d'alimentation munie d'un conducteur de protection (terre/masse).
- Ne pas régler ni remplacer les pièces internes à l'instrument.
- Ne pas faire fonctionner l'appareil si des capots ou des pièces ont été retirés.
- En cas de déversement de liquides à l'intérieur de l'appareil, débrancher celui-ci de la prise d'alimentation et contacter les Services techniques de QIAGEN.

Si l'appareil présente un danger électrique, empêcher le reste du personnel de s'en servir et contacter les Services Techniques de QIAGEN. L'appareil peut présenter un danger électrique dans les cas suivants:

- Le cordon d'alimentation présente des signes de détérioration.
- L'appareil a été stocké pendant une longue période dans des conditions non conformes à celles énoncées dans l'Annexe A.
- L'appareil a subi des chocs sévères durant le transport.

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11.4.3 Sécurité biologique

Les spécimens et les réactifs contenant des matières provenant d'êtres humains doivent être considérés comme potentiellement infectieux. Utilisez des procédures de laboratoire sûres comme décrit dans des publications telles que Biosafety in Microbiological and Biomedical Laboratories, HHS (www.cdc.gov/od/ohs/biosfty/biosfty.htm).

11.4.3.1 Échantillons

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store and dispose of such samples according to the required safety regulations.

AVERTISSEMENT



Samples containing infectious agents [W10]

Certains échantillons utilisés avec cet appareil peuvent contenir des agents infectieux. Manipulez ces échantillons avec la plus grande précaution et conformément aux règles de sécurité nécessaires. Portez toujours des lunettes de protection, deux paires de gants et une blouse de laboratoire.

La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que l'environnement de travail est sûr, que les opérateurs de l'appareil sont convenablement formés et ne sont pas exposés à des niveaux dangereux d'agents infectieux comme cela est défini dans les fiches techniques santé-sécurité (SDS) ou dans les documents de l'OSHA*, de l'ACGIH† ou du COSHH‡ applicables.

L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

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^{*} OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).

[†] ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).

[‡] COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

11.4.4 Produits chimiques

AVERTISSEMENT



Produits chimiques [W11]

Certains produits chimiques utilisés avec cet appareil peuvent être dangereux ou le devenir après l'exécution du cycle du protocole.

Toujours porter des lunettes de protection, des gants et une blouse de laboratoire.

La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que le lieu de travail environnant est sûr et que les opérateurs de l'appareil ne sont pas exposés à des niveaux dangereux de substances (chimiques ou biologiques) toxiques comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA*, de l'ACGIH[†] ou du COSHH[‡] applicables.

L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

- * OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).
- † ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).
- [‡] COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

11.4.4.1 Vapeurs toxiques

Si vous travaillez avec des solvants volatils ou des substances toxiques, vous devez disposer d'un système de ventilation de laboratoire efficace afin d'évacuer les vapeurs qui peuvent être générées.

11.4.5 Traitement des déchets

Le matériel en plastique usagé peut contenir des produits chimiques dangereux. Ces déchets doivent être convenablement collectés et mis au rebut conformément aux réglementations de sécurité locales.

Pour plus d'informations sur la mise au rebut du QIAgility, voir l'Annexe A.

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11.4.6 Dangers mécaniques

AVERTISSEMENT



Pièces mobiles [W12]

Éviter le contact avec les pièces en mouvement lorsque le QlAgility est en marche. Ne jamais placer les mains sous le bras robotisé lorsque celuici s'abaisse. Ne pas tenter de déplacer des plaques ou des tubes lorsque le bras robotisé est en marche.

AVERTISSEMENT



Pièces mobiles [W15]

Pour éviter tout contact avec des pièces en mouvement pendant le fonctionnement du QIAgility, l'appareil doit être utilisé avec le couvercle fermé.

Si le capteur de couvercle intégré ne fonctionne pas correctement, prendre contact avec les services techniques de QIAGEN.

ATTENTION



Détérioration de l'appareil [C3]

N'appliquer aucune force manuelle sur le bras Y (c'est-à-dire la tête de pipetage).

En cas de déplacement manuel accidentel du bras Y, il est fortement recommandé de redémarrer le système.

Afin que le QIAgility fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants

- Utiliser uniquement les tips et les supports de tips recommandés.
- Dans la mesure du possible, maintenir le capot de l'appareil fermé.
- Lors de la calibration des plaques capot ouvertes, toujours se tenir à l'écart de l'appareil au moment des réglages. Le dispositif de verrouillage est désactivé en mode de calibration. Par conséquent, ne pas cliquer avec la souris ni appuyer sur une des touches du clavier lors de l'observation de l'inspection visuelle de la calibration des plaques. Attendre l'arrêt complet du bras robotisé pour déplacer une plaque ou un tube.
- Ne rien poser sur le bras robotisé.

Avant de retirer des plaques ou des tubes de la table de travail, sélectionner « Pause » dans la barre d'outils du logiciel QIAgility pour mettre l'appareil en mode de pause. Attendre l'arrêt complet du bras robotisé. Cela peut prendre jusqu'à 10 s.

11.4.7 Sécurité relative à la maintenance

Procéder à la maintenance comme décrit à la <u>Maintenance procedures</u>. QIAGEN facture les réparations rendues nécessaires suite à une maintenance inappropriée.

AVERTISSEMENT/ ATTENTION



Risque d'accident corporel et de détérioration du matériel [W13]

Effectuer uniquement la maintenance spécifiquement décrite dans ce manuel.

AVERTISSEMENT/ ATTENTION



Risque de décharge électrique [W14]

Ne pas ouvrir les panneaux du QlAgility.

Risque d'accident corporel et de détérioration du matériel

Effectuer uniquement la maintenance spécifiquement décrite dans ce manuel.

ATTENTION



Détérioration de l'appareil [C4]

Ne pas utiliser de solvants ni de réactifs contenant des acides, des bases ou des composés abrasifs pour nettoyer le QlAgility.

ATTENTION



Détérioration du capot du QIAgility [C5]

Ne pas utiliser de solvants ni de réactifs contenant des acides, des bases ou des composés abrasifs pour nettoyer le capot de l'appareil. L'alcool peut endommager le capot. Employer uniquement de l'eau distillée.

ATTENTION



Détérioration des panneaux latéraux du QIAgility [C6]

Ne jamais nettoyer le capot ou les panneaux latéraux de l'appareil à l'alcool ou avec une solution à base d'alcool.

ATTENTION

Réinstallation du QIAgility [C7]

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La pièce la plus importante de l'emballage est le support en bois qui immobilise le bras Y. Ce support doit être réutilisé si vous déplacez le QIAgility.

11.4.8 Symboles sur le QIAgility

Symbole	Emplacement	Description
\triangle	Table de travail et près de la plaque signalétique	Symbole de mise en garde
	Table de travail et près de la sortie des tips	Symbole de danger biologique
	Table de travail	Symbole de risque d'écrasement
CE	Plaque signalétique à l'arrière de l'appareil	Marque CE
C Us	Plaquette à l'arrière de l'appareil	Marquage CSA pour le Canada et les Etats-Unis
	Plaquette à l'arrière de l'appareil	Fabricant légal
X	Plaque signalétique à l'arrière de l'appareil	Symbole DEEE
F©	Plaque signalétique à l'arrière de l'appareil	Label FCC de la Federal Communications Commission des États-Unis
	Plaque signalétique à l'arrière de l'appareil	Marque RCM (antérieurement marque C-Tick) pour l'Australie (identifiant du fournisseur N17965)
25)	Plaque signalétique à l'arrière de l'appareil	Label RoHS pour la Chine (restriction de l'utilisation de certaines substances dangereuses dans l'équipement électrique et électronique))
[]i	À l'arrière de l'appareil	Consulter les consignes d'utilisation

11.5 Appendix D Sicherheitshinweise

Lesen Sie dieses Handbuch sorgfältig durch, bevor Sie den QlAgility benutzen. Beachten Sie dabei insbesondere die Sicherheitshinweise. Die Gebrauchsanweisungen und Sicherheitshinweise im Handbuch müssen befolgt werden, um einen sicheren Betrieb des Geräts zu gewährleisten und das Gerät in einem sicheren Zustand zu erhalten. In diesem Handbuch werden die folgenden Kategorien von Sicherheitshinweisen verwendet:

WARNUNG



Der Begriff "WARNUNG" ("WARNING") weist Sie auf Situationen hin, in denen eine Verletzungsgefahr für Sie selbst oder andere Personen besteht.

Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

ACHTUNG



Der Begriff "ACHTUNG" ("CAUTION") weist Sie auf Situationen hin, in denen das Gerät oder andere Geräte beschädigt werden könnten.

Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

Die in diesem Handbuch enthaltenen Hinweise stellen eine Ergänzung und keinen Ersatz der üblichen Sicherheitsanforderungen dar, die im jeweiligen Land gelten.

11.5.1 Sachgemäße Handhabung

WARNUNG/ ACHTUNG



Verletzungsgefahr und Beschädigung des Geräts [W1]

Die unsachgemäße Bedienung des QIAgility kann zu einer Verletzung des Benutzers oder zur Beschädigung des Geräts führen. Der QIAgility darf nur durch qualifiziertes Personal, das entsprechend geschult wurde, bedient werden. Die Instandhaltung des QIAgility darf nur durch einen Außendienst-Mitarbeiter des QIAGEN Service durchgeführt werden.

WARNUNG/ ACHTUNG



Überhitzungsgefahr [W2]

Vergewissern Sie sich, dass zwischen Wand und QIAgility (Rückseite und Seitenteile) ein Mindestabstand von 10 cm eingehalten wird. Das gewährleistet eine ausreichende Belüftung des Gerätes. Die Lüftungsschlitze und Öffnungen,

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die für die Be- und Entlüftung des QIAgility bestimmt sind, dürfen nicht zugedeckt werden.

WARNUNG/ ACHTUNG

Verletzungsgefahr und Beschädigung des Geräts [W3]



Der QlAgility ist zu schwer, um von einer einzelnen Person gehoben zu werden. Zur Vermeidung einer Verletzung oder Beschädigung des Gerätes heben sie das Gerät nicht alleine an.

WARNUNG/ ACHTUNG

Verletzungsgefahr und Beschädigung des Geräts [W4]

Der QlAgility darf auf keinen Fall während des Betriebs fortbewegt werden.

WARNUNG/ ACHTUNG

Explosionsfähige Atmosphären [W5]



Der QIAgility ist nicht für den Gebrauch in explosionsfähigen Atmosphären vorgesehen.

WARNUNG/ ACHTUNG

Explosionsgefahr [W6]



Der QlAgility ist ausschließlich mit Reagenzien und Substanzen aus den QlAGEN Kits zu benutzen. Die Benutzung von anderen Reagenzien oder Substanzen kann Feuer oder eine Explosion auslösen.

ACHTUNG

Geräteschäden [C1]



Direktes Sonnenlicht kann zum Ausbleichen von Geräteteilen führen und Schäden an Kunststoffteilen verursachen. Der QIAgility darf nicht direkter Sonneneinstrahlung ausgeliefert werden.

ACHTUNG

Beschädigung des Geräts [C2]

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Vermeiden Sie es, Wasser oder Chemikalien auf der Oberfläche des QlAgility zu verschütten. Durch verschüttete Chemikalien oder verschüttetes Wasser verursachte Geräteschäden sind nicht durch die Garantie abgedeckt.

WARNUNG

Verletzungsgefahr [W7]



Bei der Kalibrierung der Positionen bewegt sich der Roboterarm bei geöffneter Gerätehaube. Klicken Sie nicht auf die Maus und drücken Sie nicht auf eine Taste der Tastatur, während sich Ihre Hände oder andere Körperteile im Arbeitsbereich des Roboterarms befinden.

Schalten Sie im Notfall den QlAgility aus (der Netzschalter befindet sich auf der Geräterückseite), und ziehen Sie den Netzstecker aus der Steckdose.

11.5.2 Elektrische Sicherheit

Ziehen Sie das Netzanschlusskabel aus der Steckdose, bevor Sie Wartungsarbeiten am Gerät vornehmen.

WARNUNG

Gefahr durch Stromschlag [W8]



Jede Unterbrechung des Schutzleiters (Erdungs- bzw. Masseleiter) im Gerät oder außerhalb des Geräts und jede Abtrennung des Schutzleiters am Anschluss der Netzleitung erhöht die Gefahr eines Stromschlags. Eine absichtliche Unterbrechung der Schutzleiterverbindung ist verboten.

Gefährliche Spannung im Gerät

Wenn das Gerät an die Stromversorgung angeschlossen ist, sind die Anschlussstellen spannungsführend. Durch das Öffnen der Abdeckungen oder das Entfernen von Gehäuseteilen können spannungsführende Komponenten freigelegt werden.

WARNUNG

Gefahr durch UV-Strahlung [W9]



Eine elektronisch gesteuerte Verriegelung stellt sicher, dass die Gerätehaube geschlossen sein muss, bevor die UVLampe eingeschaltet werden kann. Diese Verriegelung sollte auf keinen Fall deaktiviert werden.

Um einen zufriedenstellenden und sicheren Betrieb des QIAgility zu gewährleisten, befolgenden Sie bitte die nachstehenden Richtlinien:

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- Das Netzkabel muss an eine Wechselstrom-Steckdose mit Schutzleiter (Erdungs-/ Masseleiter) angeschlossen werden.
- Nehmen Sie im Geräteinneren keine Einstellungen an Teilen vor und wechseln Sie keine Teile aus.
- Nehmen Sie das Gerät nicht in Betrieb, wenn Abdeckungen oder Teile entfernt worden sind.
- Falls Flüssigkeit auf dem Gerät verschüttet wird und in das Gerät läuft, dann schalten Sie es sofort aus, trennen Sie es von der Netzspannung (Stecker ziehen!) und setzen Sie sich mit dem Technischen Service von QIAGEN in Verbindung.

Falls die elektrische Sicherheit bei der Bedienung des Geräts nicht mehr gewährleistet werden kann, muss das Gerät gegen unbefugte oder unabsichtliche Benutzung gesichert werden. Kontaktieren Sie anschließend den Technischen Service von QIAGEN. Die elektrische Sicherheit des Geräts ist nicht mehr gegeben, wenn:

- das Netzkabel beschädigt ist;
- das Gerät längere Zeit unter ungünstigen Bedingungen, d. h. unter anderen Bedingungen als in Anhang A angegeben, gelagert wurde;
- das Gerät unsachgemäß transportiert worden ist.

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11.5.3 Biologische Sicherheit

Bei Substanzen und Reagenzien, die humanes Untersuchungsmaterial enthalten, sollte immer von einer möglichen Infektionsgefahr ausgegangen werden. Wenden Sie nur sichere Laborverfahren an, wie sie z. B. in Veröffentlichungen wie Biosafety in Microbiological and Biomedical Laboratories (HHS, www.cdc.gov/biosafety/publications) beschrieben werden.

11.5.3.1 Proben

Proben können infektiöse Erreger enthalten. Sie sollten sich der Gesundheitsgefahr bewusst sein, die von diesen Erregern ausgeht, und derartige Proben gemäß den erforderlichen Sicherheitsbestimmungen handhaben, lagern und entsorgen.

WARNUNG

Proben, die infektiöse Erreger enthalten [W10]



Einige Proben, die mithilfe dieses Geräts verarbeitet werden, können infektiöse Erreger enthalten. Gehen Sie beim Umgang mit diesen Proben mit der größtmöglichen Vorsicht und gemäß den erforderlichen Sicherheitsbestimmungen vor.

Tragen Sie immer eine Schutzbrille, zwei Paar Laborhandschuhe und einen Laborkittel.

Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist und die Bediener des Geräts ausreichend geschult sind. Außerdem dürfen die Grenzwerte in Bezug auf infektiöse Erreger, die in den entsprechenden Sicherheitsdatenblättern (SDS) oder den Vorschriften der OSHA,* ACGIH† oder COSHH‡ festgelegt sind, nicht überschritten werden.

Beim Betrieb des Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze zu Gesundheitsschutz und Sicherheit am Arbeitsplatz auf übernationaler, nationaler und regionaler Ebene eingehalten werden.

- * OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)
- [†] ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika)
- [‡] COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

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11.5.4 Chemikalien

WARNUNG

Gefährliche Chemikalien [W11]



Einige Chemikalien, die mit diesem Gerät verwendet werden, können gefährlich sein oder nach Beendigung eines Protokolllaufs gefährlich werden.

Tragen Sie immer eine Schutzbrille, Laborhandschuhe und einen Laborkittel.

Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist. Auch dürfen die Grenzwerte in Bezug auf infektiöse Erreger, die in den entsprechenden Sicherheitsdatenblättern (SDS) oder den Vorschriften der OSHA*, ACGIH† oder COSHH‡ festgelegt sind, nicht überschritten werden.

Beim Betrieb des Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze zu Gesundheitsschutz und Sicherheit am Arbeitsplatz auf übernationaler, nationaler und regionaler Ebene eingehalten werden.

- * OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)
- † ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika)
- [‡] COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

11.5.4.1 Giftige Dämpfe

Alle Arbeiten mit flüchtigen Lösungsmitteln oder toxischen Substanzen müssen unter einem funktionierenden Laborabzugssystem durchgeführt werden, damit die möglicherweise entstehenden Dämpfe abziehen können.

11.5.5 Abfallentsorgung

Benutzte Kunststoff-Laborartikel können gefährliche Chemikalien enthalten. Derartige Abfälle müssen gemäß den geltenden regionalen Sicherheitsbestimmungen gesammelt und entsorgt werden.

Beachten Sie bei der Entsorgung des QIAgility die gesetzlichen Bestimmungen zur Entsorgung von Elektround Elektronik-Altgeräten (siehe Anhang A).

11.5.6 Gefahren durch mechanische Teile

WARNUNG

Sich bewegende Geräteteile [W12]



Vermeiden Sie jeglichen Kontakt mit sich bewegenden Geräteteilen, während der QlAgility in Betrieb ist. Auf gar keinen Fall dürfen sich Ihre Hände unter dem Roboterarm befinden, wenn dieser herunterfährt. Versuchen Sie nicht, Reaktionsgefäße oder Platten zu entnehmen oder zu bewegen, während der Roboterarm arbeitet.

WARNUNG

Sich bewegende Geräteteile [W15]



Um einen Kontakt mit sich bewegenden Teilen beim Betrieb des QIAgility zu vermeiden, darf das Gerät nur mit geschlossenem Deckel betrieben werden.

Wenn der integrierte Deckelsensor nicht ordnungsgemäß funktioniert, wenden Sie sich an den Technischen Service von QIAGEN.

ACHTUNG

Beschädigung des Geräts [C3]



Bewegen Sie den Y-Arm (den Pipettierkopf) nicht von Hand.

Wir empfehlen dringend, einen Neustart des Systems durchzuführen, falls der Y-Arm versehentlich von Hand bewegt wurde.

Um einen zufriedenstellenden und sicheren Betrieb des QlAgility zu gewährleisten, befolgen Sie bitte die nachstehenden Richtlinien:

- Verwenden Sie nur die empfohlenen Pipettenspitzen und -Racks.
- Halten Sie die Gerätehaube möglichst immer geschlossen.
- Wenn bei geöffneter Gerätehaube die Plattenpositionen kalibriert werden, achten Sie darauf, dass Sie nicht in den Arbeitsbereich des Roboterarms kommen, wenn Sie Justierungen vornehmen. Im Kalibrier-Modus ist die Verriegelung der Gerätehaube deaktiviert. Achten Sie deshalb darauf, nicht mit der Maus zu klicken oder eine der Bedienungstasten auf der Tastatur zu drücken, wenn Sie die Kalibrierungen der Plattenpositionen überprüfen. Warten Sie, bis die Bewegungsabläufe des Roboterarms beendet sind, bevor Sie Reaktionsgefäße oder Platten entnehmen oder bewegen.
- Stellen Sie keine Gegenstände auf den Roboterarm.

Wählen Sie die Option "Pause" in der Werkzeugleiste der QlAgility Software, um das Gerät anzuhalten, bevor Sie Reaktionsgefäße oder Platten von der Arbeitsplattform entfernen. Warten Sie, bis der Roboterarm die gerade ablaufende Bewegung beendet hat – dies kann bis zu 10 Sekunden dauern.

11.5.7 Sicherheitshinweise – Wartungsarbeiten

Führen Sie alle Wartungsarbeiten gemäß den Anweisungen in Abschnitt durch. QIAGEN stellt alle Reparaturen in Rechnung, die nachweislich auf eine inkorrekte Wartung zurückzuführen sind.

WARNUNG/ ACHTUNG

Verletzungsgefahr und Beschädigung des Geräts [W13]



Führen Sie nur Wartungsarbeiten durch, die ausdrücklich in dieser Gebrauchsanweisung beschrieben sind.

WARNUNG/ ACHTUNG

Gefahr durch Stromschlag [W14]

Öffnen Sie keine Gehäuseteile des QIAgility.



Verletzungsgefahr und Beschädigung des Geräts

Führen Sie nur Wartungsarbeiten durch, die ausdrücklich in dieser Gebrauchsanweisung beschrieben sind.

ACHTUNG

Beschädigung des Geräts [C4]



Verwenden Sie weder Lösungsmittel noch Reagenzien, die Säuren, Laugen oder Abrasivstoffe enthalten, um das QlAgility zu reinigen.

ACHTUNG

Beschädigung der QIAgility Gerätehaube [C5]



Verwenden Sie weder Alkohol noch Lösungsmittel oder Reagenzien, die Säuren, Laugen oder Abrasivstoffe enthalten, um die QlAgility Haube zu reinigen. Alkohol schädigt die Gerätehaube. Verwenden Sie zum Reinigen der Gerätehaube ausschließlich destilliertes Wasser.

ACHTUNG

Beschädigung der QIAgility Seitenwände [C6]



Reinigung sie niemals die Gerätehaube und Seitenwände mit Alkohol oder alkoholhaltigen Lösungen.

ACHTUNG



Standortwechsel des QIAgility [C7]

Der wichtigste Bestandteil der Verpackung ist die Holzstütze, die den Y-Arm fixiert. Diese Stütze muss verwendet werden, wenn der QlAgility an einen anderen Standort verlagert wird.

11.5.8 Symbole auf dem QIAgility

Symbol	Ort	Beschreibung
\triangle	Arbeitsplattform und nähe Typenschild	Symbol "Achtung"
	Auf der Arbeitsplattform und nähe "Tip ejector" Ausgang	Symbol "Biogefährdung"
	Arbeitsplattform	Symbol "Quetschgefahr"
C€	Typenschild auf der Rückseite des Instruments	CE-Kennzeichnung
⊕ US	Plakette auf der Rückseite des Gerätes	CSA-Zeichen für Kanada und die USA
	Typenschild auf der Rückseite des Instruments	Hersteller i. S. d. Gesetzes
Z	Typenschild auf der Rückseite des Instruments	WEEE-Kennzeichnung (gemäß europäischer Richtlinien bzw. Elektround Elektronik-Altgeräte- Verordnung)
F©	Typenschild auf der Rückseite des Instruments	FCC-Kennzeichen der Federal Communications Commission der Vereinigten Staaten
	Typenschild auf der Rückseite des Instruments	RCM-Kennung (ehemals C-Tick-Kennzeichen) für Australien (Herstellerkennung: N17965)
25	Typenschild auf der Rückseite des Instruments	Markierung gemäß RoHSRichtlinie für China (Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)
[]i	Auf der Rückseite des Instruments	Bedienungsanleitung beachten

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11.6 Appendix F

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QlAgility User Manual 02/2022

11.6.2 Version history

Date R5 05/2018	Description Previous version
R6 02/2022	Updated the Normalization and Serial Dilution workflow screen to include Kit Information. Updated the Symbols on the QIAgility section. Updated the Environmental conditions section. Added the Sample to output include premix on the Generic PCR Liquid Classes. Updated the weight information of the QIAgility. Updated the laptop specifications on the Computer section. Added a note on multiple sections to explain the new cursor

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