appliedbiosystems

QuantStudio™ Absolute Q™ Digital PCR System

INSTALLATION, USE, AND MAINTENANCE

Catalog Numbers A52864

Publication Number MAN0025621

Revision C.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0025621

Revision	Date	Description
C.0	29 September 2021	• Added an appendix documenting the use of Security, Auditing, and E-signature (SAE) v2.2 software with QuantStudio™ Absolute Q™ Digital PCR Software (Appendix C, "Use the software with Security, Auditing, and E-signature (SAE) v2.2"). • Updated the "Overview of the software" on page 9 to include Security, Auditing, and E-signature (SAE) v2.2. • Added "QuantStudio™ Absolute Q™ Digital PCR Software security" on page 15 documenting the use of the Security, Auditing, and E-signature (SAE) v2.2 software for system security. • Updated "Load the reagent mix into the MAP plate" on page 17 with revised step for placing MAP plate gasket strips on the MAP plate.
B.0	13 September 2021	• Updated graphics in the Analysis page section with optical dye channel information. • Updated "Prepare the dPCR reagent mix" on page 17 with steps to thaw or equilibrate reagents before use and to vortex Absolute Q™ DNA Digital PCR Master Mix (5X) and Digital PCR assay.
A.0	1 September 2021	New publication documenting instrument functions and data analysis features of the QuantStudio™ Absolute Q™ Digital PCR System.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The QuantStudio™ Absolute Q™ Digital PCR System enables precision quantification of target nucleic acid sequences. QuantStudio™ Absolute Q™ MAP16 Digital PCR plates (MAP plates) using patented microfluidic array technology are loaded with digital PCR reagents and then processed by the QuantStudio™ Absolute Q™ Digital PCR Instrument. Depending on the protocol, results can be provided in less than 90 minutes. The resulting data is visualized with the QuantStudio™ Absolute Q™ Digital PCR Software.

Instruments, kits, consumables, and accessories

The following table describes the products covered in this user guide.

Instrument system		
Item	**Catalog No.**	**Amount**
QuantStudio™ Absolute Q™ Digital PCR System: • QuantStudio™ Absolute Q™ Digital PCR Instrument • Dell™ OptiPlex XE3 Tower computer with monitor, keyboard, and mouse	A52864	1 instrument, 1 desktop computer and monitor
QuantStudio™ Absolute Q™ MAP16 Plate Kit includes: • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL QuantStudio™ Absolute Q™ Isolation Buffer	A52865	1
QuantStudio™ Absolute Q™ Digital PCR Starter Kit [1]	A52732	1
Absolute Q™ DNA Digital PCR Master Mix (5X)	A52490	200 reactions
QuantStudio™ Absolute Q™ Isolation Buffer	A52730	(1) 3 mL bottle

[1] The kit is required for system installation. See *QuantStudio™ Absolute Q™ Digital PCR Starter Kit User Guide* (Pub No. MAN0025653).

Digital PCR Assays

Pre-designed and custom digital PCR assays are available for use in dPCR experiments, for more information contact your local sales representative or visit **http://www.thermofisher.com**.

For information on the use of digital PCR assays, see the documentation provided with the assay.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Centrifuge, table top	MLS
Pipettes, P10, P20 and P200	MLS
Pipette tips, P10, P20 and P200	MLS

(continued)

Item	Source
Other consumables	
Microcentrifuge tubes	MLS
Microcentrifuge tube rack	MLS
Nuclease-Free Water	MLS
Microfiber or optical lens cleaning cloth	MLS
70% ethanol in water	MLS

Software description

Overview of the software

The QuantStudio™ Absolute Q™ Digital PCR System uses the following software:

- QuantStudio™ Absolute Q™ Digital PCR Software — controls the instrument, perform experiments, analyzes run files. See “QuantStudio™ Absolute Q™ Digital PCR Software” on page 9.
- Connect Transfer Software (Optional) — data transmission feature that collects instrument run data to send to Thermo Fisher Scientific to be used for improving the product and user experience. This feature is installed and configured during system installation.
- Security, Auditing, and E-signature (SAE) v2.2 (Optional) — controls security and user access to the software and specific features. See Appendix C, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

The software is installed during system installation. See “Download and install the software” on page 54.

QuantStudio™ Absolute Q™ Digital PCR Software

QuantStudio™ Absolute Q™ Digital PCR Software lets you define an experiment, control the instrument, and analyze data generated by the experiment. Parameters such as plate format, optical channels, and thermal conditions can be easily modified to start data generation quickly.

The software allows you to perform the following tasks:

- Define the experiment, including sample types, sample groups, replicates, pool sample, experiment notes, and names
- Create and edit protocols
- Run and monitor protocols
- View system status
- View data in plot and tables
- Generate run reports
- Export data and reports

- Insert and remove MAP plates
- Install the shipping lock screw for transport of the instrument

Instrument hardware description

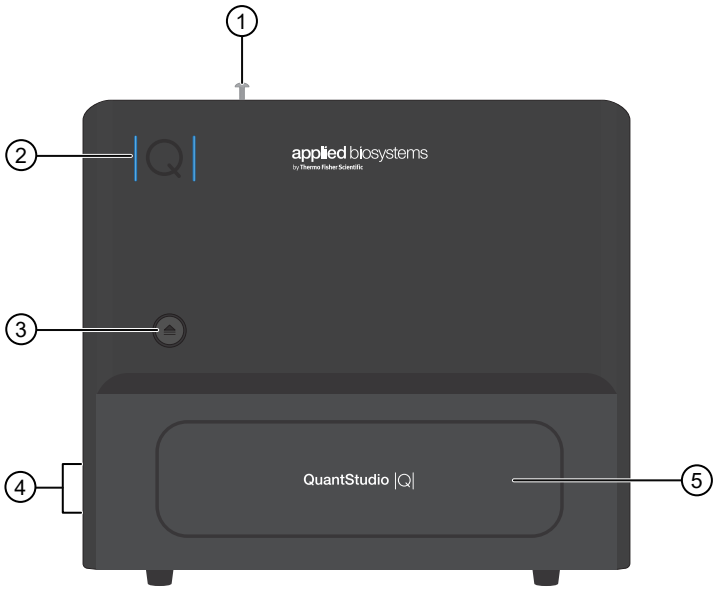
Overview of the instrument

The instrument is an integrated processing system compatible with QuantStudio™ Absolute Q™ MAP16 Digital PCR plates.

A dedicated computer provided with the instrument uses the QuantStudio™ Absolute Q™ Digital PCR Software to operate the instrument and analyze data.

For information on installing the instrument, see Appendix B, “Install and move the QuantStudio™ Absolute Q™ Digital PCR System”.

For information on maintaining the instrument, see Appendix D, “Maintain the instrument”.



1. Shipping lock screw
2. Status indicator light
3. Plate presentation tray open/close button
4. Power switch, power port, USB port
5. Plate presentation tray

The instrument has the following features and functions:

- The plate presentation tray is controlled using a button on the front panel or from within the software. Once a MAP plate is loaded into the tray, it is retracted into the instrument for automated processing.
- An internal barcode scanner verifies the barcodes on the MAP plates.
- An internal compressor and pneumatic subsystem drives the microfluidic array compartmentalization directly within the MAP plate using positive pressure.
- Liquid never contacts any parts in the instrument, so minimal maintenance and cleaning are required.
- The plate nest is thermally controlled to perform PCR thermal cycling.
- The fluorescent optical system is mounted above the MAP plate and scans the MAP plate in up to 5 optical channels before and after PCR.
- Each optical channel is associated with a color and a supported dye. See "QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Dyes" on page 14.
- A computer integrated into the instrument manages critical runtime activities and stores recent data that has not yet been analyzed.
- During an experiment run, positive pressure is applied to drive and separate the reagent mix into pico-scale microreaction chambers on the MAP plate before starting PCR. PCR occurs in parallel across the entire MAP plate; each microreaction chamber contains a discrete reaction.
- The microreaction chamber arrays are scanned for fluorescence before and after PCR and are used for data analysis.

Instrument indicator status light key

The vertical bars of the Q symbol on the front of the instrument display the instrument status.

Appearance	Color	Status	Meaning
–|Q|–	White	Flashing	On, initializing – not ready.
|Q|	White	Steady	On, not connected to software.
|Q|	Blue	Steady	On, ready.
~|Q|~	Blue	Pulsing	Running protocol.
–|Q|–	Yellow	Brief flashing	Plate door open button pushed while door is locked.
|Q|	Red	Steady	Error, see Appendix E, "Troubleshooting".

QuantStudio™ Absolute Q™ MAP16 Digital PCR plates

The QuantStudio™ Absolute Q™ Digital PCR Instrument uses QuantStudio™ Absolute Q™ MAP16 Digital PCR plates (MAP plates) for loading samples and running experiments.

IMPORTANT! When disposing of plates, follow all applicable waste regulations controlling the chemicals used in the experiment.

Each MAP plate has the following features:

- Contains 16 wells, 4 columns of 4 wells each, and each experiment must use at least one full column (4 samples).
- Contains 16 digital PCR microreaction chamber arrays that each contain 20,480 fixed volume microreaction chambers where dPCR is performed.
- Can be used in up to 4 experiments, depending on the number of columns used in each experiment. A MAP plate with unused columns can be used with subsequent experiments until all 4 columns have been used.
- Has a standard microtiter plate footprint and is compatible with most plate and liquid handlers.
- Has a label that includes a barcode, product number, and unique serial number. The instrument automatically reads the barcode when the MAP plate is inserted, and the unique serial number is tracked in the results.

Note: MAP plate gasket strips can only be used once per MAP plate.

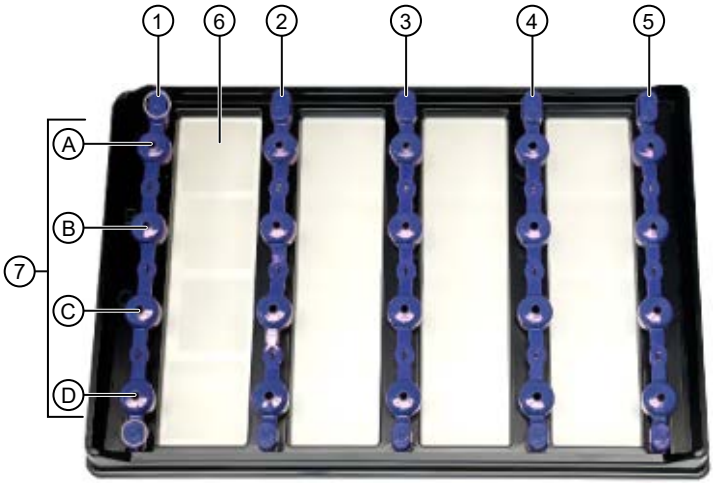


Figure 1 MAP plate layout

1. MAP plate gasket strip on column 1
2. MAP plate gasket strip on column 2
3. MAP plate gasket strip on column 3
4. MAP plate gasket strip on column 4
5. MAP plate gasket strip on column X
6. Microreaction chamber associated with well 1A
7. A–D represents wells A1–D1 associated with column 1

The following figure shows the dimensions of a MAP plate.

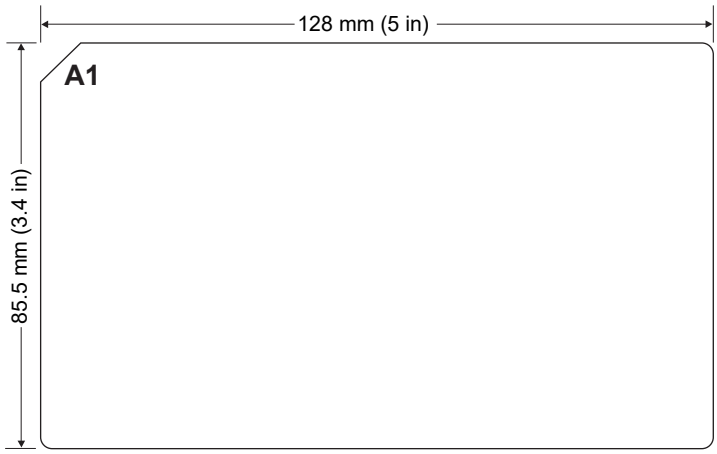


Figure 2 MAP plate dimensions

QuantStudio™ Absolute Q™ MAP16 Digital PCR plate compatibility

IMPORTANT! The instrument is only compatible with QuantStudio™ Absolute Q™ MAP16 Digital PCR plates. The instrument can malfunction with third-party plates, which could result in contamination of the instrument.

- For best results, we strongly recommend that you use Absolute Q™ DNA Digital PCR Master Mix (5X) and QuantStudio™ Absolute Q™ Isolation Buffer.
- MAP plates are made of injection molded thermoplastic commonly used in other PCR vessels and are generally compatible with most existing reagent kits and components available from third parties. Compatibility of any untested third-party reagent is not guaranteed. Contact technical support for more information on tested reagents (see Appendix H, “Documentation and support”).

QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Dyes

The following optical dyes are supported for use when selecting optical channels when analyzing experiment runs.

For more information on optical configuration, see “QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Configuration” on page 68.

Channel Color	Compatible Dyes
Blue	FAM™
Green	HEX™ VIC™
Yellow	ABY™
Red	ROX™
Dark Red	CY5™ JUN™

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

QuantStudio™ Absolute Q™ Digital PCR Software security

By default, the QuantStudio™ Absolute Q™ Digital PCR Software does not require login credentials to access the software nor does it restrict access to functions within the software.

To require login credentials and modify access by user roles, see Appendix C, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

2 Run an experiment

Workflow

This workflow represents running a single experiment on the QuantStudio™ Absolute Q™ Digital PCR Instrument. The procedure for sample preparation can vary depending on application and reagents.

Experiment workflow
Prepare the dPCR reagent mix (page 17)
Load the reagent mix into the MAP plate (page 17)
Run digital PCR (page 20)
Analyze data (page 24)
Finish

Prepare the dPCR reagent mix

Use the Absolute Q™ DNA Digital PCR Master Mix (5X) to prepare a digital PCR (dPCR) reagent mix.

Note: Other dPCR reagents are compatible with the MAP plates and can be used to create the dPCR reagent mix. For more information on specific applications, contact technical support (see Appendix H, "Documentation and support").

1. Thaw or equilibrate all reagents to room temperature before use.
2. Pulse vortex the Absolute Q™ DNA Digital PCR Master Mix (5X) and Digital PCR assay (40X or 20X) at high speed for 10 seconds.
3. Combine the following reagents in the order listed.

Reagent	Final Concentration	Volume per reaction	Volume per reaction with 10% overage[1]
Nuclease-free water	–	Fill to 9 µL	Fill to 10 µL
Absolute Q™ DNA Digital PCR Master Mix (5X)	1X	1.8 µL	2 µL
Digital PCR assay (40X or 20X)	1X[2]	0.23 µL (40 X) or 0.45 µL (20X)	0.25 µL (40 X) or 0.50 µL (20X)
DNA Sample	1–11,000 copies/ µL	Variable	Variable
Total	**–**	**9 µL**	**10 µL**

[1] After calculating the number of reactions required, prepare dPCR mix for the appropriate number of reactions and scale those components by 10% for overage. Dilute assay accordingly to avoid pipetting less than 1 µL volumes.

[2] DNA copy and dilution calculator can be found at **http://www.thermofisher.com/DNA-calculator**

4. Mix the dPCR reagents well by performing one of the following actions:
   - Pipette mix 10–20 times, or
   - Pulse vortex 3–5 times for 1 second each.
5. Using a benchtop centrifuge, centrifuge at 10,000 × g or the highest speed available for 1 minute.

Load the reagent mix into the MAP plate

At a clean lab bench gather the following materials:

- P10 or P20 pipette and tips
- Prepared dPCR reagent mix
- QuantStudio™ Absolute Q™ Isolation Buffer
- MAP plate with sufficient unused columns for the experiment
- MAP plate gasket strips (unused)

IMPORTANT! At least 1 column must be run at a time. Columns cannot be reused, but a MAP plate with unused columns can be used for subsequent experiments. When the experiment is complete, if the MAP plate has unused columns, place it back into its pouch to avoid debris accumulation.

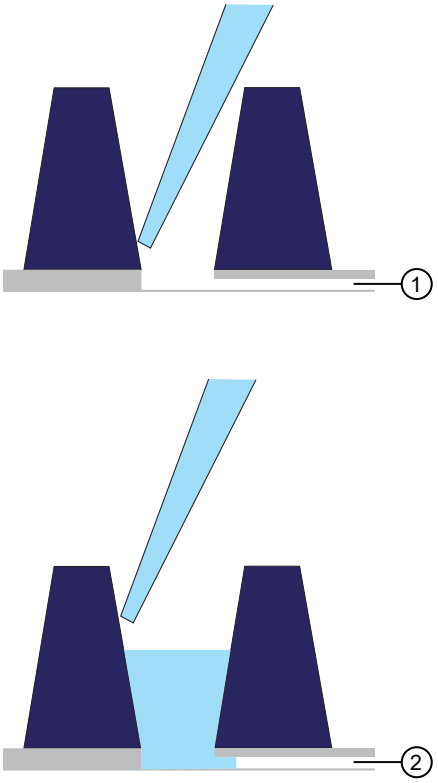
1. Open the MAP plate package.

   Note: Be careful to handle the MAP plate by its frame.

2. Place the MAP plate on a level, dust-free, dry surface.

3. Using a new pipette tip for each well, at a 45° angle, load 9 µL of the dPCR reagent mix to the bottom of the well. Pipette the mixture only to the first stop.

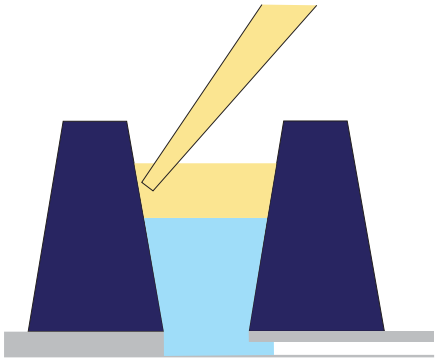
   IMPORTANT! Do not puncture the thin film at the bottom of the well.



① Microfluidic channel to the microreaction chamber array
② Reagent remains in the well until the instrument pushes it into the microreaction chamber array during the run

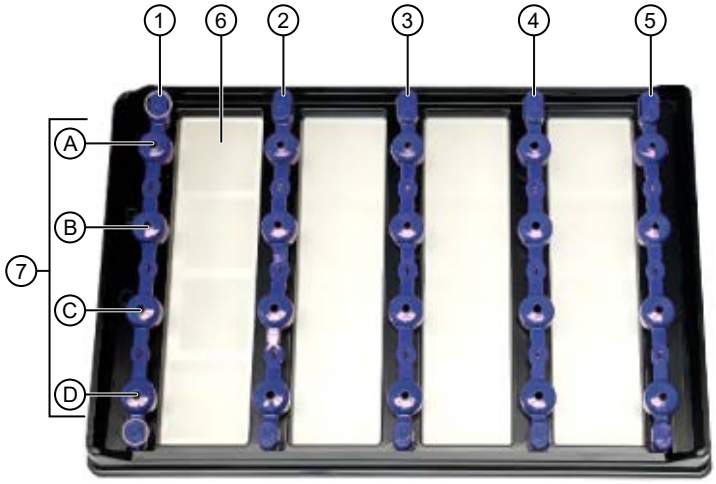
4. Using a new pipette tip for each well, at a 45° angle, load 15 µL of the Absolute Q™ Isolation Buffer overlay on the side of the well above the top of the reagent to prevent mixing or bubble formations. Pipette only to the first stop.

   Isolation buffer sits on top of the reagent, preventing contamination and evaporation.



5. Place a total of 5 MAP plate gasket strips on all 4 columns of wells and the X-shaped posts on the column X on the right side of the plate. Orient the MAP plate gasket strip so that the side labeled A–D aligns with rows A–D marked on the plate. Be sure to cover the columns completely.

IMPORTANT! MAP plate gasket strips must be placed on all columns, including unused columns. Failure to do so can produce poor results.



1. MAP plate gasket strip on column 1
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4. MAP plate gasket strip on column 4
5. MAP plate gasket strip on column X
6. Microreaction chamber associated with well 1A
7. A–D represents wells A1–D1 associated with column 1

6. Move the MAP plate to the instrument.

   IMPORTANT! Do not tip, invert, or shake the filled MAP plate.

7. Insert the MAP plate into the instrument.

Run digital PCR

Power on the instrument and computer

IMPORTANT! Prior to powering on the QuantStudio™ Absolute Q™ Digital PCR Instrument, confirm that the shipping lock screw has been removed. Failure to do so can damage the instrument. See “Uninstall the shipping lock screw” on page 57.

1. Confirm that the power cable is connected to an appropriate power source.
2. Confirm that the USB cable is connected from the instrument to the dedicated computer.
3. Power on the dedicated computer and monitor and start the software.
4. Power on the instrument by moving the power switch located on the left side near the back of the instrument to the I position.

   Note: The instrument makes a humming noise as it charges the internal compressor.

   The bars of the instrument symbol flash white to indicate that the system is initializing. This takes approximately 30 seconds.

   The instrument is ready when the status lights are a steady blue and a ready status appears under the instrument on the **Instrument** page QuantStudio™ Absolute Q™ Digital PCR Software.

Select a protocol

1. In the left pane of the QuantStudio™ Absolute Q™ Digital PCR Software, select to access the **Instrument** page.

2. Select **PROTOCOL**, then perform one of the following tasks:

Task	Actions
Select an existing protocol.	1. Select **PROTOCOL**. 2. Select a protocol from the **Protocols** list. 3. Select **LOAD**.
Edit the loaded protocol.	1. In the **PROTOCOL** pane, click . 2. Modify the optical channels and PCR parameters as needed. See Appendix A, “Modify protocols”. 3. Select **SAVE**.
Create a custom protocol	1. Select **PROTOCOL**. 2. Select a protocol from the **Protocols** list to use as a template. 3. Select **LOAD**. 4. In the **PROTOCOL** pane, click . 5. Modify the optical channels and PCR parameters as needed. See Appendix A, “Modify protocols”. 6. Enter a new name in the name field. 7. Select **SAVE**.
Import a protocol.	1. Select **PROTOCOL**. 2. In the **Protocols** list area click . 3. Select **IMPORT FILE**, then navigate to the location of the protocol file. 4. Select the file, then select **Open**. 5. Select the imported protocol from the **Protocols** list. 6. Select **LOAD**.

Load the plate and run the protocol

IMPORTANT! You must clean the plate nest before each run. See "Clean the instrument and plate nest" on page 65.

IMPORTANT! Before running the protocol, make sure your protocol parameters are defined correctly. Protocol parameters cannot be changed after the run. See "Select a protocol" on page 21 or Appendix A, "Modify protocols".

1. In the left pane, click to access the **Instrument** page.
2. In the sample plate area, use the check boxes to select the columns to be used in the run.

   IMPORTANT! Failure to deselect the columns that are not in use will prevent them from being used in a subsequent run.
3. In the **Notes** field, enter information regarding this run, then click **ADD NOTE**.
4. Click the **Start** button under the instrument icon.

   The instrument door opens to receive the loaded MAP plate.

   IMPORTANT! Confirm that gaskets are placed on all columns of the MAP plate, including unused columns. Failure to do so can produce poor results.
5. When prompted, verify that gaskets have been placed on all wells and on the column X posts on the far right as shown on the screen.

   Note: See callout 5 in the figure below for the location of column X.

6. Carefully load the MAP plate in the plate nest.

   IMPORTANT! Be sure to load the MAP plate gently to avoid damage to the plate nest.
7. Select **CLOSE DOOR**.

   The door closes and the MAP plate bar code is scanned.

Note: If the instrument cannot scan the barcode, it can be manually added in the barcode field of **Run name** dialog box.

8. When prompted, enter a **Run name**.
9. Click **RUN**.
   - The run status displays in the left sidebar.
   - While processing the run, the instrument lights slowly pulse blue.
   - When the run is complete, the instrument lights are a steady blue.
   - Data populates the **ANALYSIS** tab on the **Runs** page as it becomes available.
10. When the **Run complete** dialog displays, select the run name to view the final data in the **Runs** page.

    For more information on analyzing experiment results, see Chapter 3, “Analyze data”.

Download a protocol from the Instrument page

You can save a protocol that you have created or modified to use on another computer using the QuantStudio™ Absolute Q™ Digital PCR Software by using the download option on the **Instrument** page.

1. In the left pane, click to access the **Instrument** page.
2. Select **PROTOCOL**, then select a protocol from the **Protocols** list.
3. Above the **Channels** list, click .
4. Select one of following options.
   - **Download Protocol** to download the protocol AQUA file.
   - **Download PNG** to download a graphic representation of the protocol.
5. When prompted, navigate to the location where you want to save the file, then select **Save**.

3 Analyze data

Software screens

QuantStudio™ Absolute Q™ Digital PCR Software has the following pages:

- The **Runs** page lets you see experiment results organized by plate run and provides access to the **SETUP**, **ANALYSIS**, and **RESULTS** pages for analyzing and viewing experiment results. See “Runs page” on page 25.
- The **SETUP** page for a run provides controls for analysis options such as sample and target names, sample groups, replicate statistics, pooling, copy number calculations. See “SETUP page” on page 26.
- The **ANALYSIS** page for a run displays relevant information by samples or groups and provides different options for viewing the data. See “ANALYSIS page” on page 32.
- The **RESULTS** page for a run shows a summary of the run data and provides reporting and data exporting options. See “RESULTS page” on page 43.

Runs page

The **Runs** page lets you see experiment results organized by plate run. Recently completed runs require a few minutes to complete the data analysis. The progress of the analysis is displayed in the **Analyzed** column. A green check mark indicates that the analysis is complete.

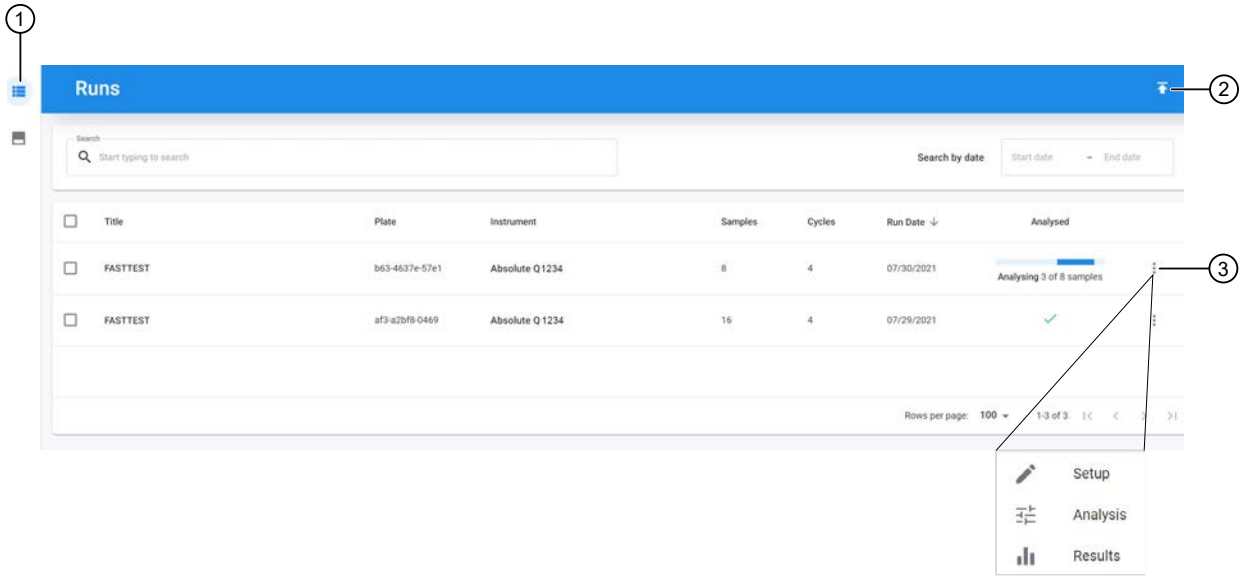


Figure 3 Runs page overview

1. Runs page
2. Import run
3. Run analysis shortcut menu

When a run is being analyzed, the **ANALYSIS** and **RESULTS** pages periodically update with new information.

Run data can be exported for analysis on another computer running the QuantStudio™ Absolute Q™ Digital PCR Software and imported from runs generated on a different instrument. See “Export and Import Runs” on page 47.

Select a run

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list.

Proceed to the **SETUP** page (see “SETUP page” on page 26), **ANALYSIS** page (see “ANALYSIS page” on page 32}, and **RESULTS** page (see “RESULTS page” on page 43) to proceed with the data analysis of the run.

SETUP page

The **SETUP** page within the run allows you to perform the following tasks:

- Name the samples and set the analysis type. See “Name a sample” on page 26.
- Create and assign groups. See “Manage groups” on page 28.
- Save or load previously defined groups. See “Save and load group sets” on page 30.
- Download the PCR thermal protocol or an image of the protocol. See “Download a protocol from the SETUP page” on page 27.

Sample names and group analysis can be done before or during the run in the **Instrument** tab or after the run in **Runs** ▸ **SETUP** tab.

Sample selection and names

Sample names are user-assigned identifiers for the contents of each loaded well of a plate.

Groups determine what type of analysis is applied to all samples within a group.

Selecting a sample displays the analysis of that single sample.

Select samples

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. In the sample area, hover over the sample to display the check box.
4. To select samples perform one of the following tasks:

Task	Actions
Select a single sample.	1. Select the sample check box. 2. Click anywhere in the sample area to select it.
Select multiple samples.	1. Click and drag through samples to select multiple samples at once.

Name a sample

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. In the sample area, hover over the sample to display the .
4. Click and enter a sample name.

5. Perform one of the following actions to save the edit:
    - Click away from the sample name field.
    - Press enter to save the edit and open to the next sample name for editing.

Hide samples

When samples are hidden, they are treated as if they were never run.

Hidden samples can be revealed. No data is lost by hiding a sample.

Hiding samples can be useful if there is an issue with the reagents, loading, or integrity of the sample.

Hiding samples has the following effect on the information that is included for display:

- No analysis results are shown for the samples.
- The samples are removed from the **RESULTS** page.
- The samples are excluded from reports.
- The samples are excluded from calculations for replicates or pooled results.

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.
3. Hover over the sample to be hidden and click to hide the sample.
4. To reveal a sample, hover over a hidden sample and click .

Download a protocol from the SETUP page

You can save a protocol that you have created or modified to use on another computer using the QuantStudio™ Absolute Q™ Digital PCR Software by using the download option on the **SETUP** page.

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. In the Protocol area, click .
4. Select one of following options.
    - **Download Protocol** to download the protocol AQUA file.
    - **Download PNG** to download a graphic representation of the protocol.
5. When prompted, navigate to the location where you want to save the file, then select **Save**.

Manage groups

After a run is completed, groups are used to define the analysis and results type for reporting for individual samples or sets of samples. Once defined, a group can be edited or deleted.

Note: Only groups without samples can be deleted.

When samples are assigned to a group, they will all have the same definition for:

- The target DNA associated with each fluorescent dye.
- The analysis type for each optical channel:
  - CNV (Copy Number Variation) – Reporting ratio of CNV/CNV Ref
  - CNV Ref (Copy Number Variation Reference) – The reference target for CNV

    Note: The reference target is a gene of known and stable copy number used to calculate the copy number for the gene of interest.
  - Signal – Absolute quantification
  - Not Used – Ignored in analysis
- Grouping options:
  - Individual – Each sample has a separate result entry.
  - Replicates – The results show the Mean, Standard Deviation, and the CV% of the concentration for all the samples in the group.
  - Pooling – The results treat all of the samples in the group as one large sample.

See the following sections for more information:

- To create groups, see “Create groups” on page 28.
- To edit groups, see “Edit groups” on page 29.
- To delete groups, see “Delete groups” on page 29.
- To add samples to groups, see “Assign samples to groups” on page 29.
- To save and load group sets, see “Save and load group sets” on page 30.

Create groups

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. Below the sample plate area, select **EDIT GROUPS**.
4. Select **CREATE** in the Group list.
5. In the **Group name** field, enter a name for the group.
6. In the **Target DNA** fields, enter the name of the DNA target for each active optical channel.
7. From the **Analysis** drop-down, select the analysis type for each optical channel.

8. From the **SAMPLES** area, select one of the following sample grouping options:
   - **Individual**
   - **Replicates**
   - **Pooling**
9. Select **SAVE**.

Edit groups

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. Below the sample plate area, select **EDIT GROUPS**.
4. Select the group in the **Name** list.
5. Edit group settings as needed.
6. Select **SAVE**.

Delete groups

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. Below the sample plate area, select **EDIT GROUPS**.
4. Select the group in the **Name** list.
5. Click .

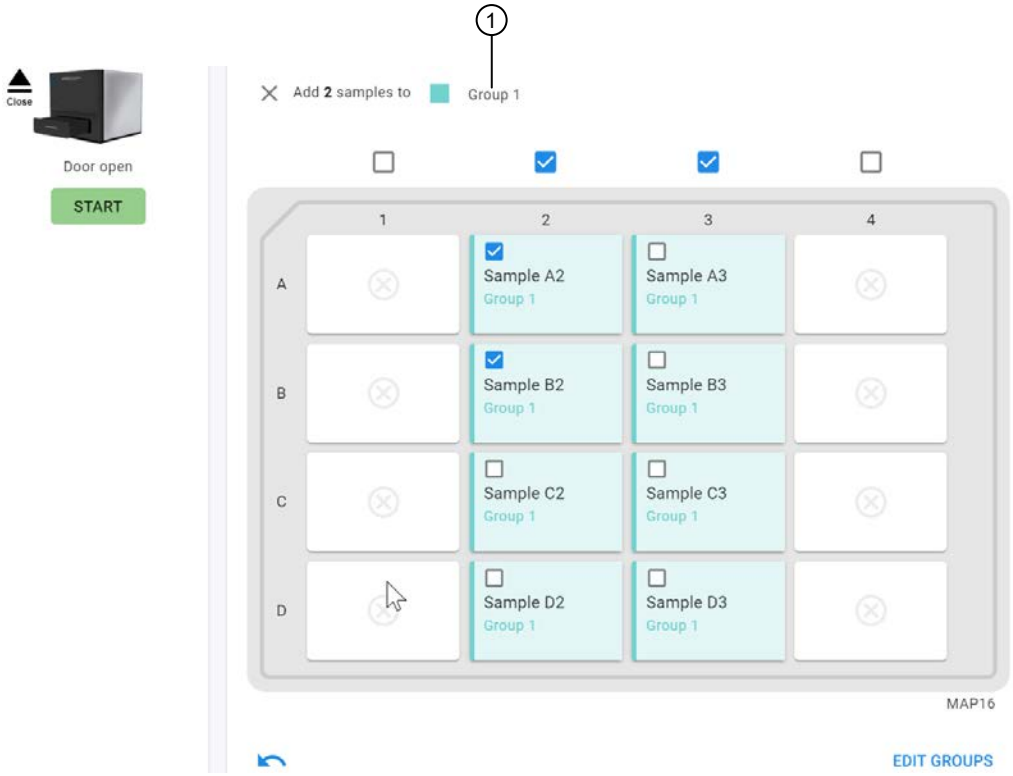
Assign samples to groups

Assigning samples to groups defines the analysis and results type for reporting for individual samples or sets of samples.

1. To access the sample plate perform one of the following actions:
   - In the left pane, click to access the **Instrument** page.
   - In the left pane, click to access the **Runs** page, then select **SETUP**.

2. In the sample plate area select one or more samples to be included in a group.

   Pre-defined groups appear above the sample plate grid.



① Group list area

3. Select the group for these samples.

Save and load group sets

All the groups defined for a plate can be saved as a named group set.

Saved group set can be loaded into other runs.

Each group set contains the following information:

- All the groups created in a run, even if they are not applied to a sample.
- The location of the samples applied to each group.
- The name and color of each group.
- The target DNA names in each group.
- The analysis type for each target.
- The analysis for samples as individual, replicates, or pooling.

Group sets do not include the sample names, and dye names which will be unaffected by loading a group set.

The QC channel in a run is never changed by loading a group set. For information on the QC channel, see “View by samples” on page 35.

To save a group set, see “Save group sets” on page 31.

To load a group set, see “Load group sets” on page 31.

Save group sets

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. From the sample plate grid, select the groups to be included in the Group Set.
4. In the upper-right corner of the page, click .
5. In the **Save Group Set** dialog, enter a name for the Group Set, then select **SAVE**.

Load group sets

When a group set is loaded:

- All existing groups in the run are replaced by the groups in the set and applied to the samples as defined by the group set.
- The targets in each group are assigned to the closest channel matching the run that the group set originated from.
- When a group set containing only a single column is applied to a multi-column run, the group pattern is repeated for each column.

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **SETUP** page.
3. In the upper-right corner of the page, click
4. In the **Load Group Set** dialog, choose a group set from the list, then select **LOAD**.

ANALYSIS page

The **ANALYSIS** page displays relevant information by samples or groups and provides different options for viewing the data.

Figure 4 ANALYSIS page overview

1. View by samples
2. View by groups
3. View QC channel
4. Export sample data
5. View data by optical channel
6. Toggle **Show Rejects** to show microreaction chambers automatically rejected from analysis and results
7. Toggle to display array view
8. Toggle between **1D Scatter plot**, **Histogram**, and **2D Scatter plot** views

Viewing by samples puts the color channel information into rows for comparison across the color channels. You can view or download data plots for each sample. See “View by samples” on page 35.

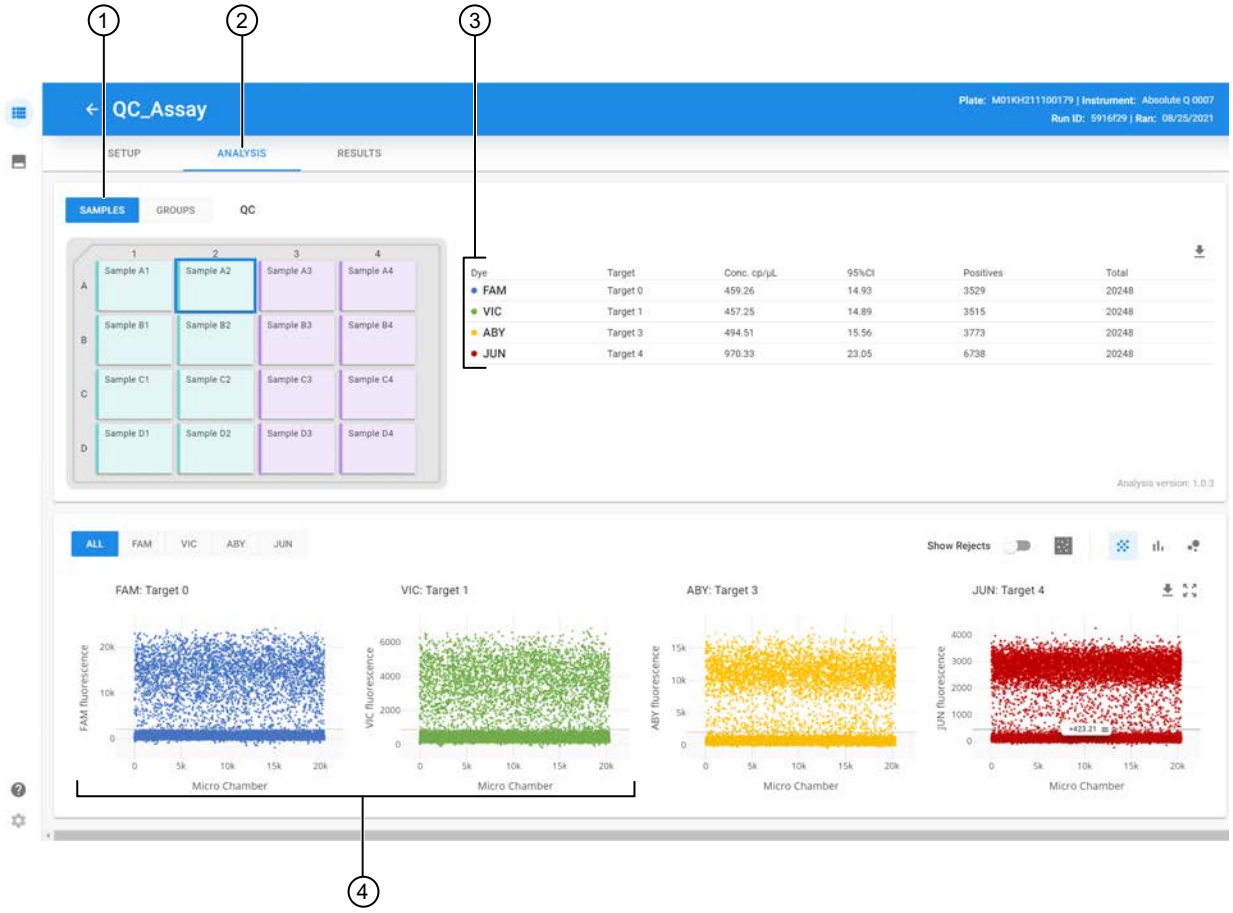


Figure 5 View by samples

① View by samples
② ANALYSIS page
③ Color channels for the selected sample
④ 1D Scatter plots with positive and negative threshold

QC channel data is provided for each sample.

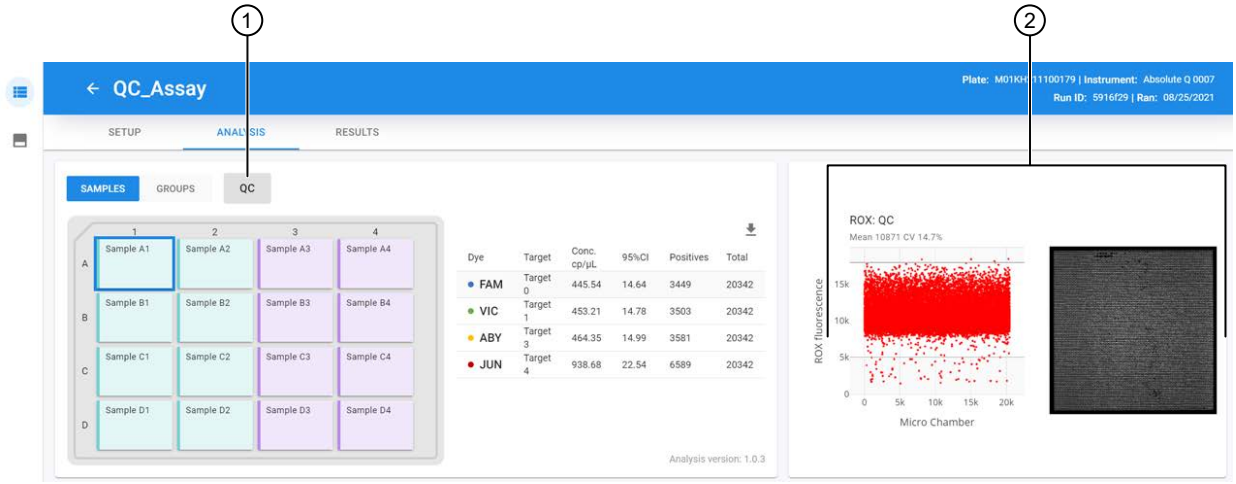


Figure 6 View QC channel data for a sample

① QC channel toggle
② QC channel window

Viewing by **GROUPS** lets you compare color channels across the sample. You can view or download data plots for each group. See “View by groups” on page 36.

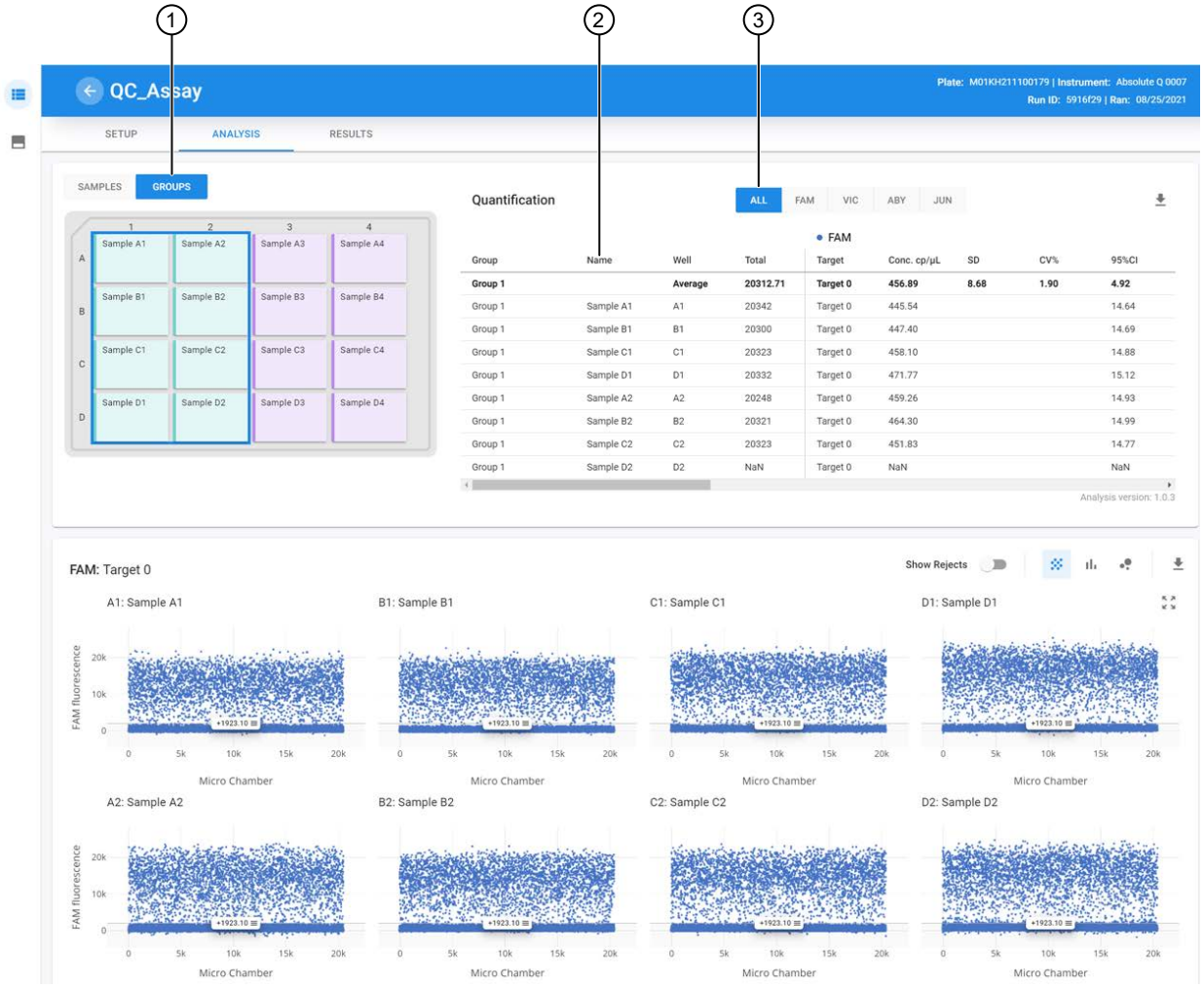


Figure 7 View by groups

① View by groups
② Row for each sample in the selected group
③ Color channel selection

View by samples

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.
3. On the **ANALYSIS** page, select **SAMPLES**.
   The information is displayed in the results area using the 1D Scatter plot type.

4. (Optional) Use these options to change the results view:

Option	Description
	1D Scatter plot type
	Show Rejects
	Histogram plot type
	2D Scatter plot type
	2D Scatter plot type with heatmap
	Show Arrays

For information on plots, see “Plot types” on page 37 and “Navigate plots” on page 38.

For information on showing arrays, see “Show array images” on page 42.

5. (Optional) Toggle the QC window by selecting the **QC** button.

   The QC window displays the plot and array image for the QC channel (usually ROX).This quality control data ensures that only properly filled microreaction chambers are used for analysis by evaluating the ROX signal for each microreaction chamber.

   The top and bottom thresholds are automatically set but can be manually adjusted. The QC plot should have a single level band indicating uniform filling.

6. (Optional) Adjust the threshold by selecting the threshold bar and dragging it to the desired position on the plot.

   For more information on thresholds, see “Set thresholds” on page 41.

View by groups

1. In the left pane, click  to access the **Runs** page.

2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.

3. On the **ANALYSIS** page, select **GROUPS**.

   The information is displayed in the results area using the 1D Scatter plot type.

4. (Optional) Use these options to change the results view:

Option	Description
	1D Scatter plot type
	Show Rejects
	Histogram plot type
	2D Scatter plot type
	2D Scatter plot type with heatmap
	Show Arrays

For more information on plots, see “Plot types” on page 37 and “Navigate plots” on page 38.

For more information on showing arrays, see “Show array images” on page 42.

5. (Optional) Adjust the threshold by selecting the threshold bar and dragging it to the desired position on the plot.

   For more information on thresholds, see “Set thresholds” on page 41.

Plot types

There are three plot options:

Option	Description
	1D scatter (signal versus index)
	Histogram
	2D Scatter, with optional heatmap (color versus color)- use the Heatmap to display scatter plots as a heatmap. Brighter areas indicate a higher density of plots.

Show Rejects (): display or hide microreaction chambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.

Figure 8 View 1D Scatter plot

① Show Rejects option
② 1D Scatter option

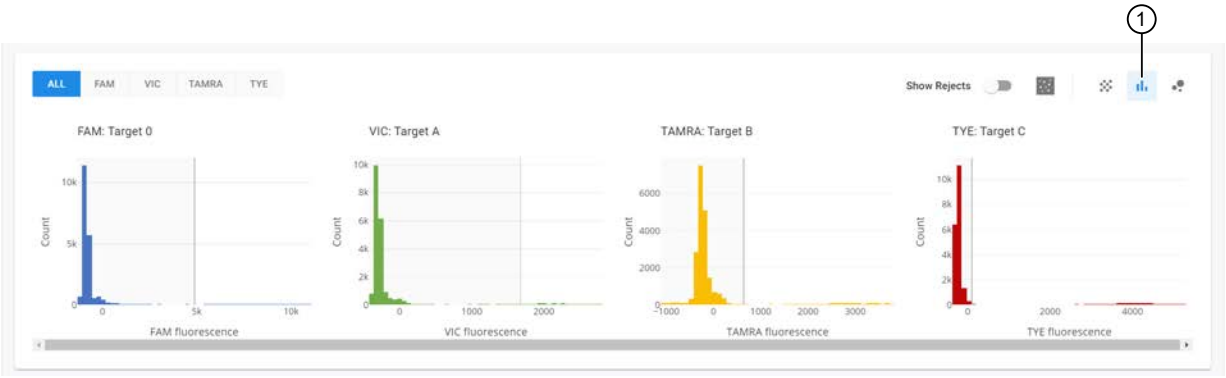


Figure 9 View Histogram plot

① Histogram plot option

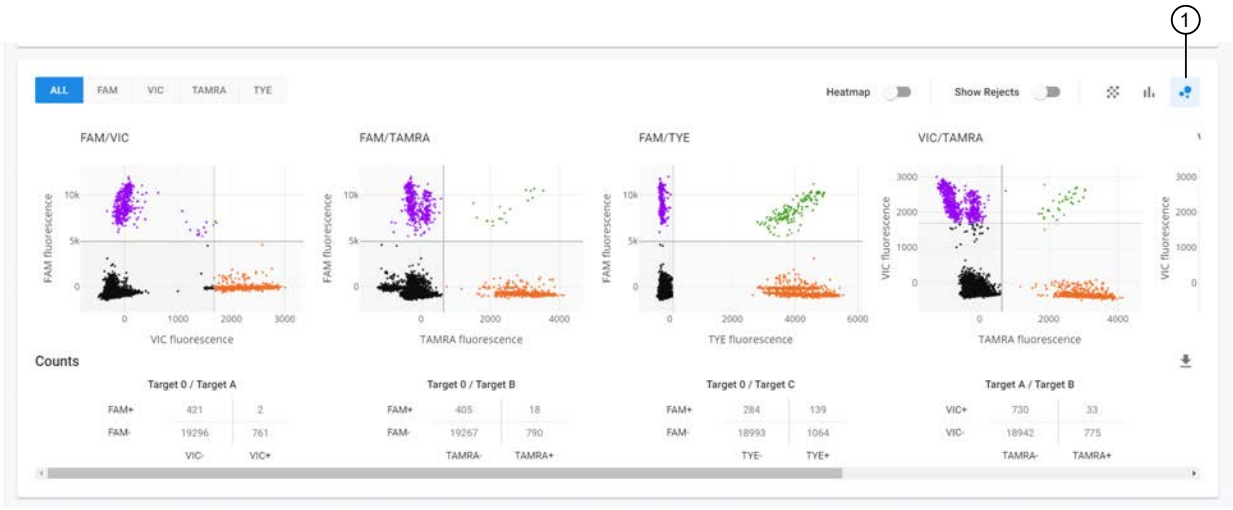


Figure 10 View 2D Scatter plot

① 2D Scatter plot option

Navigate plots

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.

3. On the **ANALYSIS** page, hover over a plot to reveal the threshold handle and buttons. Select the threshold handles and drag to adjust.

Option	Description
	Download the plot as a PNG image or download data from the plot.
	Autoscale to return the plot to return the original scale.
	Auto-threshold to return the plot to the original threshold.
	Enlarge the plot.

4. Click and drag to zoom in on a section of a plot.

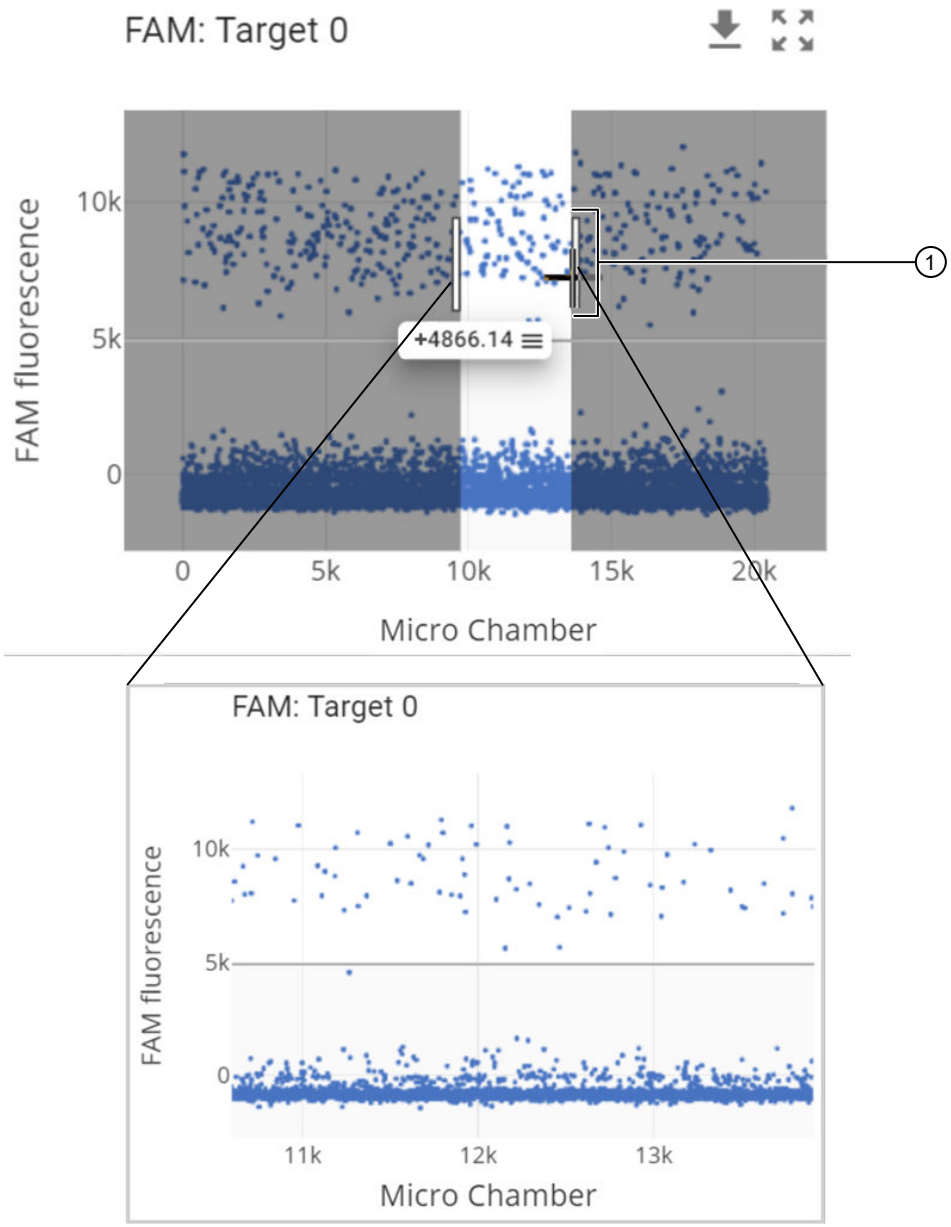


Figure 11 Use threshold handles to zoom in on a plot

① Click and drag to zoom in on a selection

5. With enlarged plot views, use the left and right arrows to navigate between the channels.

Set thresholds

Thresholds on all plots are automatically set during the initial analysis based on the data distribution.

Automatic (default) thresholds have a single grey line indicating the barrier between positive and negative data points.

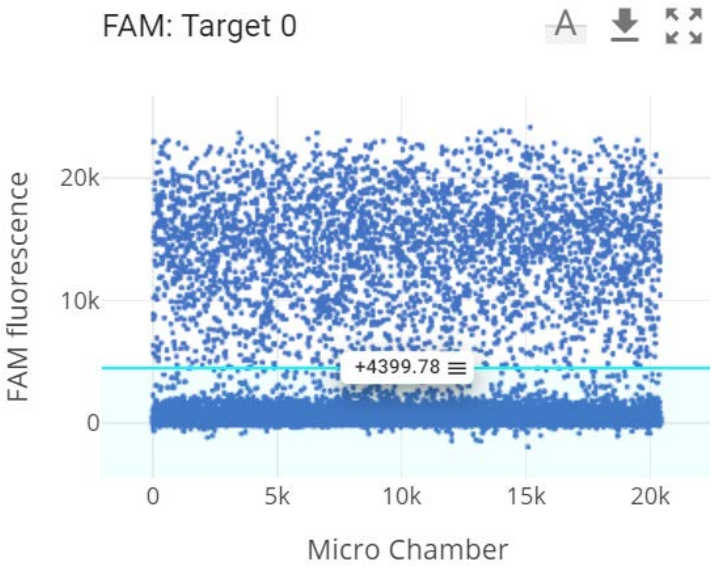


Figure 12 Adjust thresholds

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.
3. Adjust the thresholds on a plot by selecting and dragging the threshold bar to the desired position.

   Note: For samples that have been grouped, dragging a channel threshold changes the threshold value for all samples in the group to the same value.

4. To revert to the original threshold, click A.

   Note: Auto-threshold reverts all the samples that have had thresholds adjusted back to their original auto-threshold values. Samples in groups that have not been adjusted will not be changed.

Show array images

The **Show Arrays** ( ) option toggles the display of array images for all channels.

Microreaction chambers with positive data points are colored. Microreaction chambers with negative and rejected data points are gray scale. Often they have a low signal and appear black.

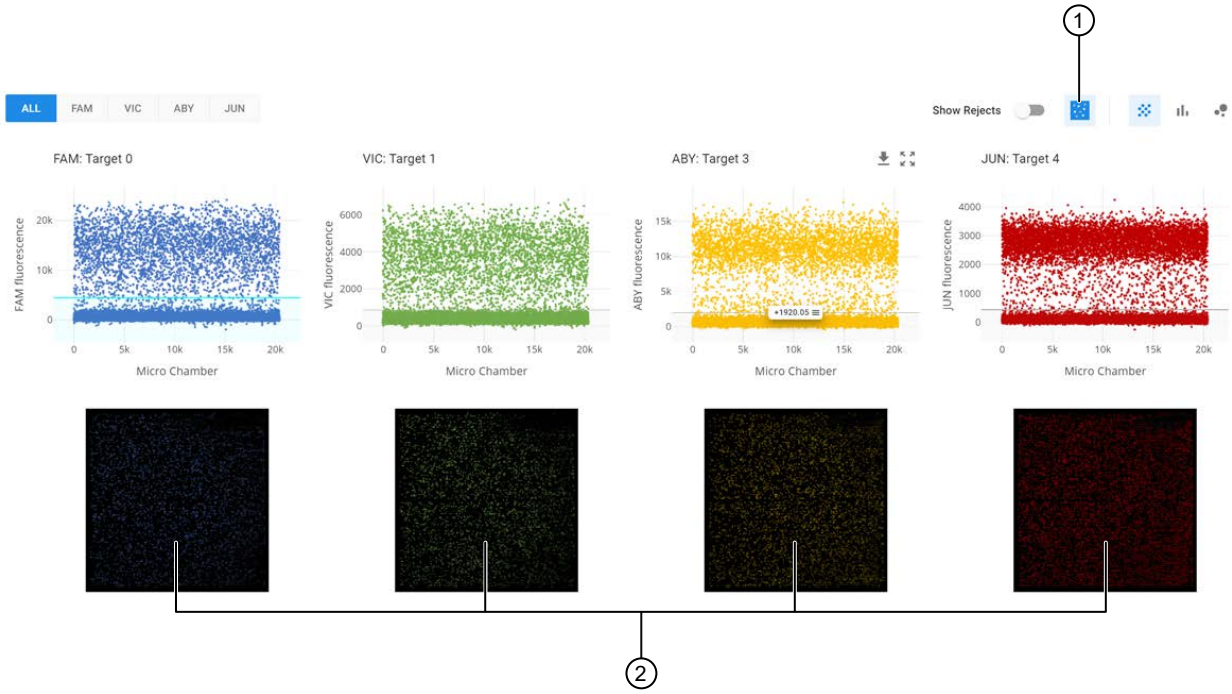


Figure 13 View channel array images

① Show arrays option
② Channel array images

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.
3. On the **ANALYSIS** page for a sample or group, click .
4. Select an array to view an enlarged image.
5. Use the left and right arrows to navigate between arrays.

Export data from the ANALYSIS page

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **ANALYSIS** page.
3. To export data, perform one of the following tasks:

Option	Actions
Export table data.	1. From the **ANALYSIS** page, in the optical channel table area, click . 2. Select an export option. • **Copy to Clipboard** to copy the data in HTML format • **Download CSV** to download the data in CSV format 3. When prompted, navigate to the location to save the file and select **Save**.
Export plot data.	1. From the **ANALYSIS** page, in the plots area, hover over a plot and click . 2. Select an export option. • **Download Plot** • **Download Data** 3. When prompted, navigate to the location to save the file and select **Save**.

RESULTS page

The **RESULTS** page displays the results for all the samples in a single table. The values are plotted together below the concentration table.

From the **RESULTS** page, you can:

- View statistical results from the run. See “View results” on page 45.
- View results plots from the run by sample or group. See “View results” on page 45.
- Copy the data to the clipboard in HTML format. See “Export data from the RESULTS page” on page 46.
- Download the data table in CSV format. See “Export data from the RESULTS page” on page 46.
- Generate data reports. See “Generate reports” on page 46.

The presentation of the **RESULTS** page is based on the groups assigned in the **SETUP** page.

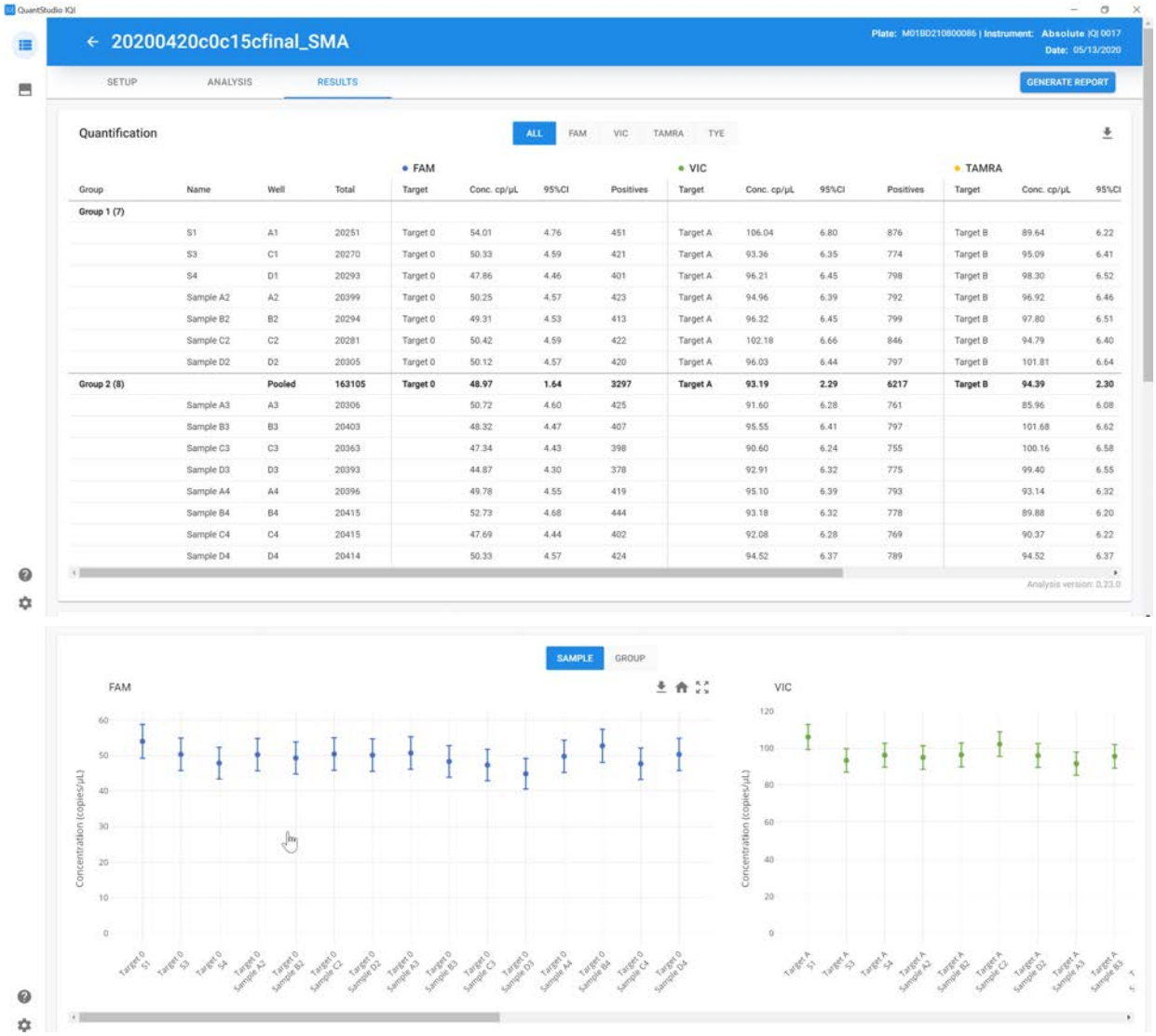


Figure 14 View Results

View results

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **RESULTS** tab.
3. To view results, perform one of the following tasks:

Option	Actions
View results for all optical channels.	1. From the list of active optical channels above the concentration table, select **ALL** to view all channels. 2. Use the scroll bar at the bottom of the concentration table to scroll through concentration table data for all channels. 3. Use the scroll bar on the side of the page to scroll down to see the plot data for all channels. 4. Toggle between **SAMPLE** and **GROUP** to change the display of the plot data. 5. Use the scroll bar at the bottom of the plot area to scroll through the plot data for all channels.
View results for a specific optical channel.	1. From the list of active optical channels above the concentration table, select the desired channel. 2. Use the scroll bar on the side of the page to scroll down to see the concentration table data for the channel. 3. Use the scroll bar on the side of the page to scroll down to see the plot data for the channel. 4. Toggle between **SAMPLE** and **GROUP** to change the display of the plot data.

Export data from the RESULTS page

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **RESULTS** page.
3. To export data, perform one of the following tasks:

Option	Actions
Export concentration data.	1. From the **RESULTS** page, in the concentration table area, click . 2. Select an export option. • **Copy to Clipboard** to copy the data in HTML format. • **Download CSV** to download the data in CSV format. • **Multi-channel CSV** 3. When prompted, navigate to the location to save the file and select **Save**.
Export plot data.	1. From the **RESULTS** page, in the plots area, hover over a plot and click . 2. Select an export option. • **Download Plot**. • **Download Data**. 3. When prompted, navigate to the location to save the file and select **Save**.

Generate reports

The **GENERATE REPORT** option uses the Report Builder to create and export reports as PDF files.

Note: Hidden samples are excluded from reports. You must unhide any samples that you need included in a report. To unhide a sample, see “Hide samples” on page 27.

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **RESULTS** page.
3. From the **RESULTS** page, select **GENERATE REPORT** to open **Report Builder**.
   The **Report Builder** dialog box opens and **All Groups** are selected by default to be included in the report.
4. (Optional) Select **Select Groups**, then from the list of groups, select the check box next to each group to be included in the report. Any combination of groups can be selected.
5. (Optional) Select **QC Channel** to include the QC channel data for all samples.

6. (Optional) Select **Arrays** to include array images for all samples.
7. Select **BUILD**.

Export and Import Runs

Runs can be exported to a ZST file that can be transferred and imported into QuantStudio™ Absolute Q™ Digital PCR Software running on a different computer.

The ZST run file contains all analysis options:

- Group details
- Sample names
- Thresholds

Run files are approximately 30 MB in size.

To export a run, see “Export a run” on page 47.

To import a run, see “Import a run” on page 47.

Export a run

1. In the left pane, click to access the **Runs** page.
2. In the list of runs, select the check boxes of the runs to be included in the export.
3. In the upper-right side of the run list, click .
4. When prompted, enter a name for the export file and navigate to the locate where you want to save the run, then select **Save**.

Import a run

1. In the left pane, click to access the **Runs** page.
2. In the upper-right corner of the **Runs** page click .
3. Perform one of the following options:

Option	Action
Drag the ZST file into the **Import your result file** window from Windows™ File Explorer.	1. Using Windows™ File Explorer, navigate to the location of the ZST file to import. 2. Drag and drop the file into the **Import your result file** window.
Navigate to the ZST file from the **Import your result file** window.	1. Select **IMPORT FILE** and navigate to the location of the ZST file to import. 2. Select the file, then select **Open**.

Delete a run

IMPORTANT! Deleting a run is permanent. You cannot restore a deleted run.

1. In the left pane, click to access the **Runs** page.
2. In the list of runs, select the check boxes of the runs to be deleted.
3. In the upper-right side of the run list, click .
4. When prompted to delete the run, select **DELETE**.

Modify protocols

The QuantStudio™ Absolute Q™ Digital PCR Software is pre-configured with a default protocol to use as a template to create custom assays. Existing protocols can be edited or used as templates to create additional custom protocols.

Protocols define the following run information:

- Dyes used in each active optical channel
- PCR parameters

1. In the left pane, click to access the **Instrument** page.
2. Select **Protocol**, then click to edit the loaded protocol.
3. Modify optical channels as needed.

Parameter	Action
Active optical channel	Select the check box for each optical channel to be used.
Target dye for active channel	For each active optical channel, select the drop-down to choose the target dye.

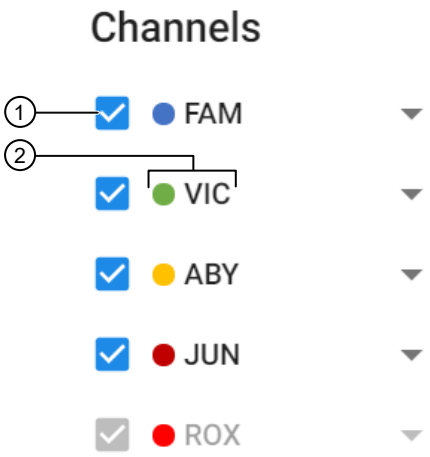


Figure 15 Optical channel dyes

① Dye channel check box
② Dye channel name

4. Modify PCR parameters as needed.

Parameter	Actions
Temperature	• Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature.
Dwell times	Enter in seconds or minutes and seconds in mm:ss format.
Cycles	Set the number of cycles by entering a value into the **Cycles** field.
RNA-RT	Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples.
Preheat	Select **Preheat** to add a preheat step. Sometimes called hot start, pre-heating the samples before PCR helps to reduce non-specific binding at lower temperatures.
Two or three-step cycling	Select the **Two Step** drop-down to select 2 or 3 step cycling.
Two-stage PCR cycle	Select **Two Stage PCR** to add a second PCR cycle stage.

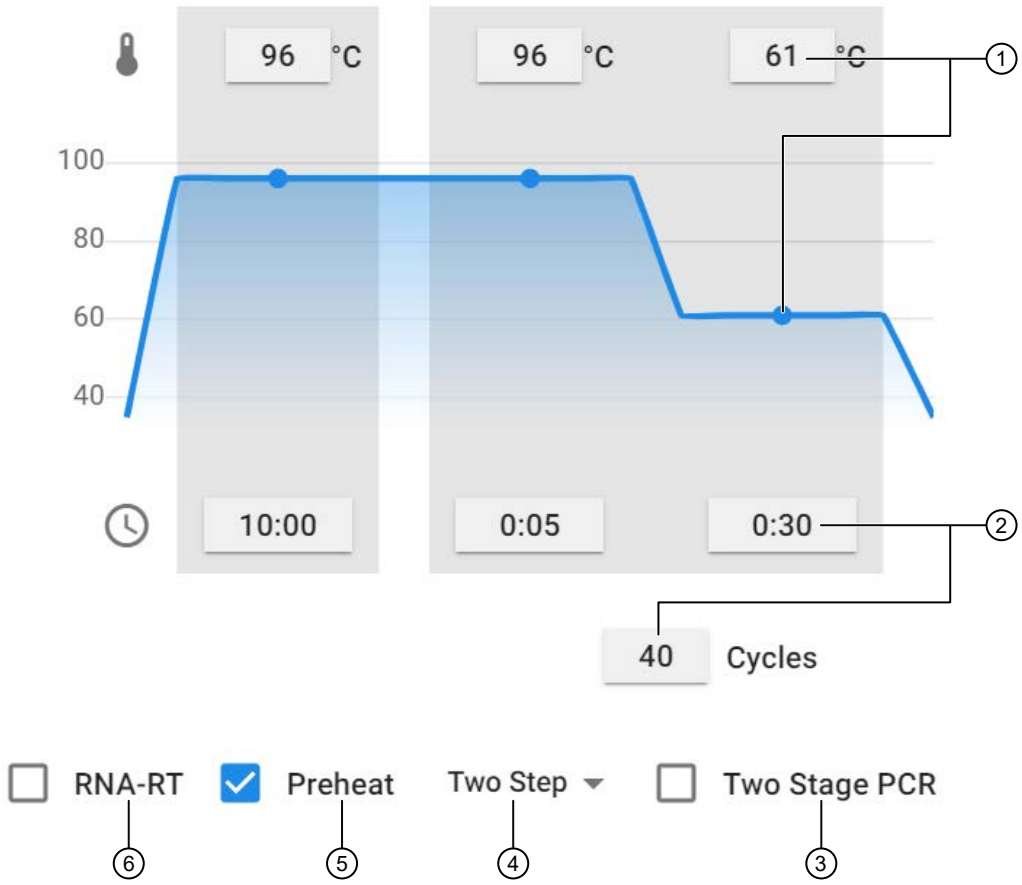


Figure 16 Protocol parameters

① Temperature settings fields and slider bar
② Time fields and cycles field
③ Two-stage PCR setting
④ Two or three step cycling option
⑤ Preheat setting
⑥ RNA-RT setting

5. Select **SAVE**.

B

Install and move the QuantStudio™ Absolute Q™ Digital PCR System

Installation and environment requirements

The room where the instrument is installed must be kept within the following operational environment conditions.

Condition	Acceptable Range
Installation site	For indoor laboratory use only (Applicable pollution degree 2)
Operating temperature and humidity	15-30°C (60-85°F), 0-80% RH
Storage temperature and humidity	5-40°C (40-105°F), 0-80% RH
Vibration	Do not place the instrument adjacent to strong vibration sources. Excessive vibration during use can affect instrument performance.
Altitude	Up to 6,500 ft (2000 m)
Input voltage tolerance	+/-10%
Over voltage category	II

- Installation time: <10 minutes
- Required materials: scissors or a strap cutter
- Space requirement: The instrument is approximately 0.6 m (2 ft) cubed. The presentation drawer must not be obstructed and extends approximately 200 mm (8 in) from the front panel of the instrument when open. The power and USB connections are on the left side near the back of the instrument.
- Ensure that the fan vents on the back and bottom of the instrument are not obstructed.

IMPORTANT! Keep all packaging materials in good condition, as they are required if the instrument needs to be returned for any reason.

WARNING! The instrument requires 2–3 people for moving. Moving the system alone may result in serious injury.

Install the QuantStudio™ Absolute Q™ Digital PCR System

IMPORTANT! Ensure that the installation location meets the power and environmental requirements specified in “Installation and environment requirements” on page 52.

1. With 2–3 people, carefully unbox the instrument by cutting the straps and lifting the top of the box off using the hand holes.

   Do not cut or damage any of the packaging. Keep all packaging as it is required for returns or service requests.

2. Carefully place the instrument on a flat, stable surface with no adjacent vibration sources.

3. Position the instrument so that there is access to the power and USB connectors on the left side of the system.

4. Once the instrument is in place, remove the shipping lock screw on the top of the instrument.
   a. With the power off, unscrew the shipping lock screw on the top of the instrument.

   b. Insert the provided white plastic cap into the screw hole.

      For more information on removing the shipping lock screw, see “Uninstall the shipping lock screw” on page 57.

   Keep the shipping lock screw in case the instrument needs to be moved or returned for service.

5. Confirm that the power switch is in the OFF, O, position and then connect the power cable to the instrument and a suitable power source.

6. Set up the dedicated computer and monitor near the instrument.

7. Use the power cable to connect the dedicated computer to a suitable power source.

8. Connect the keyboard and mouse to the back of the dedicated computer.

9. Use the USB cable to connect the instrument to the dedicated computer.

10. Turn on the power to the dedicated computer.

11. Install the software onto the dedicated computer. See “Download and install the software” on page 54.

12. When the software installation is complete, turn on the power to the instrument by moving the power switch located at the left side near the back to the I position.

    Wait approximately 30 seconds for the instrument to initialize.

13. Once connected to the software, check that there are no errors reported.

The system is ready for use.

Download and install the software

Computer requirements for the desktop software

Install the QuantStudio™ Absolute Q™ Digital PCR Software on the computer provided by Thermo Fisher Scientific, and use it to control the instrument. Thermo Fisher Scientific does not support the use of customer-provided computers to control the instrument.

However, you can install the QuantStudio™ Absolute Q™ Digital PCR Software on a customer-provided computer to use the software to import run data for analysis. Minimum requirements for a customer-provided computer are:

- Operating system—Windows™ 10 (64-bit)
- Dell™ OptiPlex XE3 Tower computer

Download the desktop software

1. Go to **https://www.thermofisher.com/us/en/home/global/forms/life-science/quantstudio-absolute-q-software.html**.

2. Download each software package.

Install the desktop software

1. Use a Windows™ Administrator account to log in to the computer on which you are installing the desktop software.

2. For each software package perform the following actions:
   a. Unzip the downloaded software.

   b. Double-click **setup.exe**

   c. Follow the **InstallShield Wizard** prompts to install the software.

   d. Select **Typical** as the setup preference, then click **Next**.

   e. Click **Finish**.

3. Start the QuantStudio™ Absolute Q™ Digital PCR Software.

4. When prompted, accept the End User License Agreement.

5. When prompted, accept or decline the Privacy Statement that allows Thermo Fisher Scientific to use the Connect Transfer Software to collect instrument run data.
   - If you accept, Connect Transfer Software data transmission is activated.
   - If you decline, Connect Transfer Software data transmission is not activated.

Note: Connect Transfer can be deactivated at any time by a user with administrator privileges within the QuantStudio™ Absolute Q™ Digital PCR Software by accessing the Help menu (?), opening the Privacy Statement, and declining data collection.

Moving the instrument

IMPORTANT! When moving the instrument the shipping lock screw must be manually installed before moving the unit, and manually removed after transport. Moving the instrument without the shipping lock screw in place can cause damage to the instrument.

IMPORTANT! When moving the instrument, make sure there is no plate in the instrument as it can become dislodged and jam mechanical parts during instrument transport.

Install the shipping lock screw

1. Power on the instrument.
2. Start the QuantStudio™ Absolute Q™ Digital PCR Software.
3. Open the plate door to ensure there is no plate loaded. If a plate is loaded, remove it.
4. Close the plate door.
5. In the left pane, select to access the **Instrument** page.
6. Click on the instrument and select **Prepare for Shipping**.
   Wait until a message stating *Ready for Shipping* appears before proceeding.
7. Remove the white plastic plug from the shipping screw hole and place it in the bag attached to the shipping screw.
8. Insert the shipping screw and screw it finger tight. Do not over tighten.

9. Close the software and power off the instrument.

① Shipping lock screw

Uninstall the shipping lock screw

IMPORTANT! Perform this task before powering on the instrument.

1. Ensure that the power is off and the instrument is not plugged into a power source.
2. Unscrew the shipping lock screw from the top of the instrument.

① Shipping lock screw

3. Insert the white plastic cap in the shipping lock screw hole.
   The instrument is now ready for power-up and use.

C Use the software with Security, Auditing, and E-signature (SAE) v2.2

The Security, Auditing, and E-signature (SAE) v2.2 software is only compatible with the QuantStudio™ Absolute Q™ Digital PCR System.

For more information on Security, Auditing, and E-signature (SAE) v2.2, including definitions of accounts and roles, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

Overview of the SAE Administrator Console components

The SAE Administrator Console includes three components:

- SAE Administrator Console that an administrator uses to configure the module.
- SAE server that stores settings, user accounts, and audit records. By default, the SAE server is installed on the same computer as the SAE Administrator Console.
- SAE screens in an application (sign in and audit that a user interacts with). QuantStudio™ Absolute Q™ Digital PCR Software is an application.

The SAE Administrator Console provides the following SAE functionality in the QuantStudio™ Absolute Q™ Digital PCR Software:

- **System security**—Controls user sign in and access to functions.
- **Auditing**—Tracks changes and actions performed by users.
- **E-signature**—Allows users to provide an electronic signature (user name and password) when performing certain functions.

Depending on the way that your SAE administrator configures these features:

- Some features and functions that are described in this guide may not be accessible to you.
- You may see dialog boxes and prompts when you use the software.

Overview of the QuantStudio™ Absolute Q™ Digital PCR Software functionality when SAE is enabled

The following features are active when SAE functions are enabled in the QuantStudio™ Absolute Q™ Digital PCR Software:

- Users must sign in with an SAE user account to use QuantStudio™ Absolute Q™ Digital PCR Software.
- Auditing functions are active (if they are enabled in the SAE Administrator Console).
- Run setup and software functions for a user are determined by the SAE application profile and user account settings.

Enable SAE functions

Workflow

Enable SAE functions

1. **Configure SAE Administrator Console application profiles** (page 59)
2. **Connect to the SAE server** (page 60)
3. **Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software** (page 60)

Configure SAE Administrator Console application profiles

Note: Configuring application profiles in the SAE Administrator Console requires an SAE administrator account.

In the SAE Administrator Console, an application profile contains default settings for an application. Before using SAE an administrator must install, then configure the profile for the QuantStudio™ Absolute Q™ Digital PCR Software.

For information on configuring application profiles, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

Connect to the SAE server

- Install the SAE Administrator Console on the dedicated computer with a static IP address (*recommended*) or a dynamic IP address.
- Install, then configure profiles for the QuantStudio™ Absolute Q™ Digital PCR Software.

For information on configuring application profiles, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

1. In the menu bar, click ⚙ **System** ▸ **SAE Connection Settings**.
2. Enter the IP address and port number of the SAE server.

   Note: If using a dynamic IP address, enter the hostname instead of the IP address to prevent the loss of a connection.

3. (*Optional*) Click **Test Connection** to confirm that the connection information is correct.
4. Click **Save**.

Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software

This procedure requires an SAE administrator account.

Before you enable SAE functions in the QuantStudio™ Absolute Q™ Digital PCR Software, you must complete the following tasks:

- Connect to the SAE server (see “Connect to the SAE server” on page 60).
- Close all protocol or analyzed run files.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select ⚙ **System** ▸ **Enable Security**.
2. Enter your SAE administrator account user name and password, then click **Sign In**.

The SAE administrator account is automatically signed into the software after SAE is enabled. The SAE user name is displayed in the upper-right corner of the software menu bar. All users must sign into the software while SAE is enabled.

To sign out of the SAE administrator account, see “Sign out of the software using an SAE account” on page 61.

Note: Signing out of the SAE administrator account does not disable SAE functions in the software. To disable SAE functions in the software, see “Disable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software” on page 64.

Sign into QuantStudio™ Absolute Q™ Digital PCR Software using an SAE account

Sign in for the QuantStudio™ Absolute Q™ Digital PCR Software is only required if SAE functions are enabled by an SAE administrator (see “Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software” on page 60).

1. In the QuantStudio™ Absolute Q™ Digital PCR Software sign in screen, enter your SAE user name and password.
2. Click **Sign In**.

The user name of the SAE account that is signed in to the software appears in the menu bar.

Sign out of the software using an SAE account

1. In the lower-left corner of the left pane, click .
2. Click **Sign Out**.

Change your SAE account password

Note: External user accounts (External/Federated LDAP repository accounts) cannot change their password in the software.

1. In the lower-left corner of the left pane, click .
2. Click **Change Password**.
3. Enter the password information, then click **OK**.

Default permissions and roles

The SAE module provides the following default permissions and roles. You can use the default roles when you create SAE user accounts or create custom roles in the Security, Auditing, and E-signature (SAE) v2.2 administrator console (see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468)).

- Administrator
- Scientist
- Technician
- Service

IMPORTANT! SAE permissions for a role apply to all user accounts that are assigned to the role.

The roles and associated user-configurable permissions are listed in the following table. You can also double-click the role in the **Roles** tab to display the list of permissions.

Note: The **No Privileges** role is used by the software when you set up user repositories. Do not assign this role to a user account.

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Miscellaneous					
Service access	Access to the instrument service menu.	No	No	No	Yes
Application administration	Access to application administration menus.	Yes	No	No	Yes
Generate report	Create analysis reports.	Yes	Yes	Yes	Yes
Instrument Control					
Edit protocol	Edit run protocols.	Yes	Yes	No	Yes
Start run	Choose a protocol and start and stop instrument runs.	Yes	Yes	Yes	Yes
Run analysis					
Change thresholds	Change channel thresholds.	Yes	Yes	No	Yes
Edit groups	Edit group definitions.	Yes	Yes	No	Yes
Rename samples	Change sample names.	Yes	Yes	Yes	Yes
Assign samples	Assign samples to set groups or load a group set.	Yes	Yes	Yes	Yes
Hide samples	Show or hide samples from an analysis.	Yes	Yes	No	Yes
Run management					
Delete run	Delete a run from the database.	Yes	No	No	Yes
e-sign run	Place an electronic signature on a run.	Yes	Yes	No	Yes

(continued)

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Import run	Import and export runs to and from ZST files.	Yes	Yes	No	Yes
Edit run	Edit run features.	Yes	Yes	No	Yes

Specify audit reason

Depending on how your SAE administrator configures the audit settings in the SAE Administrator Console, the **Enter Audit Reason** screen may be displayed when you make changes to a protocol or an analyzed run in the QuantStudio™ Absolute Q™ Digital PCR Software.

Select a reason from the drop down list, or add a custom reason.

Note: **Custom Reason** is not displayed if audit settings are configured to require users to select a reason.

View audit records

For information on viewing audit records for a protocol or an analyzed run, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

Use the following steps to view the audit record of a specific run by using the Run ID for the run.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select the desired run.
   For information on selecting a run, see “Select a run” on page 25.
2. In the upper-right corner of the **Run** screen, hover over the **Run ID** and click **Copy to clipboard**.
3. At the SAE Administrator Console perform the following steps:
   a. Select **Audit History > Application Object Records.**
   b. Select **Enable Application Objects Filtering**.
   c. In the **Object name** field, paste the Run ID that you copied in step 2.
   d. Click **Search**.

   The information regarding the run appears in results area of the **Audit History** screen.

Export audit records

For information on exporting audit records for a protocol or an analyzed run, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

Sign data in the software

1. In the left pane, click to access the **Runs** page.
2. Use the search fields to find a run or select a run from the list, then select the **RESULTS** tab.
3. In the upper-right side of the page click .
4. Select an option from the drop down list to indicate the meaning of the e-signature.
   - Reviewed and Approved Plate Results

   Note: The number of options will vary based on your configuration.
5. Enter your user name and password.
6. Click **Sign**.

If a run is signed and unmodified, the signature will show on reports that are created using **GENERATE REPORT**.

For information on viewing e-signatures in the SAE software, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

View e-signatures

For information on viewing e-signatures, see the *SAE Administrator Console v2 User Guide* (Pub. No. MAN0017468).

Disable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software

This procedure requires an SAE administrator account.

Close all plate files and data files.

1. In QuantStudio™ Absolute Q™ Digital PCR Software, select **System** ▸ **Disable Security**.
2. Enter the password of the SAE administrator account, then click **Sign In**.

D

Maintain the instrument

Clean the instrument and plate nest

All surfaces should be dry and free of dust and lint before operation.

Clean the outside of the instrument with a damp, lint-free cloth using one of the following solutions:

- Mild soap
- 70% ethanol in water

Clean the plate nest gently with a lint-free cloth (microfiber cloth or optical lens cleaning cloth) using 70% ethanol in water.

IMPORTANT! The plate nest is covered in a thin graphite sheet. This sheet is susceptible to scratches and may impact results if it is damaged. It is important to only wipe the surface with lint-free wipes or use air-dusters. Contact technical support if this surface becomes damaged (see Appendix H, “Documentation and support”.

Maintenance

For best results when using the instrument, do the following:

- The plate nest must be cleaned before each run.
- Ensure that the fan vents on the back and bottom of the instrument are not obstructed.

For information on maintenance and service plans, contact technical support (see Appendix H, “Documentation and support”).

E Troubleshooting

Observation	Possible cause	Recommended action
QuantStudio™ Absolute Q™ Digital PCR Software is not connecting, front panel LEDs are white.	Software connection error	Power cycle system using power switch on the side of the instrument.
		Uninstall and re-install the software.
QuantStudio™ Absolute Q™ Digital PCR Software is not connecting, front panel LEDs are blue.	Poor USB connection	Power off the instrument. Unplug the power and USB cables from the instrument. Wait 10 seconds. Plug the power and USB back in to the instrument and dedicated computer. Power on and connect.
Front panel LEDs are red.	Instrument error	Power cycle the instrument using the power switch.
Pressure leak error.	Missing or damaged gaskets	Make sure that all 5 columns of gaskets are present.
		Replace any damaged gaskets.
Instrument makes noise and LEDs are white one minute after power up.	Instrument firmware startup error	Power off the instrument. Unplug the power cable from the instrument. Wait 10 seconds. Plug the power cable back in and power on the instrument.
Barcode not found.	Plate in backwards	Well A1 should be at the top left of the plate tray.
	Missing or unreadable barcode label	Enter the barcode manually if it is human readable.

F

Product Specifications

QuantStudio™ Absolute Q™ Digital PCR Instrument specifications

Dimensions (unpacked)	620 mm (l) x 600 mm (w) x 540 mm (h) 24.5 in (l) x 23.5 in (w) x 21.2 in (h)
Dimensions (packaged)	860 mm (l) x 860 mm (w) x 790 mm (h) 33.5 in (l) x 34 in (w) x30 (h)
Weight	Approximately 60 kg, 132 lbs
Connections	Power, USB 3.0 (to dedicated computer)
Cooling mode	Forced convection
Illumination	Rax, Blue, Phosphor Green high-power LED
Optical channels	5 (fixed configuration)
Power input	100-240 V, 50-60Hz
Power rating	1200-1600 W
Rated current	12 A (110V), 8.5 A (230 V)
Maximum noise level	70 dB

Dedicated computer requirements

Operating system	Windows™ 10 (64-bit) or later
Computer	Dell™ OptiPlex XE3 Tower

QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Configuration

The QuantStudio™ Absolute Q™ Digital PCR Instrument comes in a single optical configuration and is pre-calibrated during manufacturing. It can be field calibrated for enhanced spectral compensation.

Note: Contact technical support to request custom calibration service (see Appendix H, "Documentation and support").

#	Color	Excitation Filter Peak	Emission Filter Peak	Compatible Dyes
1	Blue	466	520	FAM™
2	Green	514	560	HEX™ VIC™
3	Yellow	549	589	ABY™
4	Red	589	625	ROX™
5	Dark Red	630	684	CY5™ JUN™

G

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Caution, air inlet.
CAUTION! Hot surface.
CAUTION! Potential biohazard.

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Protective conductor terminal (main ground)

Conformity symbols

Conformity mark	Description
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Hot Surface

CAUTION! Hot surface. During instrument operation, the temperature of the plate nest can be as high as 100° C. The instrument has a software interlock to prevent the door from opening if the plate nest temperature is over 45° C, but if the system appears to be malfunctioning use caution when operating near the plate nest.

Air inlet

CAUTION! Air inlet. Air inlet is only suitable for atmospheric air and not pressurized gas. Do not connect flammable gas to the air inlet port. Do not restrict air inlet port.

Physical injury

CAUTION! Moving and Lifting Injury. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

Instrument component and accessory disposal

CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2011/65/EU & Commission Delegated Directive (EU) 2015/863	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment
IEC 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EMC EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Class A equipment Caution	This device complies with Part 15 of the FCC rules. 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.
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* 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.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number	Description
QuantStudio™ Absolute Q™ Digital PCR Starter Kit User Guide	MAN0025653	Describes the setup, use, and analysis of runs using the QuantStudio™ Absolute Q™ Digital PCR Starter Kit assay. (Catalog No. A52732)

Note: For additional documentation, see "Customer and technical support" on page 77.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.