Cytation 5

Imaging Multi-Mode Microplate Reader

User Manual

ERRATA NOTICE: This document contains references to BioTek. Please note that BioTek is now Agilent. For more information, go to www.agilent.com/lifesciences/biotek.

Cytation 5

Imaging Multi-Mode Microplate Reader

User Manual

ERRATA NOTICE: This document contains references to BioTek.
Please note that BioTek is now Agilent.
For more information, go to www.agilent.com/lifesciences/biotek.

Document part number 1321000N
Revision A
BioTek Instruments, Inc.
December 2021

Contents

Notices

All Rights Reserved

Restrictions and Liabilities

Information in this document is subject to change and does not represent a commitment by BioTek Instruments, Inc. Changes made to the information in this document will be incorporated in new editions of the publication. No responsibility is assumed by BioTek for the use or reliability of software or equipment that is not supplied by BioTek or its affiliated dealers.

Contact Information

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Worldwide Sales and Support

www.agilent.com/en/contact-us/page

Technical Support and Service

www.agilent.com/en/support

bio.tac@agilent.com

Instrument service and repair is available worldwide at one of our international service centers and in the field at your location.

UK Responsible Person (UKRP)

Agilent LD UK Ltd

5500 Lakeside

Cheadle Royal Business Park

Cheadle, Cheshire SK8 3GR

Intended Use Statement

The Cytation 5 is an imaging multi-mode microplate reader and intended to be used for the examination of specimens to analyze their characteristics and impact on a variety of analytes.

Quality Control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

Warranty and Product Registration

Review the warranty information that shipped with your product. Register your product to ensure you receive important information updates about the products you have purchased.

Safety Notices

Raadpleeg Bijlage C voor informatie in andere talen.

Reportez-vous à l'annexe C pour obtenir des informations dans d'autres langues.

Informationen in anderen Sprachen finden Sie in Anhang C.

Fare riferimento all'Appendice C per informazioni in altre lingue.

Consulte el Apéndice C para obtener información en otros idiomas.

Pay special attention to the following safety notices in all product documentation.

WARNING A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

CAUTION A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

Warnings and Precautions

Electrical Hazards

WARNING

Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

WARNING

Power Rating. The instrument's power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

WARNING

Electrical Grounding. Never use a plug adapter to connect primary power to the external power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.

WARNING

Service. Only qualified technical personnel should perform service procedures on internal components.

CAUTION

Power Supply. Use only the power supply shipped with the instrument, and operate it within the range of line voltages listed on it.

Chemical/Environmental

WARNING

Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemical-resistant rubber gloves and apron.

WARNING

Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, stop the program and turn off the instrument. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

CAUTION

Liquids. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

CAUTION

Environmental Conditions. Do not expose the instrument to temperature extremes. For proper operation, temperature near the instrument should remain within the range in the ***Specifications*** section of this document. Performance may be adversely affected if

temperatures fluctuate above or below this range.

CAUTION

Sodium Hypochlorite. Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

CAUTION

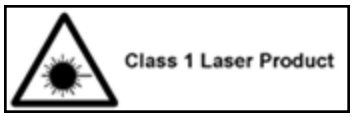
DMSO Concentration. Dimethyl sulfoxide (DMSO) vapor can coat optical surfaces, which can trigger instrument self-test errors. Using DMSO assay concentrations of 2% or below is recommended. Limit long exposure in kinetic assays or incubated assays when possible.

CAUTION

Lubricants. Do not apply lubricants to moving parts. Lubricant on components in the carrier compartment will attract dust and other particles, which may cause the instrument to produce an error.

Components

WARNING



Class 1 Laser Product. "A" models. "V" and "W"models when the optional Laser Autofocus Cube is installed.

WARNING

Pinch Hazard. Some areas of the instrument and the dispense module can present pinch hazards when the instrument is operating. The objective turret and dispense module are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.

WARNING

Two person lift. The instrument should be lifted by two people. The instrument weighs up to 36.3 kg (45.3 kg with the Peltier Cooling Module installed).

WARNING

Accessories. Only accessories that meet the manufacturer's specifications shall be used with the instrument.

CAUTION

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

CAUTION

Spare Parts. Only approved spare parts should be used for maintenance. The use of unapproved spare parts and accessories may result in a loss of warranty and potentially impair instrument performance or cause damage to the instrument.

CAUTION

Service. Only qualified technical personnel should perform service procedures on internal components.

CAUTION

Instrument Configuration. When you install or change objectives, LED cubes, imaging filter cubes, or fluorescence filter cubes you must update the instrument configuration values in Gen5 and download the information to the instrument.

CAUTION

LED cubes and filter cubes. Wear gloves when changing components to avoid contaminating them.

CAUTION

Objectives. Do not use these cleaning solvents: Methyl ethyl ketone (MEK), Dimethyl ketone (acetone).

Intended Product Use

WARNING

Software Quality Control. The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct quality control checks could result in erroneous test data.

WARNING

Data Reduction. No limits are applied to the raw measurement data. Data exported via computer control must be analyzed by the operator. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. Users must evaluate this instrument and PC-based software in conjunction with their specific assay(s). This evaluation must include the confirmation that performance characteristics for the specific assay(s) are met.

WARNING

Unspecified Use. Failure to operate equipment according to the guidelines and safeguards specified in the product user documentation could result in a hazardous condition.

CAUTION

Use of labware other than described in this document can result in positioning errors during program execution.

Symbols

Caution, consult the instructions for use for important cautionary information such as warnings and precautions.

Voorzichtig, raadpleeg de gebruiksaanwijzing voor belangrijke voorzorgsinformatie zoals waarschuwingen en voorzorgsmaatregelen.

Attention, pour des informations de mise en garde importantes telles que des avertissements et des précautions, consultez le mode d'emploi.

Achtung, lesen Sie die Gebrauchsanweisung für wichtige Vorsichtshinweise wie Warnungen und Sicherheitsvorkehrungen

Attenzione, consultare le istruzioni per l'uso per importanti informazioni cautelative come avvertenze e precauzioni.

Precaución, consulte las instrucciones de uso para obtener información importante, como advertencias y precauciones.

Warning; Biological hazard

Waarschuwing; biologisch gevaar

Avertissement : Risque biologique

Warnung; biologische Gefahr

Avvertenza, rischio biologico

Advertencia: peligro biológico

Warning; Pinch hazard

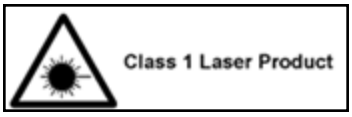
Waarschuwing; beknellingsgevaar

Avertissement : risque de pincement

Warnung; Quetschgefahr

Avvertenza, rischio di pizzicamento

Advertencia: peligro de atrapamiento



Warning; Class 1 Laser Product. “A” models. “V” and “W” models when the optional Laser Autofocus Cube is installed.

Waarschuwing; Klasse 1 laserproduct. “A” modellen. "V"- en "W"-modellen wanneer de optionele Laser Autofocus Cube is geïnstalleerd.

Avertissement; Produit laser de classe 1. Modelles « A». Modèles « V » et « W » lorsque le cube laser autofocus en option est installé.

Warnung; Laserprodukt der Klasse 1. „A“-Modelle. „V“- und „W“-Modelle, wenn der optionale Laser Autofokus Cube installiert ist.

Avvertimento; Prodotto laser di classe 1. Modelli“A”. Modelli “V” e “W” quando è installato il Laser Autofocus Cube opzionale.

Advertencia; Producto láser de clase 1. Modelos “A”. Modelos “V”

y “W” cuando está instalado el cubo de enfoque automático láser opcional.

Complies with FDA performance standards for laser products except for conformance with IEC 60825-1 Ed.3., as described in Laser Notice No.56, dated May 8, 2019.

Voldoet aan de FDA-prestatienormen voor laserproducten, behalve conformiteit met IEC 60825-1 Ed.3., zoals beschreven in Laser Notice No.56, gedateerd 8 mei 2019.

Conforme aux normes de performance de la FDA pour les produits laser, à l'exception de la conformité à la norme IEC 60825-1 Ed.3., comme décrit dans l'avis laser n°56, daté du 8 mai 2019.

Entspricht den FDA-Leistungsstandards für Laserprodukte mit Ausnahme der Konformität mit IEC 60825-1 Ed.3., wie in Laser Notice No.56 vom 8. Mai 2019 beschrieben.

Conforme agli standard di prestazione FDA per i prodotti laser, fatta eccezione per la conformità a IEC 60825-1 Ed.3., come descritto nell'avviso laser n.56, datato 8 maggio 2019.

Cumple con los estándares de rendimiento de la FDA para productos láser, excepto por la conformidad con IEC 60825-1 Ed.3., Como se describe en el Aviso sobre láser n.o 56, con fecha del 8 de mayo de 2019.

Warning: two-person lift.

Waarschuwing; Tweepersoonslift.

Avertissement; Ascenseur pour deux personnes.

Warnung; Zwei-Personen-Lift.

Avvertimento; Ascensore per due persone.

Advertencia; Elevador de dos personas.

Disposal Notice: Dispose of the instrument according to Directive 2012/19/EU, “on waste electrical and electronic equipment (WEEE)” or local ordinances

Kennisgeving van verwijdering: Verwijder het instrument volgens Richtlijn 2012/19/EU betreffende afgedankte elektrische en elektronische apparatuur (AEEA) of lokale verordeningen

Avis concernant la mise au rebut : mettez l'instrument au rebut conformément à la directive 2012/19/EU portant sur les déchets d'équipement électrique et électronique (DEEE) ou aux dispositions locales.

Entsorgungshinweis: Entsorgen Sie das Gerät gemäß der Richtlinie 2012/19/EU „für Elektro- und Elektronik-Altgeräte (WEEE)“ bzw. den Landesvorschriften.

Avviso per lo smaltimento: smaltire lo strumento in base alla Direttiva 2012/19/EU, sui "rifiuti di apparecchiature elettriche ed elettroniche (WEEE)" o le ordinanze locali

Aviso de eliminación: elimine el instrumento de conformidad con la Directiva 2012/19/UE sobre residuos de aparatos eléctricos y electrónicos (RAEE) o las ordenanzas locales

CE Marking — Indicates compliance with the requirements of the Directive 2014/30/EU on Electromagnetic Compatibility and the Directive 2014/35/EU on Low Voltage

CE-markering – Geeft aan dat wordt voldaan aan de vereisten van Richtlijn 2014/30/EU inzake elektromagnetische compatibiliteit en Richtlijn 2014/35/EU inzake laagspanning

Marquage CE – Indique la conformité aux exigences de la directive 2014/30/UE sur la compatibilité électromagnétique et de la directive 2014/35/UE sur la basse tension

CE-Kennzeichnung – Zeigt die Einhaltung der Anforderungen der Richtlinie 2014/30/EU über elektromagnetische Verträglichkeit und der Richtlinie 2014/35/EU über Niederspannung

Marcatura CE – Indica la conformità ai requisiti della Direttiva 2014/30/UE sulla Compatibilità Elettromagnetica e della Direttiva 2014/35/UE sulla Bassa Tensione

Marcado CE: indica el cumplimiento de los requisitos de la Directiva 2014/30 / UE sobre compatibilidad electromagnética y la Directiva 2014/35 / UE sobre baja tensión.

Date of manufacture

Productiedatum

Date de fabrication

Herstellungsdatum

Data di produzione

Fecha de fabricación

TÜV SÜD Certification Mark – Type tested; production monitored

TÜV SÜD certificeringsmerk - type getest; productie bewaakt

TÜV SÜD Marque de certification – Type testé ; production contrôlée

TÜV SÜD-Prüfzeichen – Typ geprüft; Produktion überwacht

Marchio di certificazione TÜV SÜD: tipo testato, produzione monitorata

Marca de certificación TÜV SÜD: tipo probado, producción controlada

This product complies with environmental protection use period as defined in People's Republic of China Electronic Industry Standard SJ/T11364-2006. Toxic or hazardous substances will not leak or mutate under normal operating conditions for 40 years.

Dit product voldoet aan de milieubeschermingsgebruiksperiode

zoals gedefinieerd in de Electronic Industry Standard SJ/T11364-2006 van de Volksrepubliek China. Giftige of gevaarlijke stoffen zullen onder normale bedrijfsomstandigheden gedurende 40 jaar niet lekken of muteren.

Ce produit est conforme à la période d'utilisation dans le cadre de la protection de l'environnement telle que définie par la norme de l'industrie électronique de la République populaire de Chine SJ/T11364-2006. Les substances toxiques ou dangereuses ne fuiront pas ou ne subiront pas de mutation dans des conditions de fonctionnement normales pendant 40 ans.

Dieses Produkt entspricht der Umweltschutz-Nutzungsdauer gemäß der Definition im Electronic Industry Standard SJ/T11364-2006 der Volksrepublik China. Giftige oder gefährliche Stoffe werden unter normalen Betriebsbedingungen 40 Jahre lang nicht austreten oder mutieren.

Questo prodotto è conforme al periodo di utilizzo della protezione ambientale come definito nello Standard del settore elettronico della Repubblica Popolare Cinese SJ/T11364-2006. Le sostanze tossiche o pericolose non fuoriescono o non subiscono mutazioni in condizioni operative normali per 40 anni.

Este producto cumple con el periodo de uso de protección ambiental según el estándar SJ/T11364-2006 de la República Popular China para la industria electrónica. Las sustancias tóxicas o peligrosas no se filtrarán ni mutarán en condiciones de funcionamiento normales durante 40 años.

UK Conformity Assessed marking is a certification mark that indicates conformity with the applicable requirements for products sold within Great Britain.

De 'UK Conformity Assessed'-markering is een certificeringsmerk dat aangeeft dat producten die in Groot-Brittannië worden verkocht, voldoen aan de toepasselijke eisen.

Le marquage UK Conformity Assessed est une marque de certification qui indique la conformité aux exigences applicables aux produits vendus en Grande-Bretagne.

Die Kennzeichnung „UK Conformity Assessed“ ist ein Zertifizierungszeichen, das die Konformität mit den geltenden Anforderungen für in Großbritannien verkaufte Produkte anzeigt.

Il marchio UKCA (conformità valutata del Regno Unito) è un marchio di certificazione che indica la conformità ai requisiti applicabili per i prodotti venduti in Gran Bretagna.

El marcado UKCA (UK Conformity Assessed) es una marca de certificación que indica la conformidad con los requisitos aplicables para los productos vendidos en Gran Bretaña.

EAC-MED is a certification mark to indicate products that conform to all the safety and quality requirements of the Eurasian Customs Union. It means that the EAC-MED marked products meet all requirements of the corresponding technical regulations and have passed all conformity assessment procedures.

EAC-MED is een certificeringsmerk om producten aan te duiden die voldoen aan alle veiligheids- en kwaliteitseisen van de Euraziatische douane-unie. Dit betekent dat de producten met een EAC-MED-markering aan alle eisen van de desbetreffende technische voorschriften voldoen en alle conformiteitsbeoordelingsprocedures hebben doorlopen.

EAC-MED est une marque de certification qui indique la conformité des produits à toutes les exigences de sécurité et de qualité de l'Union douanière eurasiatique. Cela signifie que les produits marqués EAC-MED satisfont à toutes les exigences des réglementations techniques correspondantes et ont passé toutes les procédures d'évaluation de la conformité.

EAC-MED ist ein Zertifizierungszeichen zur Kennzeichnung von Produkten, die allen Sicherheits- und Qualitätsanforderungen der Eurasischen Zollunion entsprechen. Das bedeutet, dass die EAC-MED-gekennzeichneten Produkte alle Anforderungen der entsprechenden technischen Bestimmungen erfüllen und alle Konformitätsbewertungsverfahren bestanden haben.

EAC-MED è un marchio di certificazione che indica prodotti conformi a tutti i requisiti di sicurezza e qualità dell'Unione doganale eurasiatica. Ciò significa che i prodotti con marchio EAC-MED soddisfano tutti i requisiti dei regolamenti tecnici corrispondenti e hanno superato tutte le procedure di valutazione della conformità.

EAC-MED es una marca de certificación para indicar productos que cumplen con todos los requisitos de seguridad y calidad de la Unión Aduanera Euroasiática. Significa que los productos con la marca EAC MED cumplen todos los requisitos de los reglamentos técnicos correspondientes y han superado todos los procedimientos de evaluación de conformidad.

Product complies with Australian Communications Requirements

EESS – The Regulatory Compliance Mark (RCM)

ACMA Labeling Requirements

Product voldoet aan de Australische communicatie-eisen

EESS – De markering voor naleving van de regelgeving (RCM)

ACMA-etiketteringsvoorschriften

Le produit est conforme aux exigences australiennes en matière de communication

EESS – Marque réglementaire de conformité (RCM)

Exigences en matière d’étiquetage ACMA

Das Produkt entspricht den australischen Kommunikationsanforderungen.

EESS – Kennzeichnung „Regulatory Compliance Mark“ (RCM)

ACMA-Kennzeichnungsanforderungen

Il prodotto è conforme ai requisiti Australian Communications Requirements

EESS: marchio di conformità alle normative

Requisiti di etichettatura ACMA

El producto cumple con los requisitos de comunicaciones de Australia.

EESS: marcado RCM (Regulatory Compliance Mark) de cumplimiento de la normativa.

Requisitos de etiquetado de ACMA

Korea Certification (KC) mark signifies Korea product compliance mark for safety and EMC/Radio/SAR of electrical and electronic equipment. The EMC requirements are applied to Agilent products.

Korea Certification (KC)-merkteken staat voor Korea-productconformiteitsmerk voor veiligheid en EMC/Radio/SAR van elektrische en elektronische apparatuur. De EMC-eisen worden toegepast op Agilent-producten.

La marque de certification coréenne (KC) signifie la marque de conformité des produits coréens pour la sécurité et l'EMC/Radio/SAR des équipements électriques et électroniques. Les exigences CEM s'appliquent aux produits Agilent.

Das Korea-Zertifizierungszeichen (KC) bezeichnet das koreanische Produktkonformitätszeichen für Sicherheit und EMV/Funk/SAR von elektrischen und elektronischen Geräten. Die EMV-Anforderungen gelten für Agilent-Produkte.

Il marchio Korea Certification (KC) indica il marchio di conformità del prodotto Corea per la sicurezza e EMC/Radio/SAR di apparecchiature elettriche ed elettroniche. I requisiti EMC vengono applicati ai prodotti Agilent.

La marca de certificación de Corea (KC) significa la marca de cumplimiento de productos de Corea para la seguridad y EMC / Radio / SAR de equipos eléctricos y electrónicos. Los requisitos de EMC se aplican a los productos Agilent.

Conformance to Standards

The Cytation 5 meets the requirements of the following standards:

2014/35/EU – Low Voltage Directive

2014/30/EU – EMC Directive

2011/65/EU (with exemptions) and (EU) 2015/863 – RoHS Directives

2012/19/EU – WEEE Directive as amended by (EU) 2018/849

2006/42/EC of the European Parliament and of the Council of 17 May 2006 on machinery

Standard	Description
IEC QC 080000	IEC Quality Assessment System for Electronic Components (IECQ System) - Hazardous Substance Process Management (HSPM) System Requirements
UL 61010-1	UL Standard for Safety Electrical Equipment For Measurement, Control, and Laboratory Use; Part 1: General Requirements
EN 61010-1	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 1: General Requirements
EN 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
EN 60825-1	"Safety of laser products. Part 1: Equipment classification and requirements."
CAN/CSA C22.2 No. 61010-1	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 1: General Requirements
CAN/CSA C22.2 No. 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

EMC Information and Technical Description

The Cytation 5 conforms to:

Emissions:

EN55011/CISPR 11, Class A

CFR Title 47 FCC Part 15 Subpart B, Class A

ICES-001, Issue 5, Class A (CAN ICES-001(A)/NMB-001(A))

ACMA AS/NZS CISPR 11, Class A

Immunity:

EN/IEC 61326-1 and 61326-2-6

ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL AND LABORATORY USE

PART 1: GENERAL REQUIREMENTS FOR (NON IVD) LISTED PRODUCTS

Ingress Protection Code

IP 20. Protected against solid foreign objects of 12.5 mm diameter and greater. No protection against water.

Disposal

Dispose of the instrument according to Directive 2012/19/EU, "on waste electrical and electronic equipment (WEEE)" or local ordinances.

Chapter 1

Introduction

This chapter introduces the Cytation 5 Imaging Multi-Mode Reader and describes its hardware and software features.

Product Description

The Cytation 5 is an imaging multi-mode reader. Depending on the model, Cytation 5 detection modes include fluorescence intensity (FI), fluorescence polarization (FP), time-resolved fluorescence (TRF), luminescence, UV-visible absorbance, alpha, and imaging.

The instrument is modular, and upgrade options are available; contact BioTek for more information. The reader is computer-controlled using Gen5 software for all operations, including data reduction and analysis. The Cytation 5 is robot accessible and compatible with the BioTek's BioStack 3 and BioStack 4 Microplate Stackers, and BioSpa 8 Automated Incubator. Gen5 supports OLE automation to facilitate the Cytation 5's integration into an automated system.

The Cytation 5 can perform reads using a filter cube or a monochromator.* The filter-based system can perform top fluorescence and luminescence reads. Filter fluorescence uses a xenon flash light source, along with interference filters and dichroic mirrors for wavelength specificity and a photomultiplier tube (PMT) detector. To run a fluorescence polarization protocol, the filter cube must contain polarizing filters. Luminescence is measured through an empty filter position in the filter cube; filters can be used if light filtering is necessary.

The monochromator-based system, which has both top and bottom probes, is used for absorbance, top and bottom fluorescence intensity, and luminescence spectral scans. The xenon lamp allows for both UV and visible light measurements. The monochromator provides wavelength selection from 230–999 nm in 1-nm increments. Available read methods are endpoint, area scan, spectral scanning, and pathlength correction. For luminescence reads, the Cytation 5 also has a direct-to-PMT channel (no filtering, white light only).

The alpha detection system can be used for endpoint reads when installed with the top filter system in non-synchronized plate mode. The imaging system comprises up to four LED cubes and four imaging filter cubes, up to six objectives, and a digital camera, which captures images directly through the selected objective and filter cube assembly. Brightfield imaging is also available.

The Cytation 5 has 4-Zone temperature control from 4°C over ambient to 65°C, controlled via a software-adjustable gradient. Internal plate shaking, with both linear and orbital modes, is supported to ensure that reagents are properly mixed prior to reading.

The Cytation 5 supports the reading of 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry, 1536-well microplates (for filter-based measurement and imaging only), the Take3 and Take3 Trio Multi-Volume Plates, and microscope slides, Petri dishes, and flasks (using the adapters available from BioTek).

Note: Note: Use of microplates other than those listed here can result in positioning errors during program execution.

Models with injectors support dual-reagent injection to 6-, 12-, 24-, 48-, 96-, and 384-well microplates. An external dispense module pumps fluid from the supply bottles to the two injectors located inside the instrument.

Models used with the gas controller can control the CO_2 or O_2 concentrations in the reading chamber for CO_2- or O_2-sensitive assays.

Note: See *Appendix A* for performance and technical specifications.

* This dual light path capability is protected by U.S. patent.

Package Contents

Item	Part #
Gen5 software	Various
Power supply	02285
Power cord set (varies by region): Europe (Schuko) USA/International United Kingdom Australia/New Zealand	 75010 75011 75012 75013
USB (2.0) cable	75108
Phillips screwdriver #2	01188
9/64" hex wrench	01623
Cytation 5 User Manual (delivered on USB flash drive)	1321000N
Models with the imaging module:	
USB3 components	Various
Controller (host computer)	Various
Microplate slide holder	1220548
Microplate slide holder (4 slides)	1650516
Objective calibration plate	1222531; “W” models: 1852501
3/32" hex wrench	48570

Optional Accessories

Item	Part #
Optional Dispense Module (8040036), with the following accessories:	
Injector	8040541
Inlet tubes (2) from supply bottles to syringe drives	7082121
250-µL syringes (2)	7083000
Syringe thumbscrews	19511
Priming plate	8042202
Dispense tip priming trough	8042068
Dispense module communication cable	01170
Dispense module front cover	8042197
Supply bottles (2, 30 mL)	7122609
Supply bottle holders (2)	8042193
Dispense-tip-cleaning stylus kit	2872304
Strap reagent racks (6)	7212035
Optional Gas Controller (packed separately), with the following accessories:	
Gas controller unit, CO_2/O_2 control	1210008/ 1210013
Gas Controller Unit, CO_2 only	1210007/ 1210012

Item	Part #
Joystick	1320003
Isolation table	1220521
Isolation table for use with BioStack	2010002
Isolation table for cooling module equipped instruments	1700508
Peltier Cooling Module	1700001
Absorbance Test Plate (400-800 nm) for absorbance measurement testing	7260522
Absorbance Test Plate (340 nm)[1]	7260551
Cytation 5 Product Qualification (IQ-OQ-PQ) package	1320532N
T25 Flask adapter	1222210
Petri dish holder, 35 mm, max. diam: 38.7 mm	1222240
Petri dish holder, 35 mm, max. diam: 35.5 mm	1222246
Petri dish holder, 100 mm	1222242
Petri dish holder, 60 mm	1222241
Imaging qualification plate	1222520
Objective adapter collar wrench	1222187
Take3 Micro-Volume Plate	TAKE3
Take3 Trio Micro-Volume Plate	TAKE3TRIO
PCR Tube Adapter Plates	6002072

[1]The diagnostics feature in Gen5 versions 2.08 and higher is compatible with the 340 nm Absorbance Test Plate, BTI PN# 7260551. If you are using an earlier Gen5 version, the test plate's instruction sheet explains how to manually conduct the tests and analyze results.

Item	Part #
	6002076
BioCell Quartz Vessel	7272051
BioCell Adapter Plate	7270512
Harta Luminometer Reference Microplate (includes plate carrier adapter, PN 1222205)	8030015
Fluorescence Test Plate[1]	1400501
Laser Autofocus Filter Cube[2]	1225010
Filters, fluorescence filter cubes, LED cubes, imaging filter cubes, and objectives are available for purchase. Contact BioTek for part numbers and availability.	
The Cytation 5 is compatible with the BioStack Microplate Stacker and the BioSpa Automated Incubator. Contact BioTek or visit our website to learn more.	

Materials for Liquid Tests	Part #
Absorbance Liquid Test Solutions:	
BioTek Wetting Agent Solution	7773002
BioTek QC Check Solution No. 1 (25 mL)	7120779
BioTek QC Check Solution No. 1 (125 mL)	7120782
Phosphate-Buffered Saline (PBS) tablets (pH 7.2–7.6)	Sigma #P4417
β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, reduced form)	BioTek PN 98233 or Sigma #N6785-10VL
Fluorescence Liquid Tests	

[1]Requires Gen5 version 2.06 or higher
[2]Requires Gen5 version 2.08 or higher.

Materials for Liquid Tests	Part #
Test Kits	
Kit with microplates and test solutions for conducting Corners/Sensitivity/Linearity (FI) tests using Sodium Fluorescein, Methylumbelliferone, and Time-Resolved Fluorescence (TRF) tests using Europium	7160010 (contains 7160012, 7160013, and 7160011 described below)
Kit for FI tests using Sodium Fluorescein	7160013
Kit for FI tests using Methylumbelliferone	7160012
Kit for TRF tests using Europium	7160011
Kit for Fluorescence Polarization (FP) test	7160014 or Invitrogen #P3088
Individual Materials	
Sodium Fluorescein Powder, 1-mg vial	98155
Methylumbelliferone (MUB), 10-mg vial	98156
Carbonate-Bicarbonate Buffer (CBB) capsules	Sigma #3041
Phosphate-Buffered Saline (PBS) tablets, pH 7.2-7.6	Sigma #P4417
Sodium Borate, pH 9.18	Fisher Scientific #159532, or equivalent
Injection System Tests:	
BioTek Green Test Dye	7773003

Technical Support

Note: See also Contact Information on page viii.

Please be prepared to provide the following information:

- Your name and company information, daytime phone number and/or e-mail address.
- The product name, model, and serial number.
- The onboard software part number and basecode version (available via Gen5): Select **System > Instrument Configuration**, select your instrument, then click **View/Modify > Setup**, select the **Basecode tab**, then click **Get Basecode Information**.
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed in Gen5.
- A text file of the diagnostic history of the instrument (available via Gen5 by selecting **System > Diagnostics > History**, then selecting the appropriate file and clicking **Export**).

Chapter 2

Installation

This chapter includes instructions for unpacking and setting up the Cytation 5 and, if applicable, the external dispenser. Instructions are also included for preparing the instrument and dispenser for shipment.

Important Information

Two person lift. The instrument should be lifted by two people. The instrument with all available modules weighs up to 36.3 kg (45.3 kg with the Peltier Cooling Module installed).

CAUTION

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

- This chapter contains installation and setup tasks for the Cytation 5 and accessories. Perform the tasks in the order presented.
- Save all packaging materials. Be sure to use packaging materials supplied by the manufacturer when shipping the instrument. Using other forms of commercially available packaging, or failing to follow the repackaging instructions, may void your warranty.
- During the unpacking process, inspect the packaging, instrument, and accessories for shipping damage. If the instrument is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection.

Unpack and Inspect the Instrument

Two person lift. The instrument should be lifted by two people. The instrument with all available modules weighs up to 36.3 kg (45.3 kg with the Peltier Cooling Module installed).

1. Open the shipping box, remove the instrument from the box, and place it on a level, stable surface.

   One unpacking option is to carefully cut the tape sealing the bottom seam of the outer box, and then lift the box off the inner box. The inner box sides fold out.
2. Place the packaging materials back into the shipping box for reuse if the instrument needs to be shipped again.
3. *For instruments with imaging:* Open the accessories box, and remove the isolation table, if purchased.

Note: Accessories ship in their own box. As applicable, compare the objectives, LED cubes, and filter cubes against the sales order.

Unpack and Inspect the Dispenser

Applies only to instruments with a dispenser.

1. Open the shipping box. Remove the accessories box and foam insert containing the injector tubing and bottle holders.
2. Lift out the dispenser and place it on a level surface.
3. Open the accessories box and remove its contents.
4. Place all packaging materials into the shipping box for reuse if the dispenser needs to be shipped.

Unpack and Inspect the Gas Controller

Applies only to instruments with a gas controller.

1. Open the shipping box.
2. Lift out the accessories (power supply, tubing, and manual), and set them aside.
3. Lift out the gas controller, and place it on a level surface.

4. Place all packaging materials into the shipping box for reuse if the gas controller needs to be shipped.

Select an Appropriate Location

Install the instrument on a level, stable surface in an area where temperatures between 18°C (64°F) and 30°C (86°F) can be maintained.

Leave at least six inches of space between the instrument's rear panel and any other object. This space ensures proper air flow in and out of the instrument.

The instrument is sensitive to extreme environmental conditions. Avoid the following:

- *Excessive humidity.* Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks. The humidity must be in the range of 10–85%, non-condensing.
- *Excessive ambient light.* Bright light may affect the reader's optics and readings, reducing its linear range.
- *Dust.* Readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.
- *Vibration.* The instrument should be installed in a vibration-free environment. Be sure to position the instrument away from other devices that could potentially create vibration during the read process.

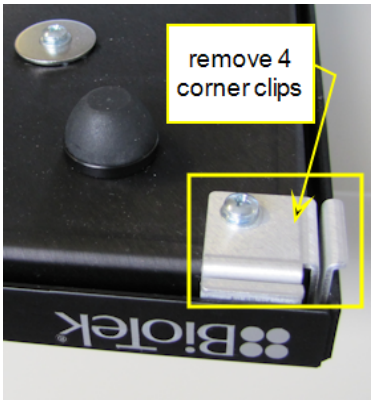
Note: If you are installing a BioStack for operation with the Cytation 5, you may wish to seat the instruments in their alignment plates now. Refer to the BioStack User Manual for more information.

Installing the Isolation Table

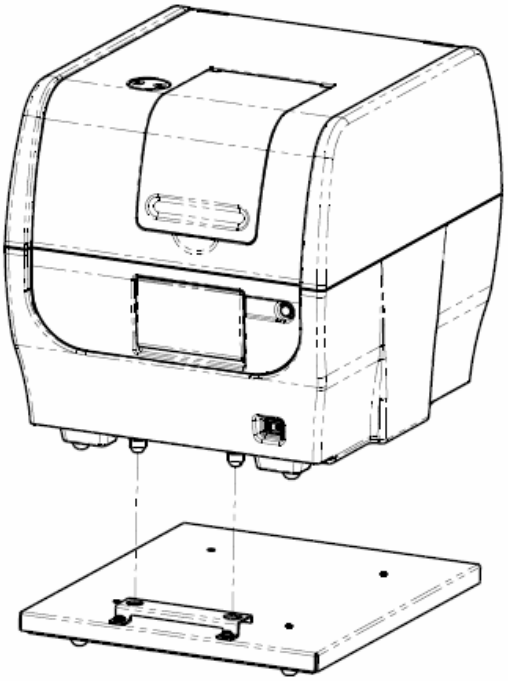
Cytation 5 models with imaging can be used with an isolation table to help eliminate vibration during image reads.

Note: If you are installing isolation table part number 2010002 for use with the BioStack, refer to the BioStack User Manual.

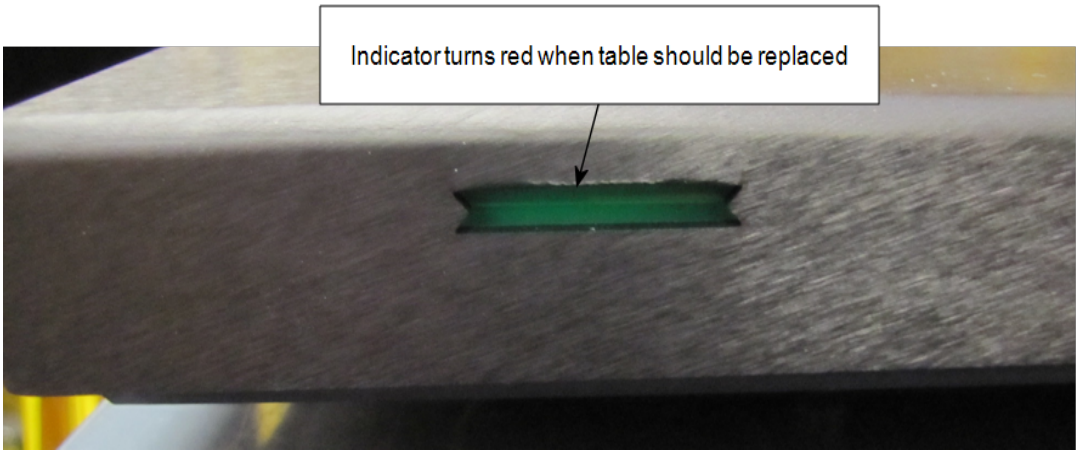
1. Flip the isolation table over and use a Philips screwdriver to remove the four corner clips used to prevent its compression during shipping.
2. Place the isolation table in the selected location.



3. Place the instrument on the table as shown right.



The isolation table contains material that dampens vibration. Over time, this material becomes compressed and can lose effectiveness. The isolation table has a color indicator that turns from green to red to show when the table should be replaced because the dampening material has been compressed.



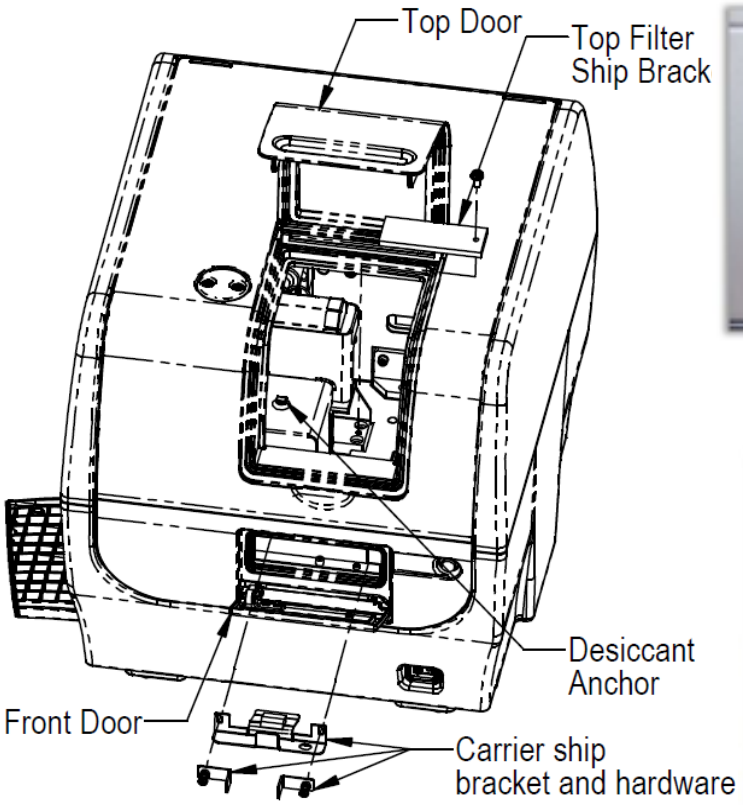
Note: BioTek offers a specially designed isolation table (PN 1700508) for use with the Cytation 5 when the Peltier Cooling Module is installed. The special isolation table accommodates the extra weight of the cooling module.

Remove the Shipping Hardware

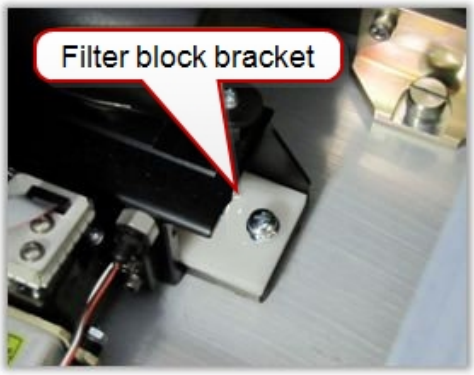
CAUTION **Shipping Hardware.** All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

1. Locate the shipping hardware, shown in the next two figures. Note: most screws are "captive."

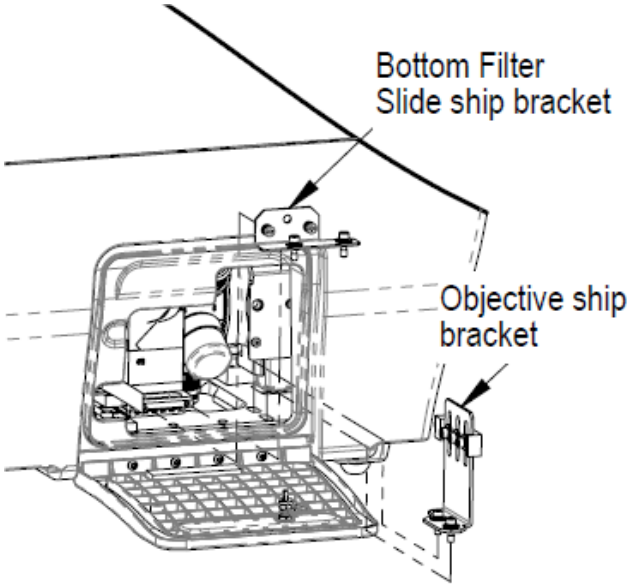
Note: The figures below depict a Cytation 5 with the filter module and imaging module.

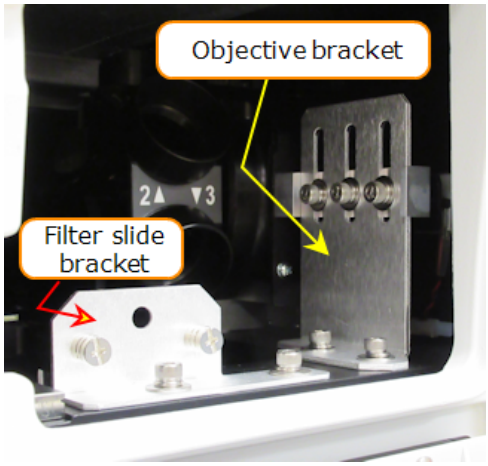


Carrier shipping bracket



Top filter shipping bracket





Bottom and objective brackets accessed via the side door

2. Open the top door, and remove the reusable zip tie and desiccant packet from the desiccant anchor.
3. If equipped, use the supplied screwdriver to remove the top filter shipping bracket.
4. Open the front door, and using the supplied screwdriver, remove the carrier shipping bracket.
5. Imaging models: use the 9/64" hex wrench to remove the bottom filter slide ship bracket
6. Push the filter slide back, and remove the two-piece objective shipping brackets.
7. Store the shipping hardware in a safe location, in case the instrument needs to be shipped again.

Install the Power Supply

WARNING **Power Rating.** The instrument's power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

WARNING **Electrical Grounding.** Never use a plug adapter to connect primary power to the external power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.

CAUTION **Power Supply.** Use only the power supply shipped with the instrument and operate it within the range of line voltages listed on it.

1. Locate the power inlet on the back of the reader.
2. Examine the power supply's plug. It has a small groove that lines up with a tab inside the power inlet.
3. Insert the plug into the power inlet and plug the power supply's cord into an appropriate power receptacle.

Important: Do **not** plug the power supply into a power receptacle until after the power supply is connected to the instrument.

Install the Gas Controller (if applicable)

Applies only to an instrument used with a gas controller.

The gas controller is an external module to control CO_2 and O_2 concentrations inside the reading chamber. If you purchased the module for operation with the Cytation 5, refer to the *Gas Controller User Guide* for installation and setup instructions.

Install the Peltier Cooling Module (if applicable)

The optional Peltier Cooling Module is attached to the back of the reader to:

- accelerate cooling of the reading chamber following an incubation session, and
- limit temperature rise in the reading chamber when running without incubation.

If you purchased the accessory, refer to the *Peltier Cooling Module Quick Reference* for installation instructions.

Important: Leave sufficient room around the cooling module to guarantee a fresh supply of circulating air (to dissipate heat and keep it cool).

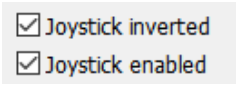
Note: BioTek offers a specially designed isolation table (PN 1700508) for use with the Cytation 5 when the Peltier Cooling Module is installed. The special isolation table accommodates the extra weight of the cooling module.

Install the Joystick (if applicable)

If applicable, install the joystick:

1. Remove the joystick from its shipping box, and place it on a level surface.
2. Place all packaging materials into the shipping box for reuse if the joystick needs to be shipped.
3. Locate the joystick cable. Plug one end into the port on the top of the joystick. Plug the other end into the joystick port on the rear of the instrument.

Note: If the joystick is connected after the instrument has been turned on: restart the instrument and run a system test or "Test Communication" to establish communication between the joystick and the instrument.

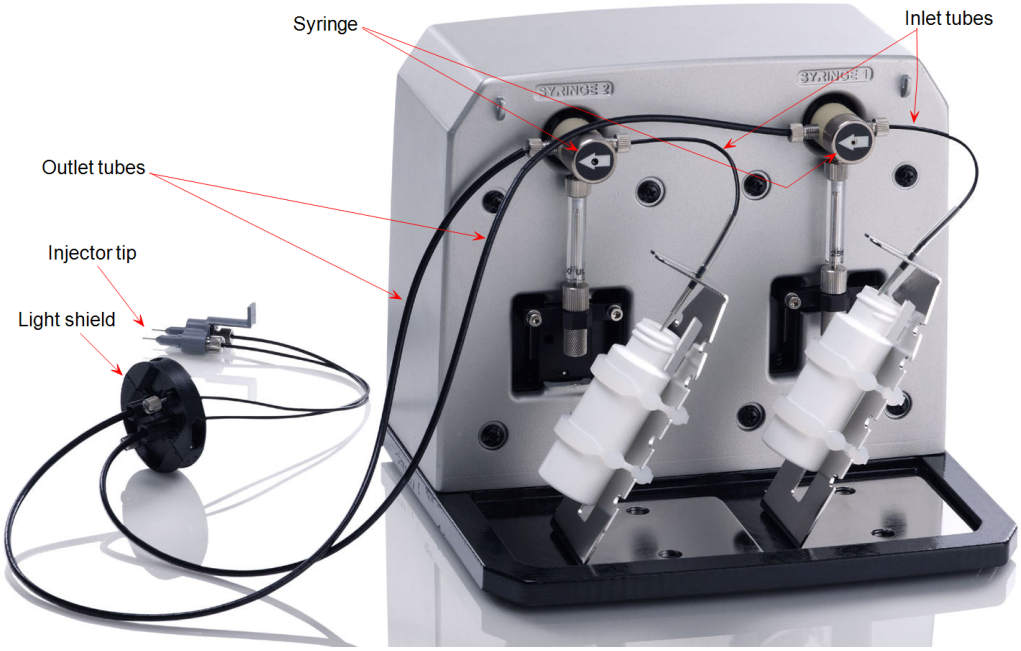


Gen5 recognizes the joystick when it is installed. On the Stage Movement panel, when the joystick is connected, two checkboxes are offered, including **Joystick enabled**. As needed, click the checkbox to use the joystick rather than the computer controls. Choose **Joystick inverted** to reverse the direction settings.

Install the Dispenser (Optional)

Applies only to instruments with a dispenser.

Note: Place the dispense module on top of the reader or on top of the gas controller (if equipped). Do not place the dispenser next to the reader.



1. Open the plastic bag containing the injector tube and tips. Remove the clear plastic shrouds from the tubes.
2. Remove the two inlet tubes from their plastic canisters.

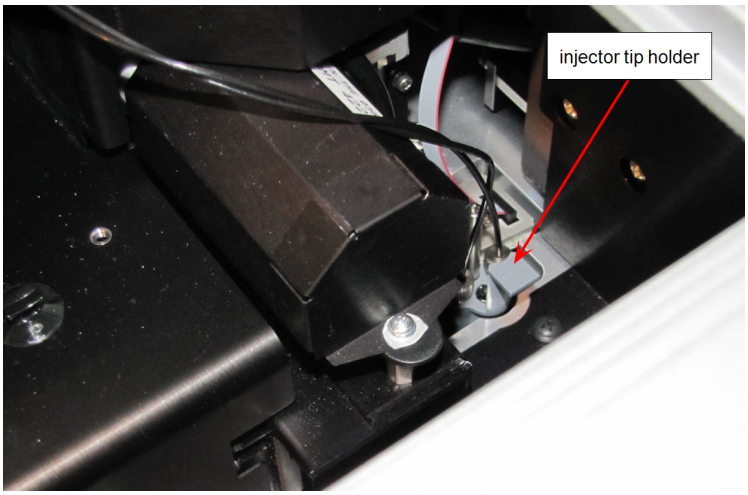
3. Identify the two syringe valves on the dispense module. Each is labeled with a left-pointing arrow.

> **Note:** When installing the inlet and outlet tubes, do not use any tools. Finger-tighten only!

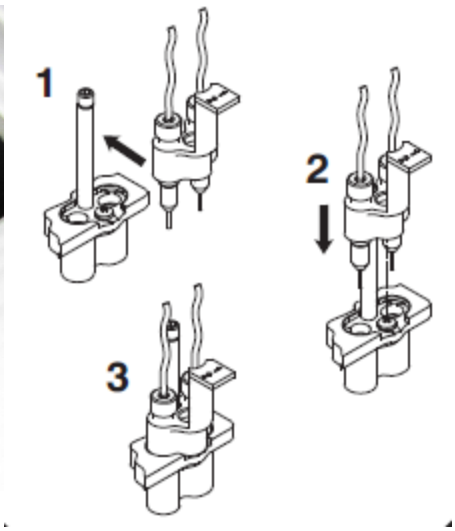
4. Screw the fitting of one inlet tube into the right side of the Syringe 1 valve.
5. Identify the #1 outlet tube, and screw it into the left side of the Syringe 1 valve.
6. Repeat these steps to attach the inlet and outlet tubing for Syringe 2.

> **Note:** It is critical that the tubing is installed in the correct ports. Otherwise, injected fluid may miss the intended well.

7.  Remove the (round) tubing feed-through cover from the top of the reader (2 screws). Store the cover and screws with the shipping hardware in case the reader needs to be shipped again.
8. Thread the injector tip holder, with outlet tubing connected to both ports, through the hole in the top of the reader.
9. Open the reader's top door, and, holding the injector tip holder by the tab, insert the injector tips into the appropriate holes inside the reader.



The injector tip holder in its socket

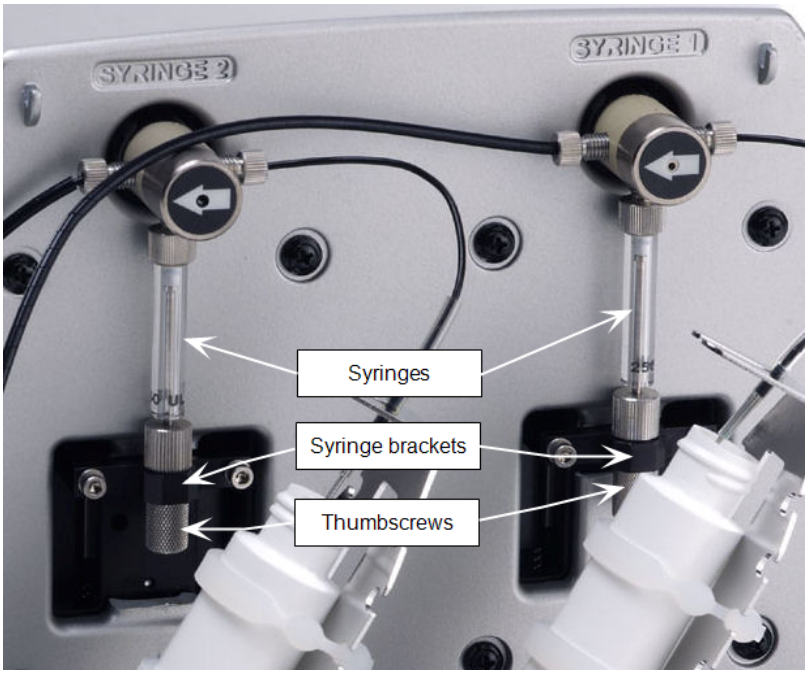


Injector tip installation

> **Note:** A magnet located between the injector tips helps to guide the tips into place and secures them in the reader.

10. Place the tubing feed-through cover over the hole in the top of the reader and finger-tighten the thumbscrews to secure it.

11. Remove the two syringes from their protective boxes. They are identical and interchangeable.
12. Install both syringes.
    - Hold the syringe vertically with the threaded end at the top.
    - Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
    - Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
    - Pass a thumbscrew up through this hole and thread it into the bottom of the syringe. Hold the syringe to prevent it from rotating while tightening the thumbscrew. Finger-tighten only.



13. Locate the dispenser cable. Plug one end into the port on the left side of the dispenser. Plug the other end into the "Dispenser Port" on the rear of the reader.
14. Locate the injector tip-cleaning stylus, packaged in a small cylinder. Attach the cylinder to the back of the dispenser for storage.

Note: Perform a visual inspection or a Performance Qualification test after reconnecting the tubes.

Install the Supplied Controller (Host Computer)

Install the controller provided by BioTek, which is the recommended host computer for imaging applications. Follow installation instructions provided with the controller. If you are not using a BioTek-supplied controller, refer to the Gen5 Getting Started guide for system requirements.

Essential Information:

> **Important:** Maintaining Gen5 data file integrity is the user's responsibility. Work with your IT experts to establish a secure file management system, e.g. daily file backups via Ethernet to the network.

- The controller supports Ethernet connections to a local network; consult your IT experts about connecting to your organization's network.
- One controller is provided with each instrument and this is the recommended way to use them.
- The controller does not require a password.

Install Gen5 and Connect the Cytation 5

> **Note:** The Cytation 5 is controlled by Gen5 software running on the controller or host computer. A certain sequence of events must be followed to ensure the software and drivers are properly installed and configured.

> **Important:** Do not connect the instrument to the controller until the USB driver shipped with Gen5 is installed.

- Gen5 software versions 3.11 and higher require Windows 10.
- You must have administrator privileges to install Gen5. Log in to Windows as "Administrator" or consult your IT department for assistance.

Install Gen5: Turn on the controller and insert the Gen5 USB flash drive into a port. Consult the information provided with Gen5 to install the software.

After installing Gen5:

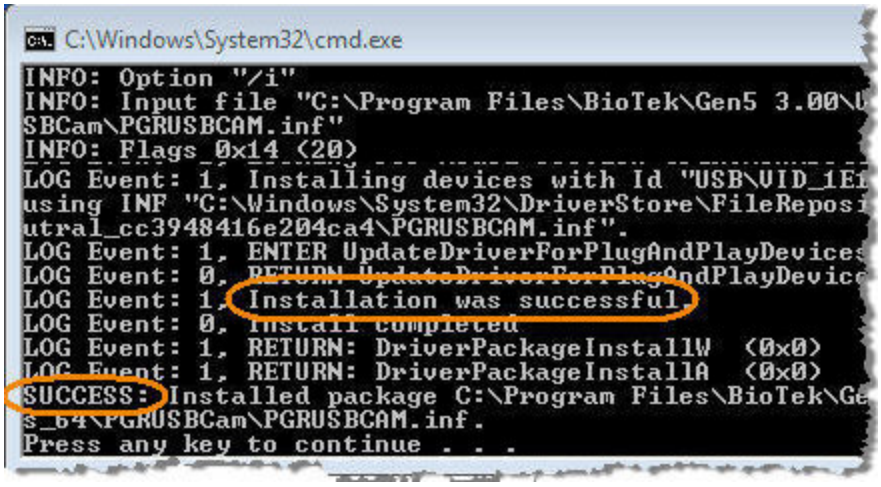
Essential Information

Two software drivers must be installed on the controller:

- (All models) A **USB Driver** that is shipped with the Gen5 software on a USB flash drive. This driver is used for connecting the instrument to the controller.
- (Imaging "V" and "W" models) A **USB3 Driver** that is copied to the controller when Gen5 is installed. This driver is used to communicate with the camera for use with imaging applications.

Installation Procedure

1. Find and install the USB driver shipped on the **Gen5 USB flash drive** in the **USB Driver** folder. Open the folder and **right-click** the **CDM<version info>.exe** file and select **Run as Administrator**.
2. Connect the Cytation 5 to the controller using the USB cable. For non-imaging models, skip to step 8.
3. Connect the camera cable to a USB3 port on the rear panel of the controller.
4. Navigate to the Gen5 program files on the controller, for example, C:\Program Files\BioTek\Gen5 3.11.
5. Open the **USB3 Drivers** folder, and then the Windows_64 folder.
6. Open the driver folder **PGRUSBCam**.
7. Right-click **InstallPGRDriver.bat** and select **Run as Administrator** to install the driver. When finished, a message displays: "SUCCESS: Installed package <path to package>". If you do not see this message, contact Technical Support.



8. After installing the drivers, restart the controller.

Turn on the Instrument

If you have not already done so, connect the supplied USB cable between the USB port on the back of the instrument and an available USB port on the controller/computer.

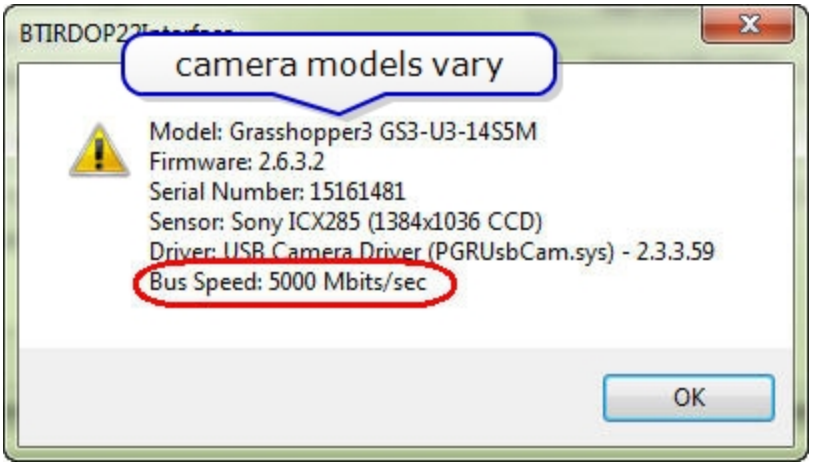
The instrument's power switch is located on the lower-right corner of the front panel. The instrument performs a system test. When the test is completed, the instrument extends the microplate carrier.

Note: The carrier button above the power switch ejects and retracts the microplate carrier.

Establish Communications

1. From the Gen5 main screen, select **System > Instrument Configuration** and click **Add Reader**.
2. Set the Reader Type to **Cytation 5**, and click **OK** to continue.
3. Select **Plug & Play**.

   Note: A **Cytation 5** must be connected via USB to the controller/host computer and turned on to appear in the Available Plug & Play Readers list.
4. Test that Gen5 can communicate with the instrument: click **Test Communications**. If successful, Gen5 displays a success message.
5. **Imaging models:** Click **Camera Information**. If communication is successful, Gen5 displays the camera information.



Imaging models only:

Check the "Bus Speed," which should say 5000 Mbits/sec. If a significantly lower bus speed is reported, review troubleshooting information.

Troubleshooting Software Drivers

Here are some suggestions for troubleshooting communication with the camera or if the Bus Speed is significantly lower than 5000 Mbits/sec.

- Reboot the controller/host computer.
- Disconnect and reconnect the USB3 cable from/to the controller/host computer. Make sure the USB3 cable is connected to a USB3 port.
- Re-run the camera driver installation, as described above under Install Gen5.
- If BioTek's controller is not in use, make sure the computer meets the recommended requirements, including an Intel 8 USB Chipset or higher.

If problems persist, ask your IT group for support or contact Technical Support.

Communication Errors

If the communication attempt is not successful, try the following:

- Did you install the USB driver?
- Is the instrument connected to the power supply and turned on?
- Is the communication cable firmly attached to both the instrument and the controller/computer?
- Is the USB3 camera cable properly connected to the controller/computer?

Note: If you cannot get Gen5 and the instrument to communicate with each other, contact Technical Support.

Install the Imaging Module

Applies only to Cytation 5 imaging models.

Several steps are required to install the imaging module.

1. First, if you are not using the BioTek-provided "controller," the host computer must support the Cytation 5's communication method. Get assistance from your IT group, if needed, to set up the **USB 3** communication components required to connect the computer to the instrument and camera.

Note: Instruments with s/n 151221xx and lower (equipped with FireWire camera) or host computers that do not meet the recommended configuration: Contact Technical Support for alternate instructions.

2. Update Gen5 imaging settings.
3. Install the objectives, LED cubes, and imaging filter cubes, and run Auto Calibration.

Set Up Gen5 for Imaging

Important: Perform this procedure in the specified order to prevent damage to the LED cubes.

To prevent overpowering the LED cubes, the Cytation 5 needs to know which LED cubes will be installed. You must enter the configuration information in Gen5 **before** physically installing the optical components. Gen5 will update the settings onboard the instrument. **The LED cubes and imaging filter cubes must be installed with the instrument turned off.**

Follow the installation steps in this order:

1. In Gen5, select **System > Instrument Configuration > Cytation 5 > View/Modify > Setup**.
2. On the Imaging Configuration tab, input the objective and LED cube and filter cube configurations. Click **Send Values** to send the information to the instrument. Turn off the instrument.

   Note: Laser AutoFocus cubes perform best in position 1: See Install the LED Cubes and Filter Cubes on page 31.

3. Install the objectives in their defined locations. See "Install the Objectives" below.
4. Install the LED cubes and imaging filter cubes in their defined locations (Install the LED Cubes and Filter Cubes on page 31) in their defined locations. Close the side access door.

Note: Leave Gen5 open to the Reader Setup dialog while performing the next steps.

5. Turn on the instrument. After the self-test, the instrument will beep and the carrier button LED will be flashing red, indicating that the objectives must be calibrated.
6. Press the carrier button to stop the beeping.
7. Click **Auto Calibration**.

Important: Cytation 5 "W" models and "V" models with high-power objectives (40X - 60X objectives) require an "objective setup plate" during Auto Calibration: "W" models must use PN 1852501 (which supports FL Illumination Correction); other models can use any objective setup plate, including PN 1222531, to calibrate their high-power objectives.

After the calibration procedure is finished, the instrument is ready to use.

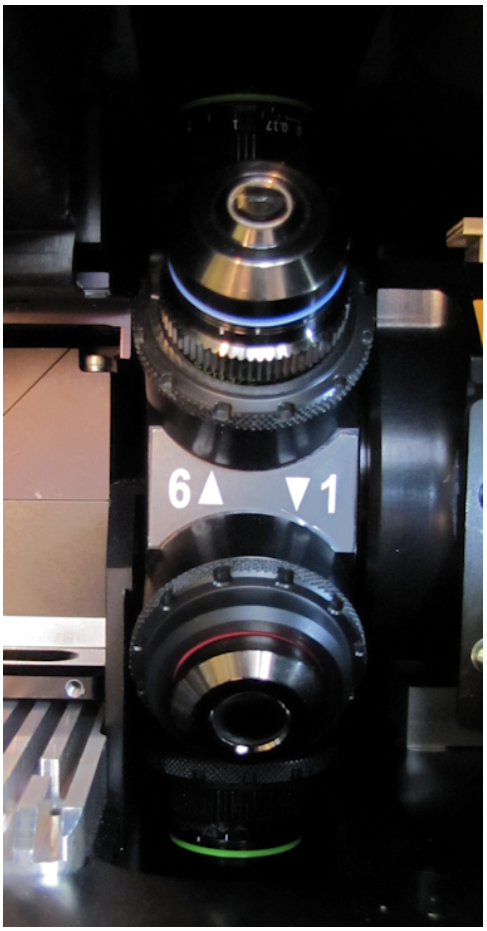
Note: The Auto Calibration process can take up to 15 minutes on an instrument with six objectives installed.

Note: Phase contrast components are calibrated in the factory before shipment. You do not need to run the Phase Ring Configuration and Calibration routines

Install the Objectives

Note: Before installing a 20X, 40X, or 60X air objective, either phase or standard, set its correction collar to match your plate type. When installing an objective with a correction collar, be sure to grasp the objective by the adapter, not by the correction collar, to avoid changing the correction collar settings.

After defining the objectives and their locations in Gen5:



Objective turret, showing positions 6 and 1

1. Open the access door.
2. Screw each objective into its defined position. Do not overtighten the objectives.

Generally it is best to install your lowest power objective, beginning with 4X, in position #1, and higher power objectives in the subsequent positions. Except objectives with power lower than 4X should be installed in the last positions. For example:

- 4X
- 20X
- 40X
- 60X
- 2.5X
- 1.25X

Especially with high-power objectives, pay close attention during installation to make sure the objective's adapter threads properly into the turret. If you feel a stop or misalignment, remove the objective and start again. Verify proper installation by making sure there is no gap between the adapter and turret.

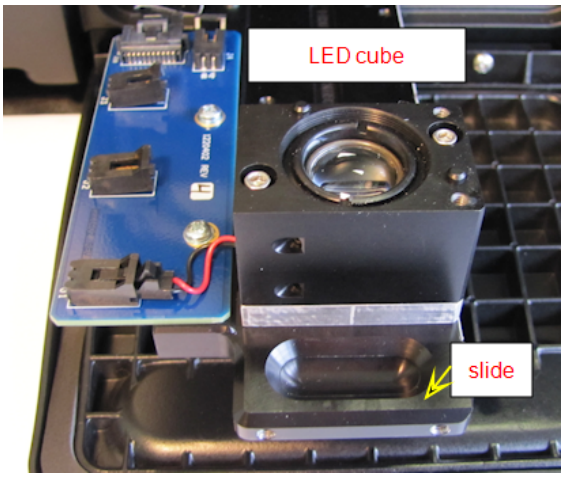
Install the LED Cubes and Filter Cubes

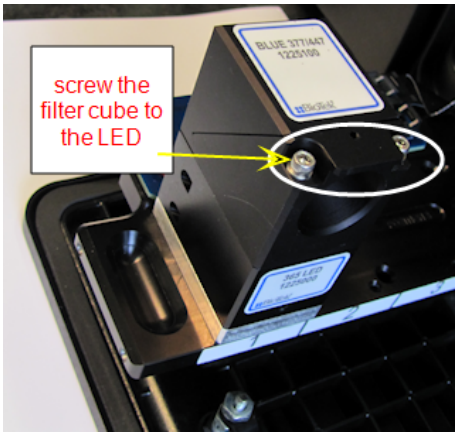
Tools: 3/32 Hex wrench

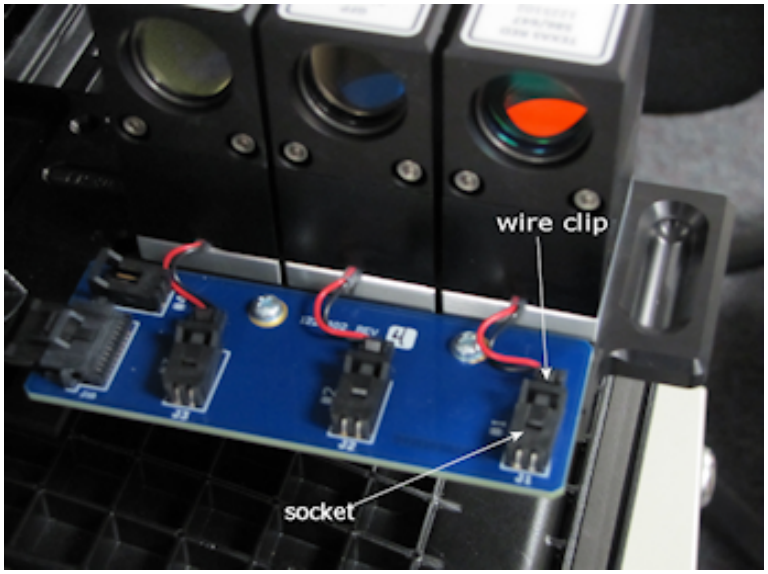
Important: If used, the Laser AutoFocus cube (PN 1225010) must be in Position 1. See the next topic.

After defining the LED and Filter cubes and their location in Gen5:

1. Reach in and pull out the filter slide.
2. Place the new LED cube in the defined position (Position 1, 2, 3, or 4).
3. Use the hex wrench to screw the LED cube into the filter slide.
4. Plug the LED cube's wire clip into the socket on the carrier.
5. Place the imaging filter cube on top of the LED cube and screw it to the LED cube.

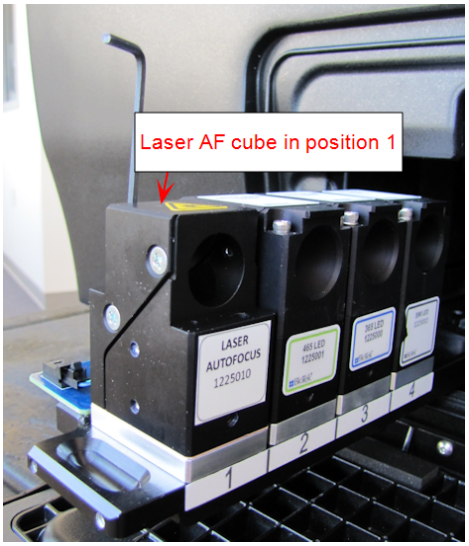






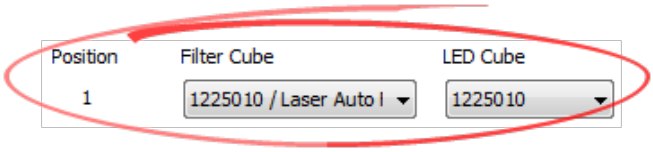
Install the Laser Autofocus Cube (optional)

Class 1 Laser Product. "A" models. "V" and "W"models when the optional Laser Autofocus Cube is installed.



Laser Autofocus - Optional Accessory

Install the Laser Autofocus cube (PN 1225010) in Position 1 on the slide (if you purchased this accessory).



Use the 3/32 hex wrench to tighten the cube's captive screws.

Plug the cube's wire clip into the socket.

Run Auto Calibration

After physically installing the LED cubes, imaging filter cubes, and objectives, you must run Auto Calibration. This process can take up to 15 minutes on an instrument with six objectives installed.

1. Turn on your instrument, and allow the self-test to run. The instrument will beep, indicating that the self-test has failed.
2. Press the carrier eject button to stop the beeping.
3. If the Reader Setup dialog is not already visible on your screen, go to **System > Instrument Configuration**, select **Cytation 5**, then click **View/Modify > Setup**.
4. On the Imaging Configuration tab, click **Auto Calibration**.
5. For all "W" model Cytation 5s and when a 40X or 60X objective is installed, Gen5 prompts you to place the objective setup plate on the carrier. After you place the jig on the carrier, click **OK**.

Important: Cytation 5 "W" models and "V" models with high-power objectives (40X - 60X objectives) require an "objective setup plate" during Auto Calibration: "W" models must use PN 1852501 (which supports FL Illumination Correction); other models can use any objective setup plate, including PN 1222531, to calibrate their high-power objectives.

After the calibration procedure is finished, the instrument is ready to use.

Troubleshooting Auto Calibration

If error messages indicate a problem with the imaging Auto Calibration process, make sure the objectives are in their assigned locations.

Rerun Auto Calibration:

1. In Gen5, click **System > Instrument Configuration > Cytation 5 > View Modify > Setup**.
2. On the Imaging Configuration tab, set the value of all objectives to **none**, and then click **Send Values**.
3. Set the correct values for all objectives, and then click **Send Values**.
4. Click **Auto Calibration**.

If problems persist, contact Technical Support.

Run a System Test

Running a system test will confirm that the instrument is set up and running properly, or will provide an error code if a problem is detected.

1. Turn on the incubator:
   - In Gen5, select **Incubate** from the **Instrument Control** tab.
   - Enter a Requested temperature of at least 37°C and click **On**.
   - Wait until the incubator temperature reaches the set point before continuing.
2. Return to Gen5's main view and select **System > Diagnostics > Run System Test**. If prompted to select an instrument, select **Cytation 5** and click **OK**.
3. When the test is completed, a dialog requesting additional information appears. Enter the information and click **OK**.

   Note: If a message appears that a pending system test is waiting from the initial power-up self-test, view the pending test results and repeat steps 2 and 3.

4. The results report appears. The text should read "SYSTEM TEST PASS."
   - You may wish to print the report and store it with your records.
   - The Gen5 software stores system test information in its database; you can retrieve it at any time.
   - Sign and date the report, and store it with your test documentation.

> **Note:** If an error code is displayed, look up the code in Appendix B . If the problem is something you can fix, do so now and rerun the system test. If the problem is something you cannot fix, or if the test continues to fail, contact Technical Support.

5. Turn off the incubator: select **Incubate** from the **Instrument Control** tab and click **Off**.

Set Dispenser Calibration Values

Applies only to instruments used with a dispenser.

Before using the dispenser, you must enter its calibration values in Gen5.

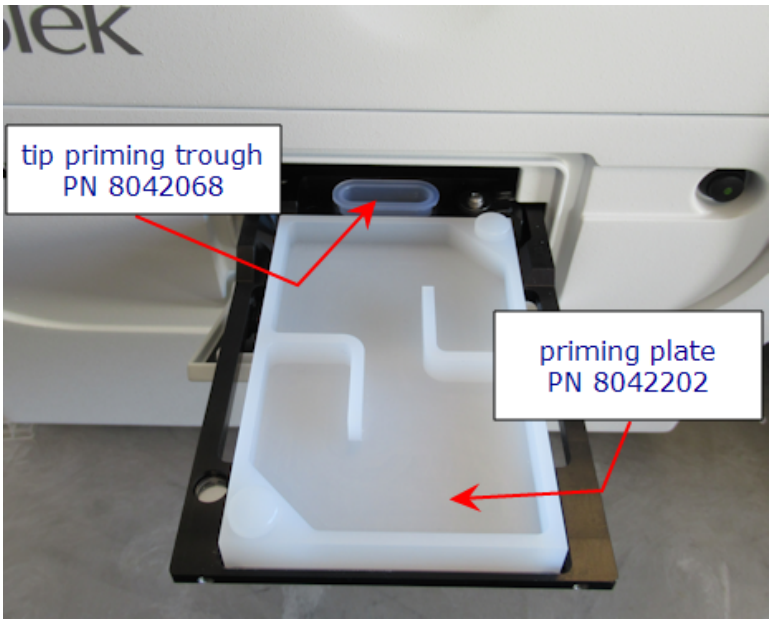
The calibration values for both dispensers (#1 and #2) are printed on labels affixed to the rear of the dispense module. Each label lists six target calibration values (e.g., 200, 80, 40) with their actual measured values (e.g., 199.3, 79.7, 39.9). **Enter the measured calibration values into Gen5.**

1. In Gen5, go to **System > Instrument Configuration**, select your instrument, and click **View/Modify**.
2. Click **Setup**, and then select the **Dispenser 1 tab**.
3. On the keyboard, press **CTRL+SHIFT+M** to enter maintenance mode for the Dispenser 1 window.
4. Enter the syringe calibration values from the label on the rear of the dispenser box.
5. Click **Send Volumes**, and then click **Get Volumes** to verify that the entered values were sent to the instrument.
6. Select the **Dispenser 2 tab**, and repeat steps 3 through 5 for Dispenser 2.

Test the Dispenser

Applies only to instruments with a dispenser.

1. If necessary, press the carrier eject button to extend the microplate carrier.
2. Place the tip priming trough in the rear pocket of the carrier.
3. Place the priming plate on the carrier.



4. Fill the two reagent bottles with distilled or deionized water. Place the bottles in their holders, and place the holders directly in front of the syringes. Insert the inlet tubes into the bottles.
5. In Gen5, use the **Instrument Control** tab (or select it from the **System** menu).
6. Select 

.
7. Click the **Prime** tab.
8. With Dispenser set to **1**, set the Volume to **5000 µL** and click **Prime**.

   The syringe should move down and up repeatedly, drawing fluid from the bottle. The fluid should pump through the tubing and dispense into the priming plate. Examine the fittings; no leaks should be detected. If leaks are detected, tighten all fittings and repeat the prime. If leaks are still detected, contact Technical Support.
9. When the prime finishes, set Volume to **2000 µL** and click **Purge** to clear the fluid lines.
10. Set Dispenser to **2** and repeat steps 8 and 9.
11. When finished, remove and empty the priming plate.

Operational/Performance Qualification

Your Cytation 5 was fully tested at BioTek prior to shipment and should operate properly following the successful completion of the installation and setup procedures described in this chapter.

If you suspect that problems occurred during shipment, if you received the reader back from BioTek following service or repair, or if regulatory requirements dictate that

Operational/Performance Qualification is necessary, see Instrument Qualification Overview on page 116.

Note: A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Cytation 5 is available for purchase (PN 1320532N). Contact your local BioTek dealer for more information.

Packing and Shipping Instructions

Two person lift. The instrument should be lifted by two people. The instrument weighs up to 36.3 kg (45.3 kg with the Peltier Cooling Module installed).

CAUTION

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

Important! Please read all of the information provided below before preparing the Cytation 5 for shipment.

- Contact Technical Support before returning equipment for service.
- Decontamination prior to shipment is required by the U.S. Department of Transportation regulations.
- If the instrument has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the instrument during shipping, handling, and servicing. The Maintenance chapter contains decontamination instructions.
- Ensure the carrier/stage is empty. Spilled fluids can contaminate the optics and damage the instrument.
- Install the shipping hardware (see next section).
- The instrument's packaging design is subject to change. If the instructions in this document do not apply to the packaging materials you are using, contact Technical Support for guidance. Replace the shipping hardware before repackaging the reader. Please contact BioTek if you have misplaced any of these items.
    - Carrier shipping bracket (PN 1220510)
    - Top filter shipping bracket (PN 8042187)
    - Bottom Filter Slide shipping bracket (PN 1380501)
    - Objective shipping bracket (PN 1380503)
- Be sure to use packaging materials supplied by the manufacturer. Other forms of commercially available packaging are not recommended and can void the warranty.

Prepare the Instrument for Shipment

1. Contact Technical Support for instructions before returning equipment for service.
2. Decontaminate the reader and, if attached, the dispense module, according to the instructions provided in the *As-Needed Maintenance* chapter.

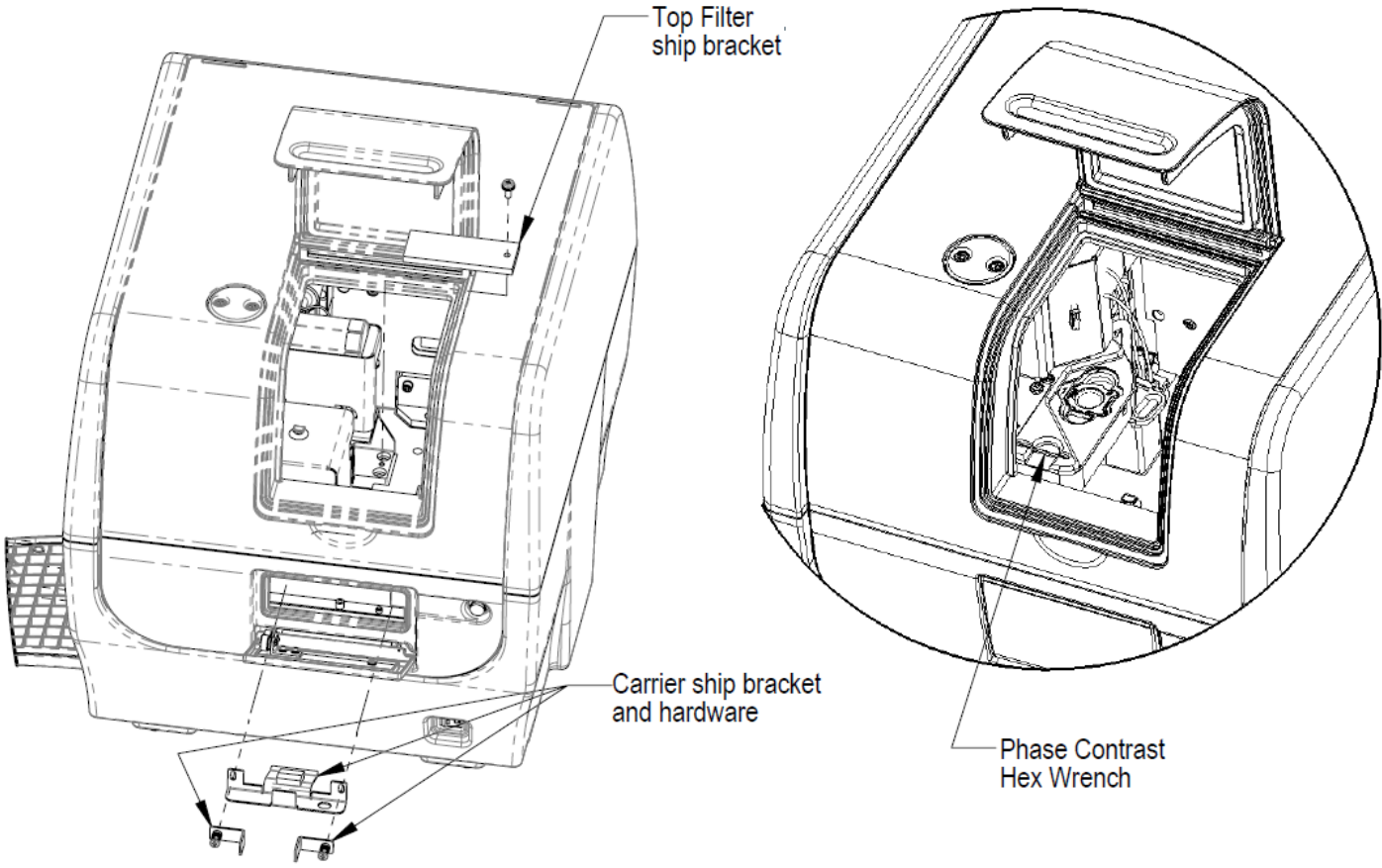
3. If applicable, disconnect and uninstall the dispense module. See *Preparing the Dispenser for Shipment* on page 45.
4. If applicable, disconnect the gas controller and store it.
5. If you have the imaging module, you must uninstall the objectives and move the objective turret into position to install the objective shipping bracket.
   a. From the Gen5 main screen, go to **System > Instrument Configuration**, select **Cytation 5**, and click **View/Modify > Setup**.
   b. In the Objective Configuration area of the Imaging Configuration tab, click **Move to Access Position**.
   c. Uninstall and pack the objectives in their accessory case.

   The imaging LED-Filter cubes are safe to ship installed. Alternatively, uninstall them and pack in their accessory case.

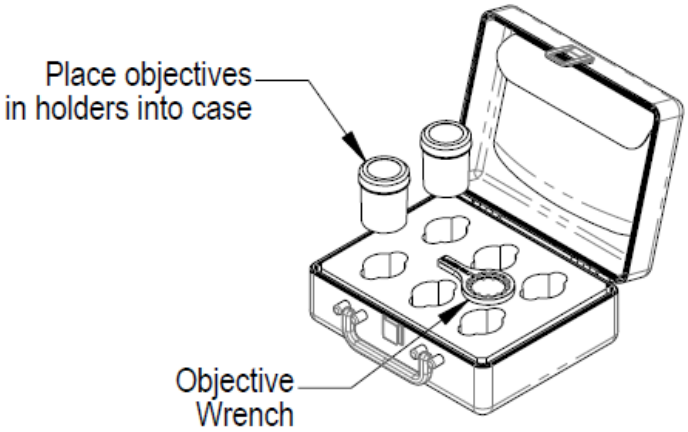
   Note: If the objective shipping bracket does not fit easily, reset the objective turret's position: turn off the instrument if it is powered on. Turn on the instrument. After the carrier is ejected and then retracted, turn it off and install the objective shipping bracket.
6. If you have not already done so, retract the microplate carrier and then turn off and unplug the reader.
7. Install all the shipping brackets, i.e. reverse the installation steps: See "Remove the Shipping Hardware" on page 17 for descriptions of this hardware.
8. Place the accessories in the accessories box, then seal the accessories box with tape.
9. Place the instrument in the large plastic bag, then place it in the interior box, surrounded by foam boards. Then, place the interior shipping box, surrounded by the foam corners, into the external shipping box. See drawings below.

Reinstalling the Shipping Hardware

1. Locate the shipping hardware.
2. If equipped, remove the fluorescence filter cube from its chamber, and place it into a clean plastic bag.
3. If equipped, install the top filter ship bracket.
4. If equipped, place the phase contrast hex wrench in the storage space, and cover it with tape.



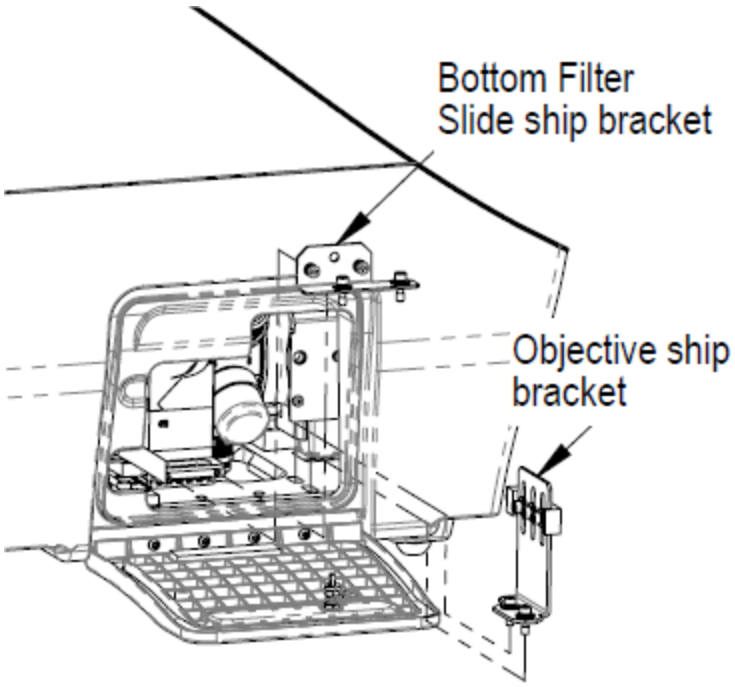
5. If equipped, remove the LED cubes and imaging filter cubes.
6. If equipped, remove the objectives, place them in their holders, and then insert the holders into the objective case. Make sure the objective wrench is also in the case.



7. If equipped, install the objective ship bracket.

> **Note:** If the objective ship bracket does not fit easily into place, turn on the Cytation 5. After the carrier is ejected and drawn back into the instrument, turn off the instrument. The objective turret is now positioned correctly to install the objective ship bracket.

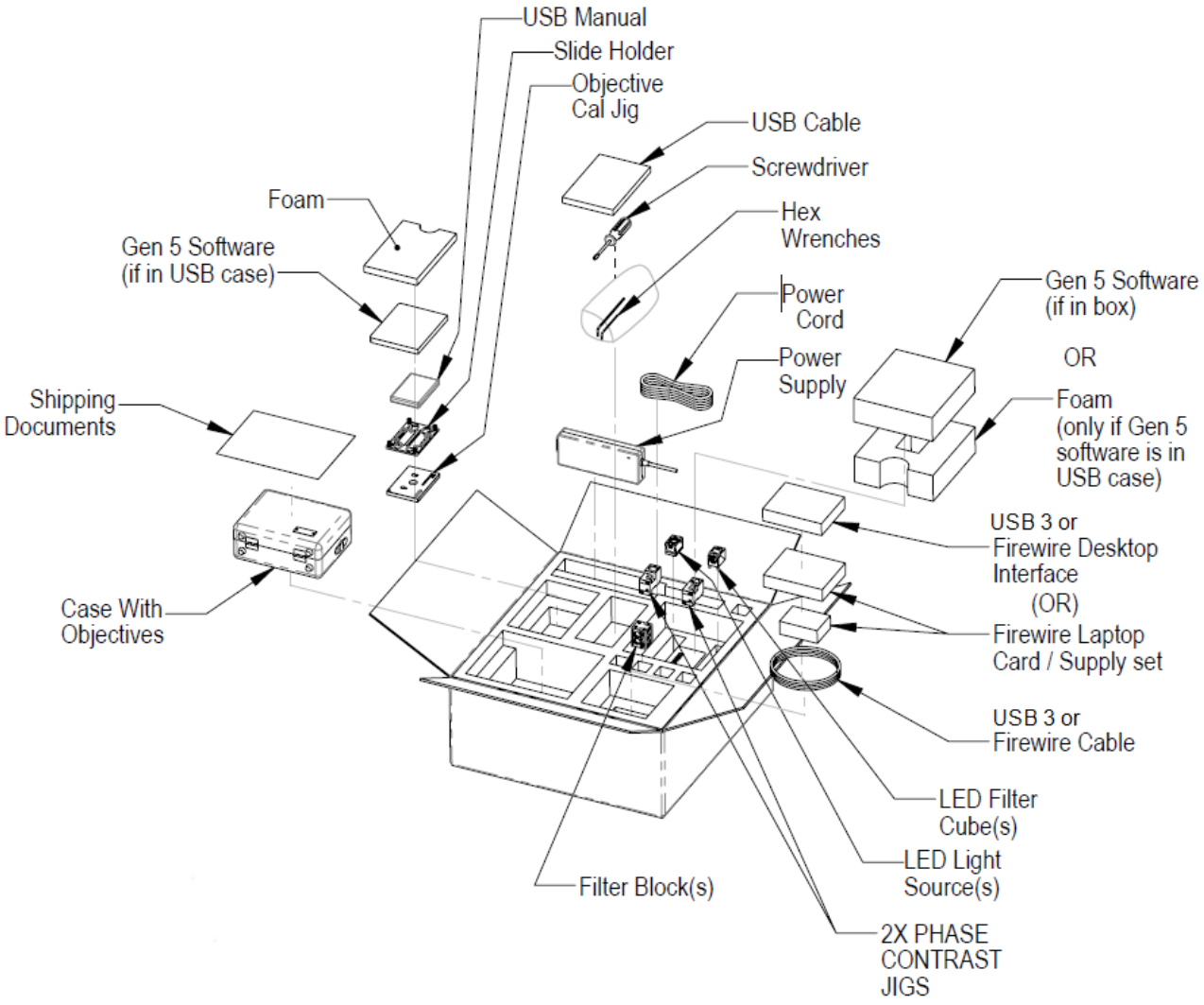
8. If equipped, install the bottom filter slide ship bracket.

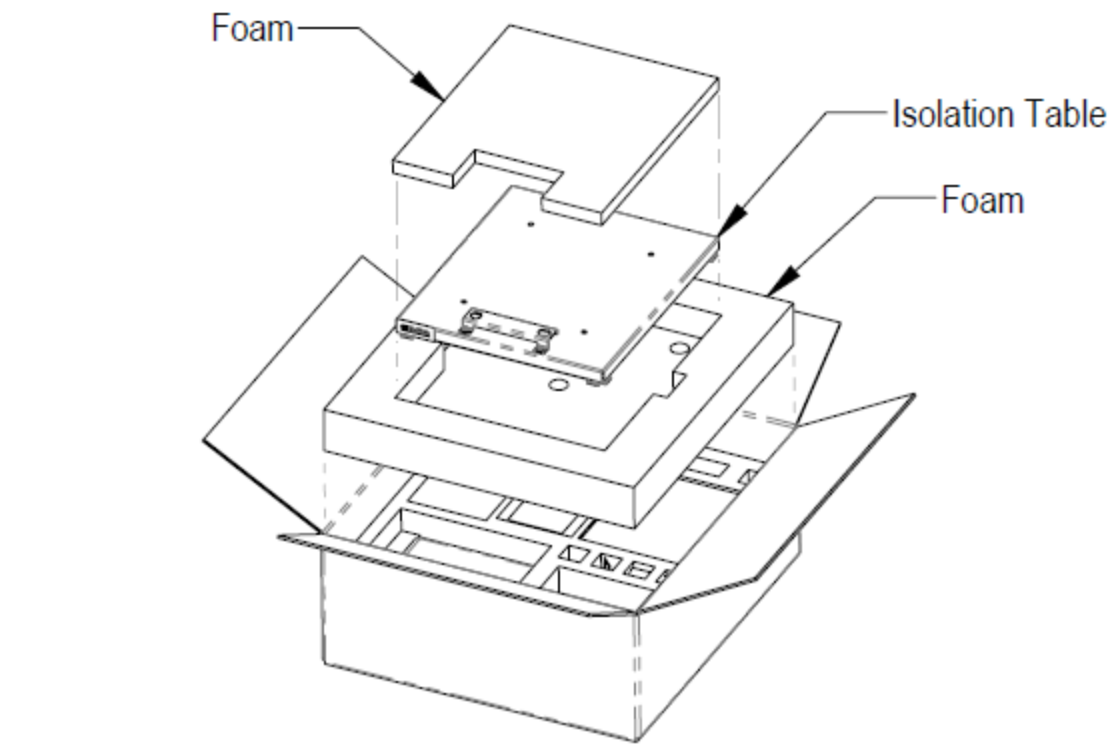


9. Install the carrier ship bracket. See figure on page 39.

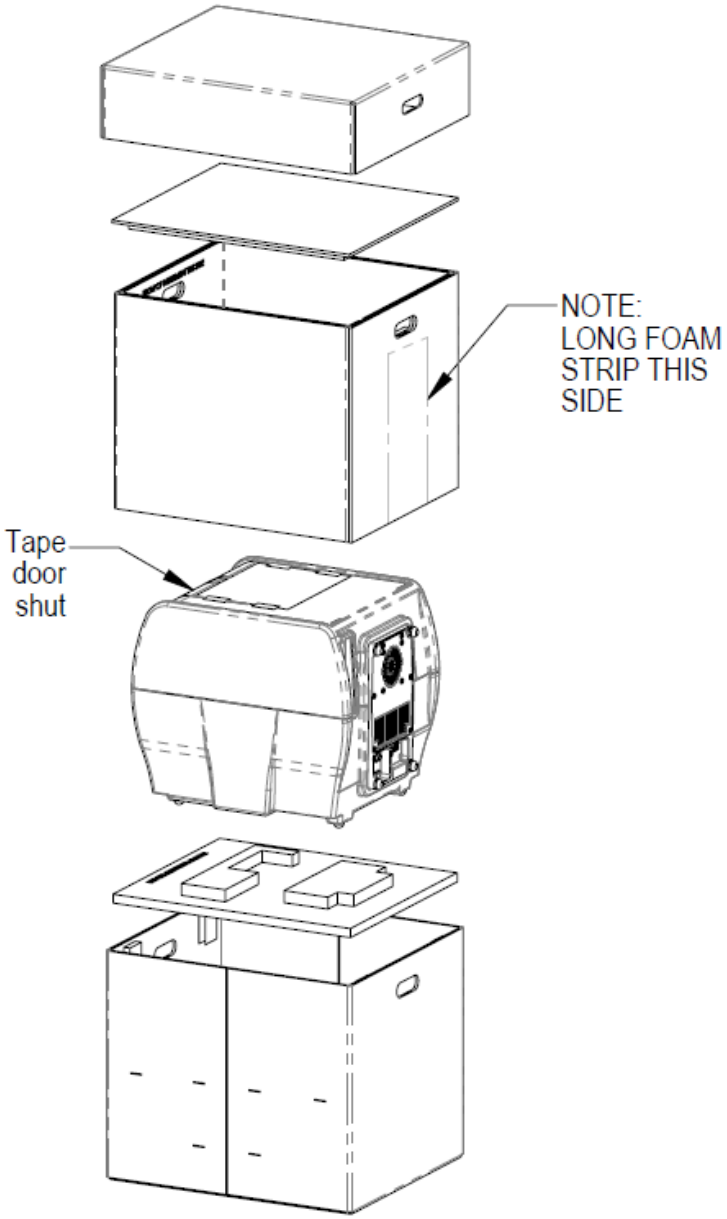
Pack the Shipping Box

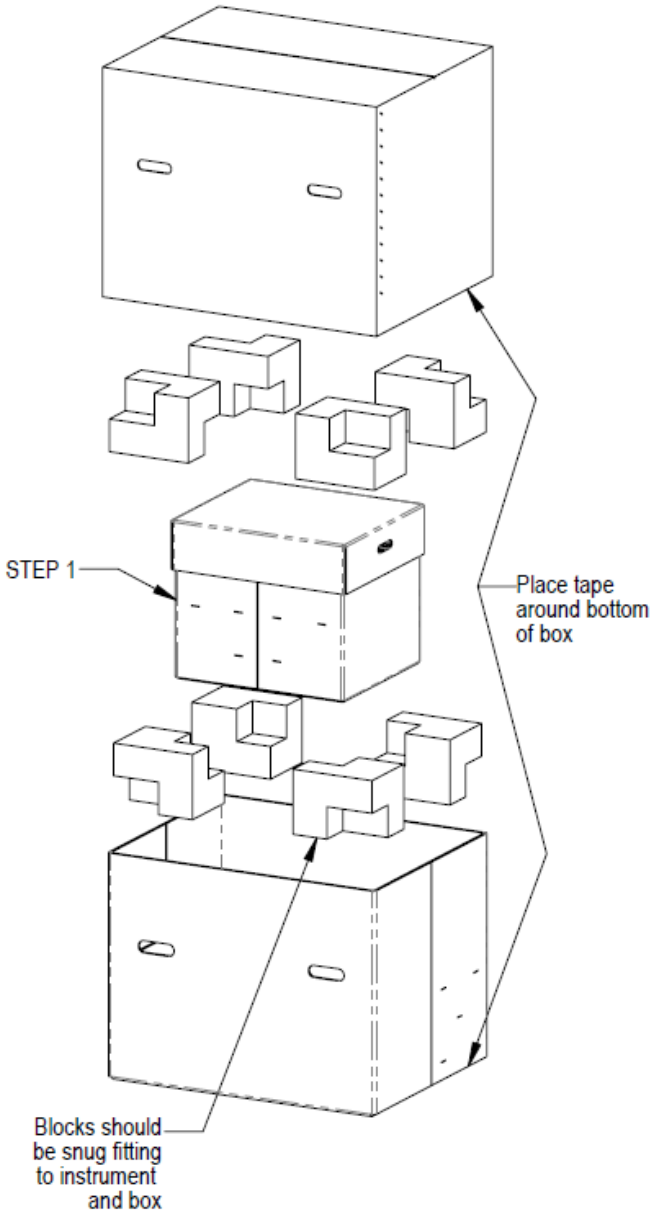
1. Place accessories into compartments, as shown below.
2. Close the accessories box, and tape it shut.


USB Manual
Slide Holder
Objective Cal Jig
USB Cable
Screwdriver
Foam
Hex Wrenches
Gen 5 Software (if in USB case)
Power Cord
Gen 5 Software (if in box)
OR
Power Supply
Shipping Documents
Foam (only if Gen 5 software is in USB case)
USB 3 or Firewire Desktop Interface
(OR)
Firewire Laptop Card / Supply set
Case With Objectives
USB 3 or Firewire Cable
LED Filter Cube(s)
LED Light Source(s)
Filter Block(s)
2X PHASE CONTRAST JIGS



3. Tape the filter cube access door shut, place the instrument in a large plastic bag, then place it in the interior shipping box, and tape the box shut.
4. Referring to the figure below, place the foam corners and interior shipping box in the exterior shipping box, then tape the bottoms of the exterior shipping box to secure them.


NOTE:
LONG FOAM
STRIP THIS
SIDE
Tape
door
shut


STEP 1
Place tape around bottom of box
Blocks should be snug fitting to instrument and box

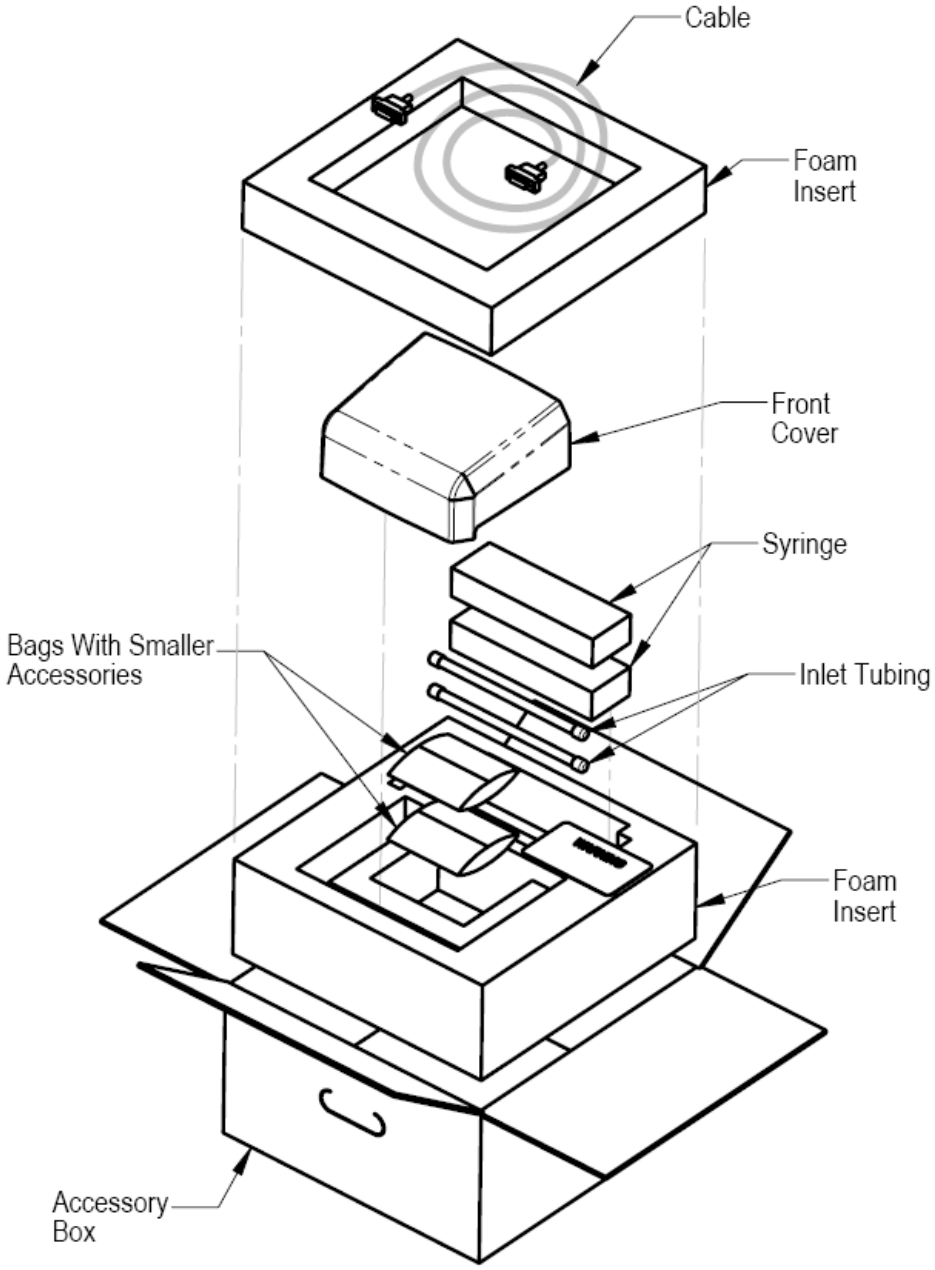
Preparing the Dispenser for Shipment

Note: Decontaminate the dispenser before shipping if it has been used with hazardous materials. Follow the instructions in Decontamination on page 108.

Important: Purge any fluid from the tubing before beginning this procedure.

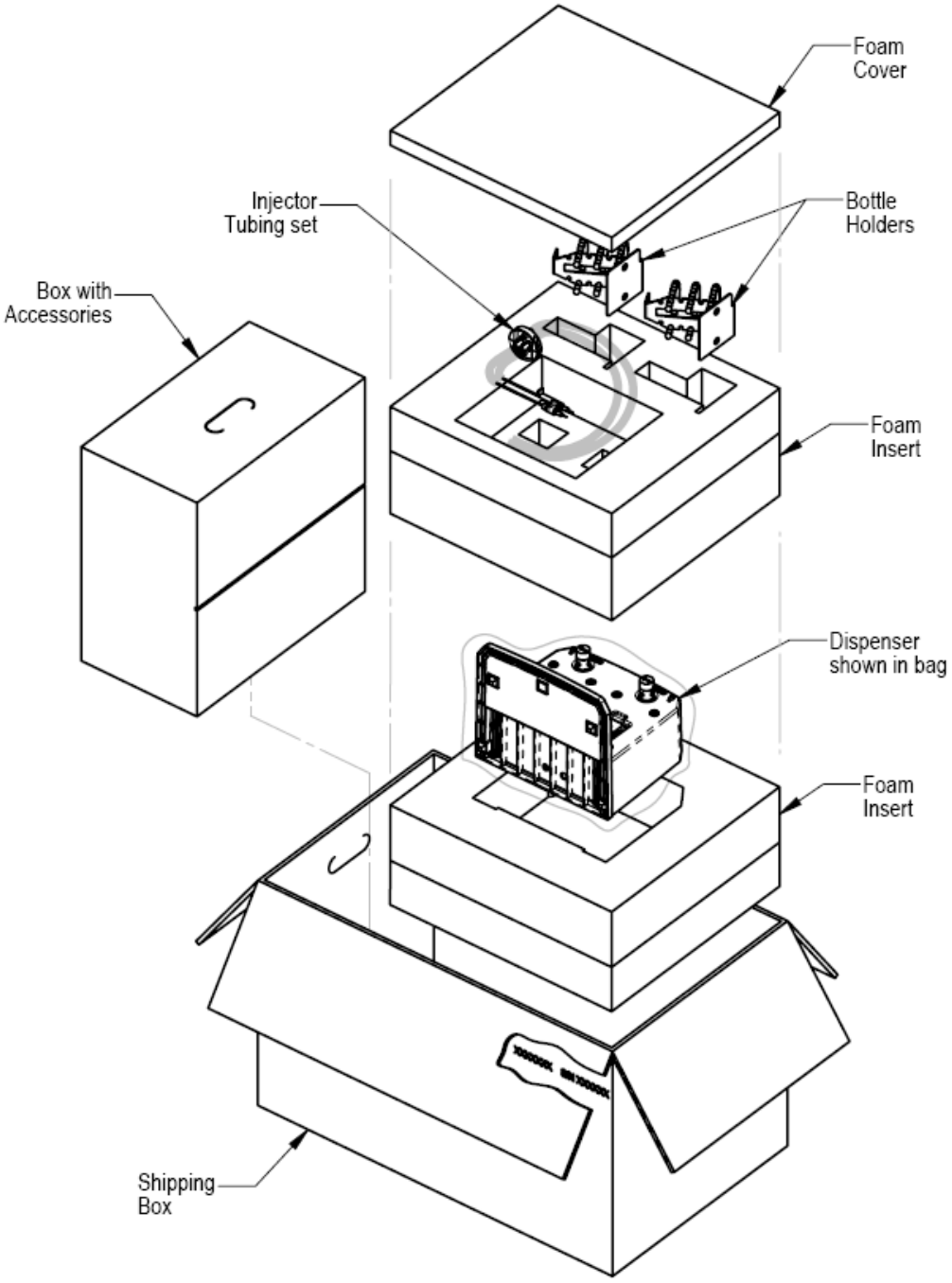
1. Remove the front cover of the dispenser.
2. With the reader on, start Gen5 and perform these steps for each syringe, 1 and 2: select **System > Instrument Control > Cytation 5**.
3. On the Prime tab, select the dispenser number (1 or 2), and click **Maintenance**. The syringe bracket lowers.
4. Remove the thumbscrew from underneath the bracket.
5. Carefully unscrew the top of the syringe from the syringe valve. Lift out the syringe and store it in its original box.
6. Remove the injector tubing from the reader, and replace the light shield.
7. Remove the two inlet tubes from the syringe valves, and store them in their plastic canisters.
8. Remove the two outlet tubes, attach their plastic shrouds to the fittings, and place them in a plastic bag.

9. Put the parts in the foam insert in the bottom of the accessory box.



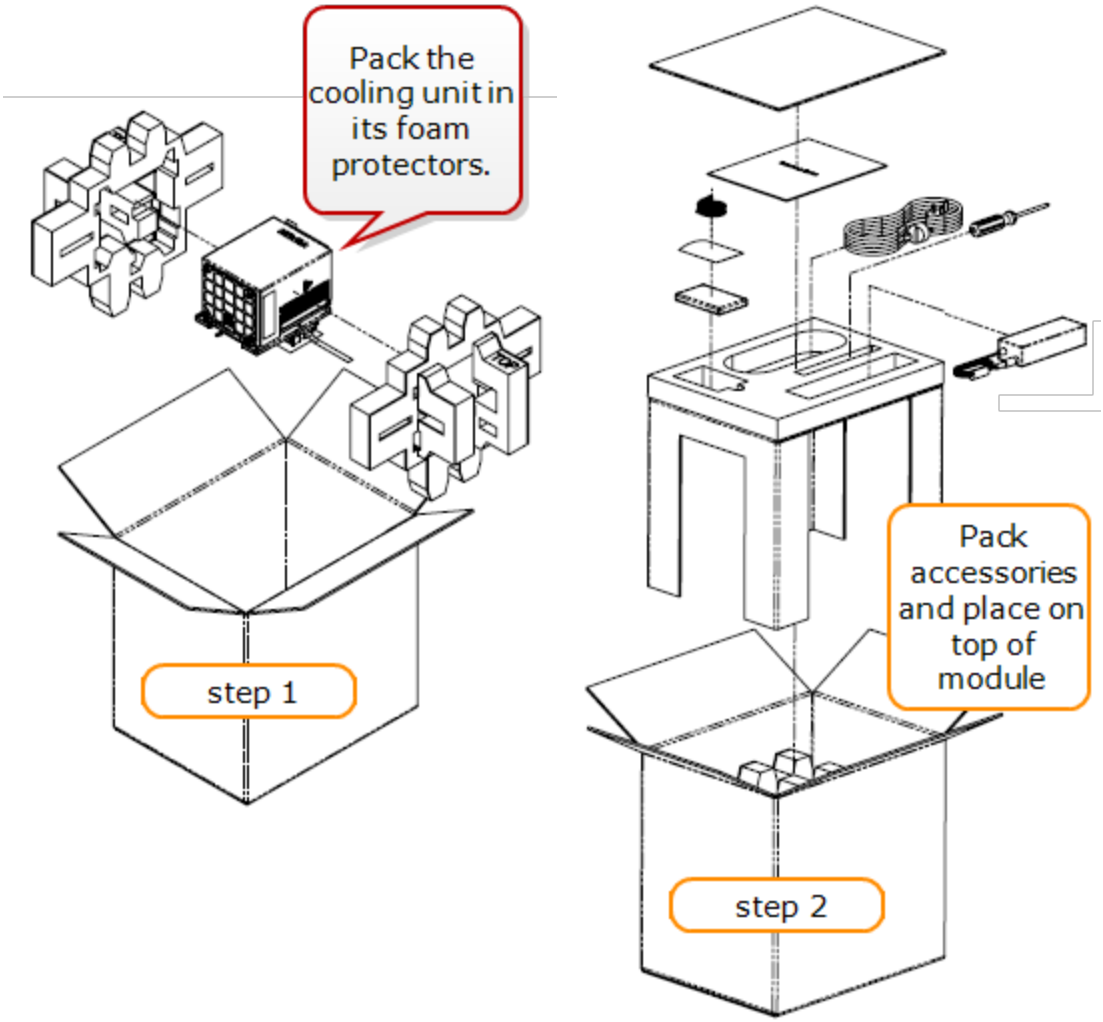
10. Cover the accessory parts with the top foam insert, and put the serial cable in the recess of the top insert.
11. Put the dispenser in a bag and then into its foam insert.
12. Add the rest of the accessories, including the box containing the accessories, to the shipping box.
13. Tape the box closed.

Note: Remove the tip priming trough and priming plate from the instrument before packing it.



Repacking the Peltier Cooling Module

Reuse the original shipping materials to repack and ship the Peltier Cooling Module, when necessary.



Chapter 3

Getting Started

This chapter describes some of the Cytation 5's key components, and provides an introduction to using BioTek Gen5 software to control the instrument.

Modular Design

The Cytation 5 is an imaging multi-mode microplate reader, with a design that allows you to initially purchase only the detection capabilities you need and then upgrade later as your requirements expand. Please contact BioTek to learn more about your upgrade options.

Gen5 software is used to control the reader. If the reader is connected and turned on, Gen5 will present you with only those options that apply to your reader model. For example, if your model is not equipped with the monochromator system, Gen5 will not provide the option to create this type of read in your assay protocol.

The module letters form the part number for each model, indicated on a label on the reader.

Part Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CYT5FV-SN	•	•	•			•	•	•	•					
CYT5FW-SN	•	•	•		•	•	•	•	•					
CYT5FAV-SN	•	•	•			•	•	•	•	•				
CYT5FAW-SN	•	•	•		•	•	•	•	•	•				
CYT5MV-SN	•	•	•								•	•	•	•
CYT5MW-SN	•	•	•		•						•	•	•	•
CYT5MPV-SN	•	•	•	•							•	•	•	•
CYT5MPW-SN	•	•	•	•	•						•	•	•	•
CYT5MFV-SN	•	•	•			•	•	•	•		•	•	•	•
CYT5MFW-SN	•	•	•		•	•	•	•	•		•	•	•	•
CYT5MFAV-SN	•	•	•			•	•	•	•	•	•	•	•	•
CYT5MFAW-SN	•	•	•		•	•	•	•	•	•	•	•	•	•
CYT5V-SN	•	•	•											
CYT5W-SN	•	•	•		•									
CYT5PV-SN	•	•	•	•										
CYT5PW-SN	•	•	•	•	•									
CYT5MF-SN						•	•	•	•		•	•	•	•
CYT5MFA-SN						•	•	•	•	•	•	•	•	•
CYT5F-SN						•	•	•	•					
CYT5FA-SN						•	•	•	•	•				

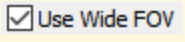
Part Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CYT5M-SN											•	•	•	•

1. Fluorescence imaging
2. Brightfield imaging
3. Color brightfield imaging
4. Phase contrast imaging
5. Wide field of view (WFOV) camera
6. Filter-based fluorescence intensity
7. Filter-based fluorescence polarization
8. Filter-based time resolved fluorescence
9. Filter-based luminescence
10. Filter-based Alpha detection
11. Monochromator-based UV-Vis absorbance
12. Monochromator-based fluorescence intensity
13. Monochromator-based time resolved fluorescence (secondary)
14. Monochromator-based Luminescence

"W" model limitations:

During Auto Calibration of the imager, Gen5 captures **FL Illumination Correction** images used to correct light fall off at the edges of wide field-of-view acquired images. But the following LED-Filter cubes are not supported with this method:

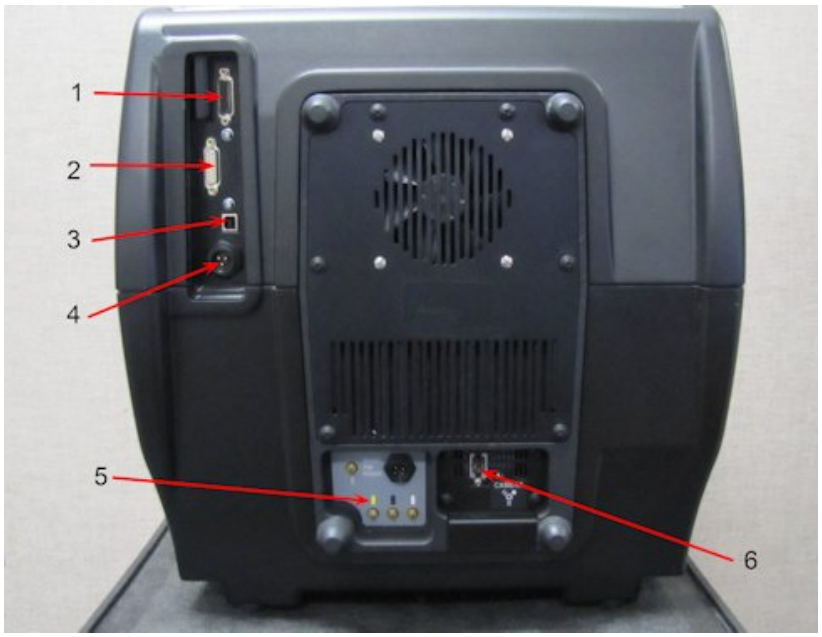
LED-Filter Cube Name	PNs	EX/EM
Cy5.5	1225114 - 1225008	647/794
Cy7	1225106 - 1225006	716/809
CFP/YFP FRET v2	1225119 - 1225011	400/550
PE	1225113 - 1225001	469/593



De-select the **Use WFOV** option to capture images using the unsupported imaging components listed above. Images can be successfully captured with a reduced field of view. [Expand the **Control** panel in imager manual mode for this option.]

External Components

1	Dispense tubing feed-through (for Dual Injector dispense module)
2	Top door (fluorescence filter cube or phase annulus access)
3	Microplate carrier eject button
4	Light-blocking microplate carrier access door
5	Power switch
6	LED cubes, imaging filter cubes, and objectives access door



1	Dispenser port
2	Joystick port
3	USB port
4	Power inlet
5	Gas controller hookup
6	USB 3 cable for camera

Internal Components

Component	Description	Page
Filter Cube	**Models with filter fluorescence.** The filter cube can contain excitation and emission filters, mirrors, and polarizing filters. Preconfigured cubes are available from BioTek, or you can change the filters and mirrors yourself. *Note:* Do not confuse these cubes with the imaging LED cubes and filter cubes.	page 54
Imaging System	**Models with the imaging module.** The imaging system, comprising a digital camera, objectives, LED cubes, and filter cubes, allows you to run experiments with imaging reads as well as view images in live mode.	page 65
Injector System	**Models with the dispense module.** The syringes may	page

Component	Description	Page
	require replacement over time. The tubing and injectors require cleaning at regular intervals.	56

Filter Cube

F-model (fluorescence filter) instruments are equipped with a filter cube that contains excitation and emission filters, mirrors, and, if required, polarizing filters. Each filter cube contains two filter sets, each of which contains one excitation filter, one mirror, and one emission filter. The filter cube is accessed via the top door of the instrument.

BioTek ships the Cytation 5 with the default filter cube configuration defined in its onboard software (or basecode). The default filter cube configuration is shown below. Your sales order lists the filter cubes shipped separately with the instrument. Verify that your cubes contain the expected/ordered filters and mirrors. Contact BioTek or your supplier if the reader is not equipped with the expected filters.

Default Fluorescence Filter Cube Configuration

	Position 1	Position 2
Excitation	360/40	485/20
Emission	460/40	528/20
Mirror	400	510

CAUTION **Instrument Configuration.** When you install or change objectives, LED cubes, imaging filter cubes, or fluorescence filter cubes you must update the instrument configuration values in Gen5 and download the information to the instrument.

Note: Review the recommendations for managing multiple filter cubes, including changing filter cubes, beginning with Filters and Mirrors Overview on page 72.

Important: Do not open the top door or any compartment during instrument operation. Doing so may result in invalid data.

Configuring the System for Luminescence Measurements

If your tests require that light emitted from the samples remain unfiltered, the Emission filter position in the filter cube should be empty. Installing or Removing a Filter Cube on page 74.

Injector System

Applies only to instruments equipped with injectors.

Important

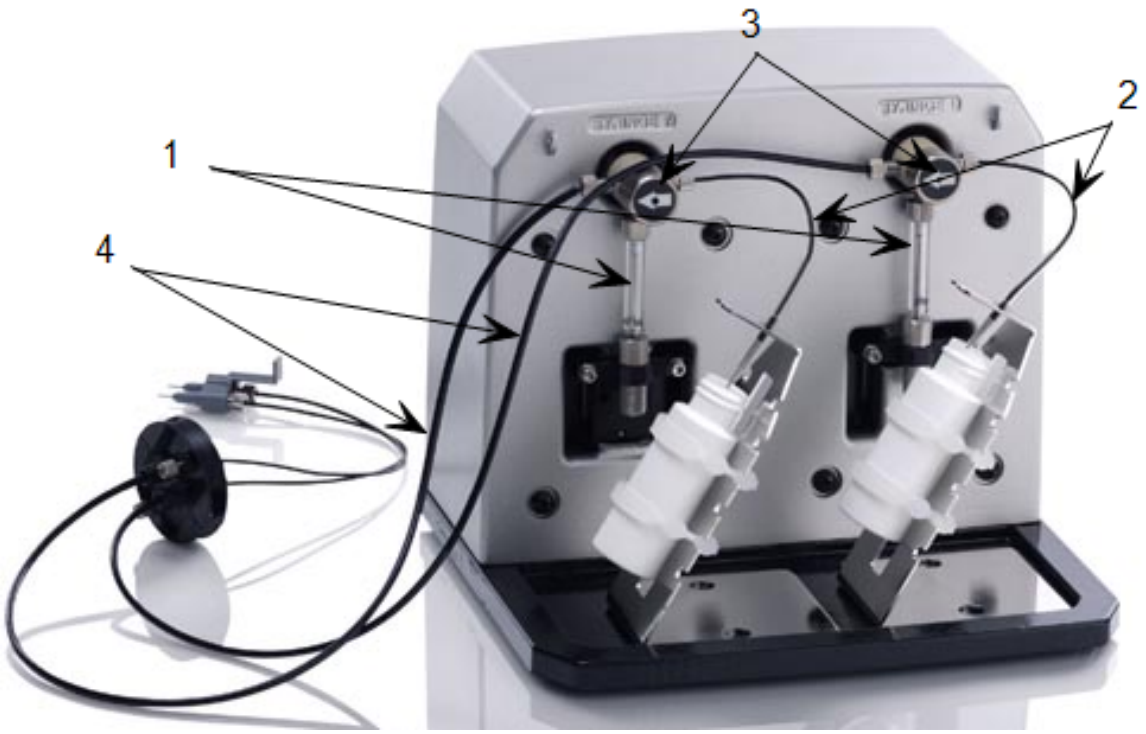
- Clean the tubing and injectors at least every three months. PM-4: Flush/Purge the Fluid Path on page 99.
- Inspect the injector system daily for leaks, preferably immediately after priming and whenever tubing changes have been made.
- If a syringe is leaking, it may need to be replaced. Dispenser Syringe Replacement on page 113.

Dispense Module

WARNING

Pinch Hazard. Some areas of the instrument and the dispense module can present pinch hazards when the instrument is operating. The objective turret and dispense module are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.

The dispense module sits on top of the reader or gas controller and pumps fluid from the reagent bottles to injector located inside the instrument. Fluid is injected into one well at a time. The injectors support plate types from 6- to 384-well plates.



1 Two 250 µL syringes draw fluid from the supply bottles

2 Inlet tubes transport fluid from the supply bottles to the syringes. These tubes are short pieces of opaque PTFE (Teflon) tubing connected to stainless-steel probes on one end and threaded fittings on the other end.

3 Solenoid valves allow the fluid drawn from the supply bottles by the syringe pumps to flow into the outlet tubes.

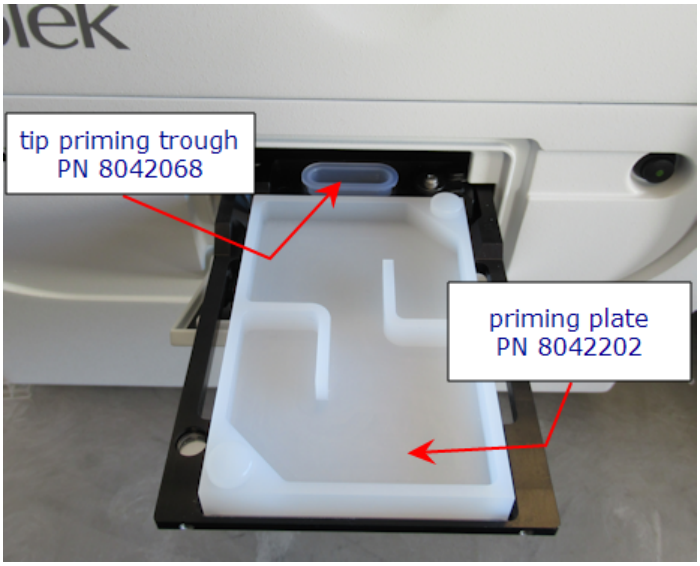
4 Outlet tubes transport fluid from the syringes into the instrument, through the tubing ports on the Cytation 5's top cover. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

Note: Avoid continuous contact with harsh chemicals. Rinse the fluid path with deionized water after contact with any strong acid, base, or solvent.

For information on the materials used in the injection system refer to Dispenser Module: Injection System Chemical Compatibility on page 1.

Priming the Injector System

Before running a dispense assay, prime the system with the reagent or dispensing fluid. In addition, tip priming can be performed at the start of an assay and, sometimes, just before each dispense to a well. The tip prime compensates for any fluid loss at the injector tip due to evaporation since the last dispense. All priming activities are controlled via Gen5.



Note: If the injector system is not primed adequately, air bubbles can get trapped in the system and affect injection volumes. Air bubbles in the system can also result in fluid spraying or scattering inside the reader.

Both types of primes require a fluid reservoir to be present on the microplate carrier.

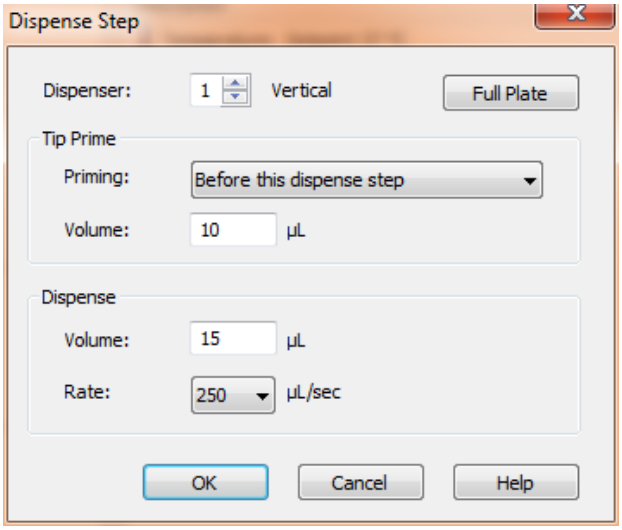
- The priming plate is placed on the microplate carrier for a Prime operation (to prime the dispense system with fluid).
- The tip priming trough is placed in the rear pocket of the carrier to catch the Tip Prime before dispensing. The trough holds up to 1.5 mL of liquid and must be periodically emptied and cleaned by the user.

Important: *Do not perform tip priming when using tall plates.* Generally, plates with fewer than 96 wells are too tall for error-free tip priming, and tip priming is rarely required for these larger-volume plates.

The priming plate should be empty before priming and contain fluid after priming.

Dispense Module Control

This section applies only to models with injectors.



Gen5 performs several dispense functions, such as initialize, dispense, prime, and purge. A Dispense Step in the protocol injects reagent into specified wells prior to reading the well.

Note: Priming and purging routines are used to clean the fluid paths. PM-4: Flush/Purge the Fluid Path on page 99.

The Prime and Purge functions are introduced here. See the Gen5 Help system for more information.

Prime

Before running an experiment with a dispense step, prime the system with the fluid to be used.

1. Place the priming plate on the carrier.
2. Fill the supply bottle with a sufficient volume of the fluid to be used for the prime and the assay. Insert the appropriate inlet tube into the bottle.
3. In Gen5, use the **Instrument Control** tab (or select it from the **System** menu) to select **Prime/Dispense** and select the **Prime** tab.
4. Select the **Dispenser** number (1 or 2) associated with the supply bottle.
5. Enter the **Volume** to be used for the prime. The minimum recommended prime volume is 2000 µL.
6. Select a prime **Rate**, in µL/second.
7. Click **Prime** to start the process.
8. When finished, carefully remove the priming plate from the carrier and empty it.

Note: If the priming plate is empty, the prime volume was too low.

Purge

To save reagent, Gen5 provides the option to purge fluid from the system back into the supply bottle.

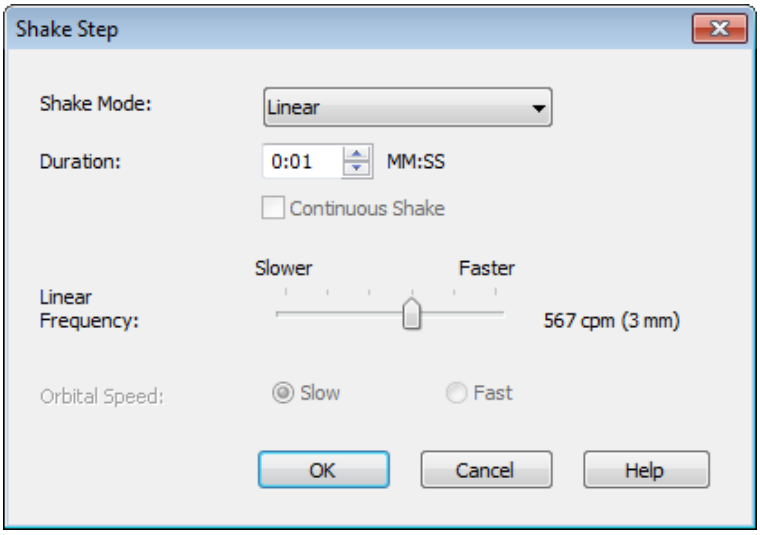
1. In Gen5, use the **Instrument Control** tab (or select it from the **System** menu) and click the **Prime** tab.
2. Select the **Dispenser** number (1 or 2) associated with the supply bottle.
3. Enter the desired purge **Volume** in µL (e.g., 2000).

4. Select a prime **Rate** in µL/second.
5. Click **Purge** to start the process.

Plate Shaking Options

Three shake modes are available for selection in Gen5:

- Linear: a linear shake moves the carrier back and forth in the y-axis.
- Orbital: an orbital shake moves the carrier in both x- and y-axes to scribe a circle.
- Double Orbital: the carrier moves in a figure-eight pattern.



For any mode, a slider bar in the software allows you to adjust the shake frequency from "Slower" to "Faster." With each adjustment, the corresponding cycles per minute (CPM) is displayed. For either orbital mode, you can further expand the frequency options by clicking a Slow or Fast button.

Plate Shaking Specifications

Option	Amplitude/Displacement	Frequency
Linear	1 mm to 6 mm in 1-mm steps	~18 Hz to ~6 Hz
Orbital Slow	1 mm to 6 mm in 1-mm steps	~10 Hz to ~3 Hz
Orbital Fast	1 mm to 6 mm in 1-mm steps	~14 Hz to ~5 Hz
Double Orbital Slow	1 mm to 6 mm in 1-mm steps	~10 Hz to ~3 Hz
Double Orbital Fast	1 mm to 6 mm in 1-mm steps	~14 Hz to ~5 Hz
Frequency is based on the selected amplitude or displacement.		

Peltier Cooling Module Described

Applicable only when the optional Peltier Cooling Module is installed

The optional, add-on cooling module significantly speeds up the Cytation 5's ability to return to ambient temperature after incubation. Strategies to minimize condensation in the instrument are built into the cooling module's behavior. In long-term room-temperature experiments the cooling module helps maintain ambient temperature inside the reader. Otherwise, the cooling module does not control temperature in the reader.

Cytation 5 models with this serial number and higher are compatible with the Peltier Cooling Module:

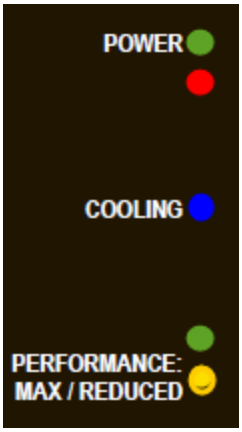
- S/N 1808023

Follow the installation instructions provided with the cooling module.

Important: Leave sufficient room around the cooling module to guarantee a fresh supply of circulating air (to dissipate heat and keep it cool).

Note: BioTek offers a specially designed isolation table (PN 1700508) for use with the Cytation 5 when the Peltier Cooling Module is installed. The special isolation table accommodates the extra weight of the cooling module.

When the cooling module is installed and powered-up, the Cytation 5 controls its performance, telling it when to turn on and off. An LED panel on the side of the cooling module shows its current status:



Power	Green = power on, ready Red = error state No color = no power
Cooling	Blue = running No color = no demand for cooling (or no power)
Performance	Green = Max energy for fastest cooling possible Yellow = Reduced energy to prevent condensation[1] No color = no demand for cooling (or no power)

Error State

When an error is detected, the Power LED turns red and an alarm sounds:

[1]Max performance requires Relative Humidity to be 50% or less, otherwise performance is reduced to prevent condensation buildup.

1. Press the **Silence Alarm** button to turn off the alarm.
2. Unplug the cooling module to power it off.
3. Clear any obstructions to the air flow around the cooling module, especially any objects underneath it.
4. Plug in the cooling module, wait a few minutes to determine if the error state persists. Contact Technical Support if the error reoccurs.

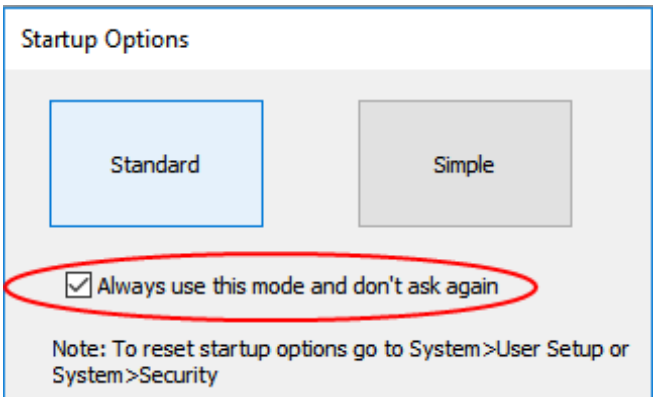
Maintenance

PM-10: Clean Peltier Cooling Module Filter on page 106.

Gen5 Software

Use Gen5 to:

- control the instrument, dispense module, and the stacker (if applicable)
- perform data reduction and analysis on the measurement values
- print or export results; and more



After the splash screen disappears, Gen5 presents "Startup options." Choose **Standard** to install the instrument and perform IQOQPQ procedures.

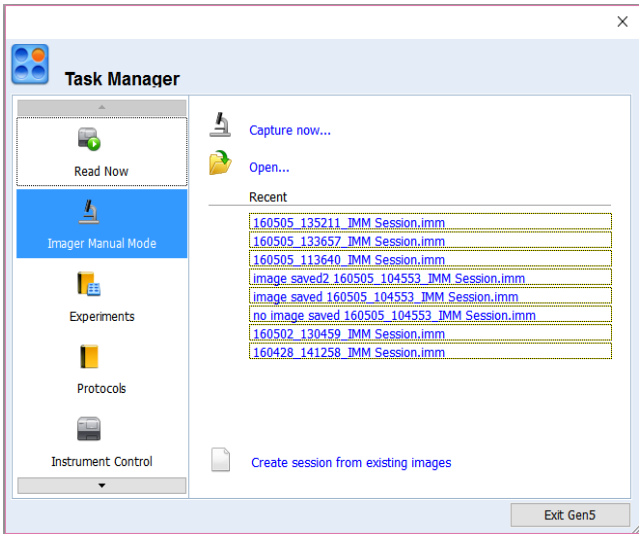
- Standard mode is recommended for imaging;
- Use Simple mode for performing single-read-step absorbance and fluorescence protocols.

- Choose an option and fill the checkbox to skip this step in future.

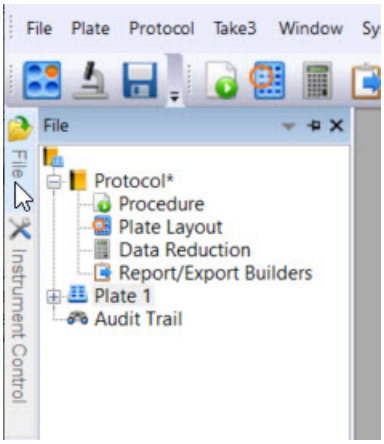
Protocols and Experiments

This section provides brief instructions for working with Gen5 to create protocols and experiments and read plates. Refer to information provided with the software and the Gen5 help system for more information. Access the help system by selecting **Help > Help Topics** or by clicking the **Help** button in any dialog.

In Gen5, a protocol contains instructions for controlling the instrument and analyzing the data retrieved from the instrument. A protocol specifies how to process the assay, including: reading parameters, incubation settings, injection volume, etc., and the data reduction steps to perform on the collected data. In Gen5, every experiment contains a protocol (to be more precise, every experiment contains an embedded copy of the protocol). You run the "experiment" to execute the protocol to read plates and analyze the acquired data.



1. From the Task Manger, select **Create New** (or **Open** a) protocol/experiment.
2. Numerous protocol options are available. Choose the type that best fits your assay requirements.



3. Hover your mouse over the **File** tab to expose the protocol controls.
4. Define the **Procedure**. At the prompt, select the Cytation 5 and click **OK**.
5. Select the **Plate Type** that matches your vessel.
6. Define all the steps needed for your assay, including the Plate Layout (or plate map), and data reduction steps you want to perform.
7. Finally, set up Microsoft Excel reports and/or export the data.
8.  Create an experiment based on the protocol, if you started with protocol creation. Gen5 keeps a copy of the original protocol for future experiments. Meanwhile, in an experiment, you can optimize the protocol parameters without affecting the original protocol.
9.  Click **Read Now** to read the plate.

Note: Gen5 stores the dimensions and other characteristics of various plate types in a database. It is essential that you select (or define) a plate type to match your assay plate/vessel. Otherwise, results may be invalid. For imaging reads, you must also define the **Bottom Elevation** parameter for the plate or slide. See the "Plate Type Database" topic in the Gen5 Help for instructions.

Imaging System

Imaging instruments can:

- fine-tune Autofocus settings and capture images in manual mode,
- perform "image" reads in experiment mode,
- save the images for later analysis using Gen5 or a third-party software.

The imaging module comprises up to four LED cubes and four imaging filter cubes, up to six objectives, and a digital camera, which captures images directly through the selected objective and filter cube assembly.

Camera

Gen5 controls the camera via USB3. Using Gen5, you can focus the camera, determine exposure settings, and capture images.

LED Cubes and Imaging Filter Cubes

The LED cubes and filter cubes are located in the compartment accessed by the left door of the instrument and are user-changeable. Changing LED Cubes and Imaging Filter Cubes on page 81 for more information.

Objectives

The objectives are located next to the LED cubes and imaging filter cubes in an objective turret. Gen5 supports the installation of up to six user-changeable objectives.

Imaging Modes

The Gen5 imaging module provides two modes of use: manual and experiment.

- **Manual mode** allows you to view, capture, and analyze images outside of a protocol or experiment. Images are displayed in real time. You can also retrieve previously captured and saved images to analyze in manual mode.
- In **experiment mode**, you can perform an image read step as an endpoint read or include it in a kinetic block in your procedures and experiments. An imaging procedure can include additional steps (as supported by the reader), such as dispense, shake, incubate, gas recharge, and different detection modes.

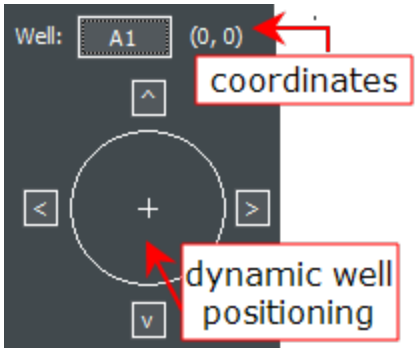
Using the Slide Holder and Other Labware

The BioTek-provided slide holder (PN 1220548) has two well positions. Position the holder on the plate carrier aligning the "A1" etching in the holder with the A1 mark on the carrier.

For best results, because the objectives read the slide from below, put the slide in the holder with its cover plate facing down, if possible.

Manual Mode

1. When prompted, select the **Slide Holder All Mag. (w/A1 mark)** as your plate type.
2. Select the objective and color channel. Begin with your lowest-power objective.
3. If needed, click the **Well** button to select the position that contains the slide you want to image.



4. By default, the middle of the selected slide is positioned above the objective. If the sample on your slide is in a different location, use the dynamic well schematic and/or the arrow buttons to move the slide around until you find your sample.
5. After finding your sample, you can change to a higher power objective, if desired.

Experiment Mode

1. In an experiment, select **Slide Holder All Mag. (w/A1 mark)** as the plate type, and create an **Image** read step.
2. Clear the **Auto** box to turn off Auto Exposure, then click  to enter manual mode.
3. Perform step 4 in the manual mode procedure (above) to find your samples, if necessary.

4. In manual mode, switch between Auto Focus and Auto Exposure to determine optimal settings, and when satisfied, click **Save settings**. Gen5 imports your exposure and focus settings and the Horizontal and Vertical offsets into the read step.

Recommendations for Optimum Performance

General

- Use clean microplates, free of dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.
- Use flat-bottomed vessels when possible. Although the Cytation 5 supports standard flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with flat-bottomed wells when running in Absorbance mode. See *Appendix A, Specifications* for more information on the supported plates.
- Check for non-uniformity in the microplate's optical density by reading an empty microplate. Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Dual-wavelength readings can eliminate this problem or even out the variation in readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially when using small volumes. For best results in most cases, use at least 100 µL per well in a 96-well plate and 25 µL in a 384-well plate.
- Pipetting solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies. For best results, however, remove the air bubbles by degassing the plate in a vacuum chamber or spinning the plate in a centrifuge before reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Shake the microplate before reading to help bring it within acceptable limits. Use Tween 20, if possible (or some other wetting agent) to normalize the meniscus for absorbance measurements. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change. The meniscus shape will stabilize over time.
- It is the user's responsibility to understand the volumetric limits of the plate type in use as it applies to the assay being run.
- Use of liquids with concentrations of acids, corrosives, or solvents of 3% or greater can corrode the materials inside the instrument's chamber. Running multiple plates with concentrations <3% in long kinetic assays may also have a destructive effect. If the experiment is incubated, it will accelerate the deterioration of chamber components. When in doubt about the use of acids, corrosives, or solvents, please contact Technical Support.

Note: Dimethyl sulfoxide (DMSO) vapor can create a removable deposit on optical surfaces, which can trigger instrument self-test errors. Using **DMSO assay concentrations of 2% or below** is recommended. Limit long exposure in kinetic assays or incubated assays when possible. For questions about the use of DMSO, please contact Technical Support.

Read Direction

The Cytation 5 performs most reads in a row-wise direction, that is, moving from well A1 to A2, then A3, and so on.

Luminescence Measurements

For highly sensitive luminescence assays using white plates, add a Delay step to your procedure to "dark adapt" the plates in the Cytation 5's reading chamber before taking measurements.

Monochromator-Based Fluorescence Systems

Although Time-Resolved Fluorescence can be performed with the monochromator, the filter-based fluorescence system is more sensitive for TRF and is the better choice.

Models with Dispenser (Injectors)

- To keep the dispense system in top condition, flush and purge the fluid lines with deionized (DI) water every day or upon completion of an assay run, whichever is more frequent. Some reagents may crystallize or harden after use, clogging the fluid passageways. Flushing the tubing at the end of each day, letting the DI water soak, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. See the *Periodic Maintenance* chapter for more information.
- When dispensing volumes less than or equal to 20 µL/well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20 µL/well, we recommend a tip prime volume of 20 µL.
- To avoid spillage and possible contamination of the instrument, empty the tip prime trough frequently and do not exceed the total fluid volume of the plate well when dispensing.

Using 384-Well Microplates

When using a 384-well microplate, you can use the Gen5 Auto Map feature to ensure you are using an accurate plate map for your reads. See the Gen5 Help for more information.

Incubation and Partial Plates

Reduce the effects of evaporation on your samples in a partial plate read that includes incubation by:

- Using microplate lids.
- Filling unused wells with fluid.
- Clustering your sample wells rather than spacing them throughout the plate.
- Placing your sample wells in the center of the plate instead of the edges of the plate.

Imaging

- For the best imaging results use Gen5's default settings. BioTek developed the default settings after extensive testing with each magnification or objective and LED cube and filter cube combination.
- Be sure to Define your vessel's Bottom Elevation on page 91.
- When using high-power objectives: Adjust the Correction Collar on page 87 to match your sample vessel.

Chapter 4

Fluorescence Filter Cubes, and Imaging Cubes & Objectives

The *Getting Started* chapter provided an overview of the filters, mirrors, and objectives and LED cubes installed in some Cytation 5 models. This chapter provides more detailed information on working with these components.

Filters and Mirrors Overview

Cytation 5 is equipped with excitation and emission filters for obtaining fluorescence and luminescence measurements. The excitation filter selects the band of light to which the sample will be exposed. The emission filter selects the band of light with the maximum fluorescence signal of the sample, to be measured by the photomultiplier tube (PMT).

For filter-based, top-reading fluorescence analysis, the Cytation 5 uses mirrors to direct the excitation and emission light paths. Mirrors are required for fluorescence polarization (FP) measurements to direct light to the sample, because fibers cannot carry polarized light. Mirrors also provide increased sensitivity for fluorescence intensity (FI) and time-resolved fluorescence (TRF) measurements.

Filters and mirrors are stored in a filter cube. You can replace the entire cube with a different one; this is the BioTek-recommended option. Alternatively, you can install different filters or mirrors in the cube. Contact BioTek for more information on purchasing additional filters and mirrors.

Fluorescence Filter Cubes

Note: For information about the imaging LED cubes and filter cubes, see Imaging LED Cubes and Filter Cubes on page 81.

Filter Cubes Available from BioTek

Preconfigured filter cubes, LED cubes, and imaging filter cubes are available for purchase from BioTek. Please note that part numbers are subject to change, and new cubes may become available. Custom filters are also available. Contact BioTek with any questions.

Overview

Most Cytation 5 models are equipped with excitation and emission filters and mirrors for obtaining fluorescence and luminescence measurements. Each filter cube has two filter sets, each of which contains one excitation filter, one emission filter, and one mirror. The filter cube is accessed through the top door of the instrument. You can mark the label on the front of the filter cube with the contents of the cube.

Filter cubes are easily reconfigured to meet your assay requirements. However, if you regularly need to change the filters or mirrors, consider purchasing additional filter cubes from BioTek to make the process easier and faster.

Note: If you have more than one filter cube, use the Gen5 Filter Cube Library to save time when changing cubes. Adding a Filter Cube to the Optics Library on page 79.

The Synergy H1 filter cubes are interchangeable with the Cytation 5 filter cubes.

The default filter cube configuration is shown below; any changes are reflected in the sales order.

Default Fluorescence Filter Cube Configuration

	Position 1	Position 2
Excitation	360/40	485/20
Emission	460/40	528/20
Mirror	400	510

Filters are not specific to either excitation or emission. Filter direction within the filter cube is important, and the direction differs depending on the filter type. Each filter has its central wavelength and band pass values printed on its side, with an arrow to indicate the proper direction of light through the filter.

The filter cube can hold up to two half-size or one full-size dichroic or 50% mirror. The mirror positions are labeled "1" and "2" to coordinate with EX1/EM1 and EX2/EM2.

For Cytation 5 models with FP capability, the cube is equipped with up to four polarizers of the following types:

- Excitation polarizer (2): visible-range and UV-range
- Emission polarizer: parallel to excitation polarizer
- Emission polarizer: perpendicular to excitation polarizer

Two types of excitation (EX) polarizers are available: visible-range (400 nm and above, the default) or UV-range (300 nm and above, available from BioTek). The polarizers, if used, are placed below the excitation filters and above the emission filters.

Cleaning the Filters and Mirrors

Instructions are provided in the *Periodic Maintenance* chapter.

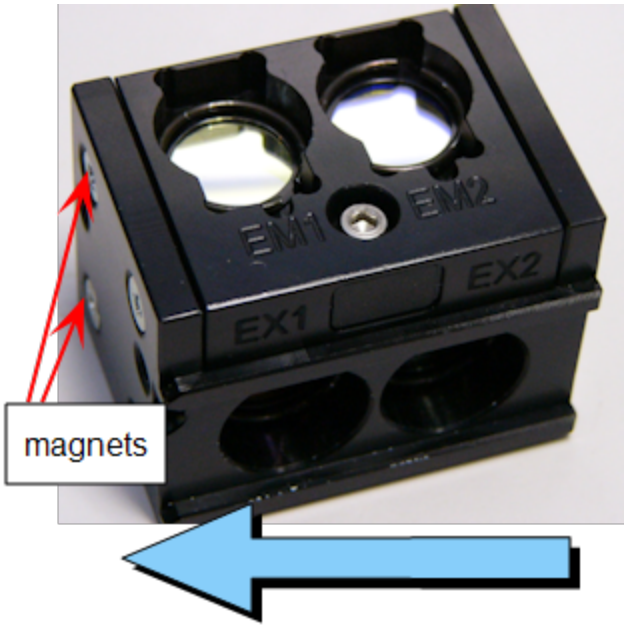
Installing or Removing a Filter Cube

Important: Do not open the top door of the instrument during operation. Doing so may affect measurements.

Turn on the reader to move the filter cube to an accessible position (if necessary).

Lift up the top door on front of the reader:

- To install a filter cube: Position the cube with its magnets and pin holes facing left. Slide it gently into its chamber. The magnets hold it in place inside the reader.
- To remove a filter cube, slide it out of its chamber.



When changing filter cubes, you must tell Gen5 and the reader which cube you are installing.

CAUTION

Instrument Configuration. When you install or change objectives, LED cubes, imaging filter cubes, or fluorescence filter cubes you must update the instrument configuration values in Gen5 and download the information to the instrument.

First, create a record in the Optics Library describing the filter cube, then update the reader using that record:

- Adding a Filter Cube to the Optics Library on page 79
- Choose the update method in the Optics Library: most users opt to let Gen5 automatically update the reader when a filter cube is selected in the protocol.

Defining Fluorescence Filter Cubes in Gen5

For "F" models (filter fluorescence), the filter cube's characteristics must be entered into Gen5 and downloaded to the reader. Perform these steps before using the reader for the first time, and again if you change the filter cube's contents or switch to a different cube.

1. Select **System > Instrument Configuration**. Highlight the **Cytation 5**, and click **View/Modify**.
2. Click **Setup**, and then click the **Filter Cube** tab.
3. Enter a name for the filter cube.
4. Select **Fluorescence Polarization Cube**, if applicable.
5. Enter a filter set name for Filter Set 1, and define the excitation, mirror, and emission settings:
    - *Band Pass*, a standard interference filter with a defined central wavelength and bandwidth.
    - *Long Pass*, cutoff filters that transmit longer wavelengths and block shorter wavelengths.
    - *Short Pass*, cutoff filters that transmit shorter wavelengths and block longer wavelengths.
    - *Plug* indicates the presence of a plug.
    - *Hole* indicates an empty location.
6. Click **Send Values** to download the data to the instrument.
7. Repeat steps for Filter Set 2.

Changing the Filters and Mirrors in a Filter Cube

You need the following tools to change a filter or mirror in a filter cube:

- 7/64" hex wrench
- Lens paper
- Cotton swab
- Linen or cloth gloves

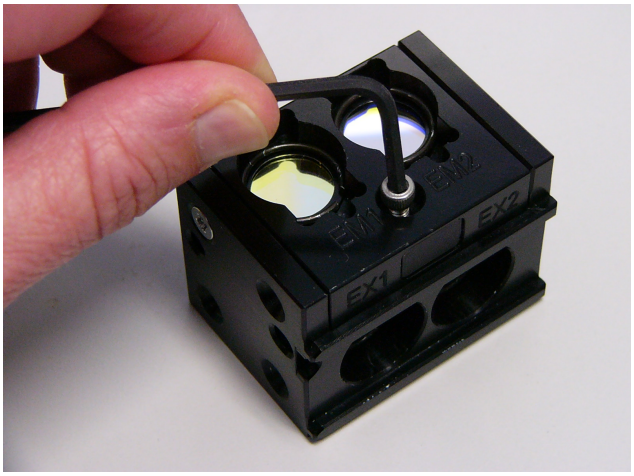
CAUTION **Instrument Configuration.** When you install or change objectives, LED cubes, imaging filter cubes, or fluorescence filter cubes you must update the instrument configuration values in Gen5 and download the information to the instrument.

Note: If you accidentally touch a mirror or polarizing filter, see PM-3: Inspect/Clean Mirrors on page 98.

To remove a filter, plug, or mirror

Important: After you remove the filter cube top, the mirrors will fall out of the cube if the cube is not on a stable, flat surface.

1. Remove the filter cube.
2. Set the cube on the work surface.
3. Using a 7/64" hex wrench, remove the screw and washer located between the emission filter positions (as shown right).
4. Carefully lift the filter cube top from the cube. The top contains the emission filters.



Remove the screw from between EM1 and EM2

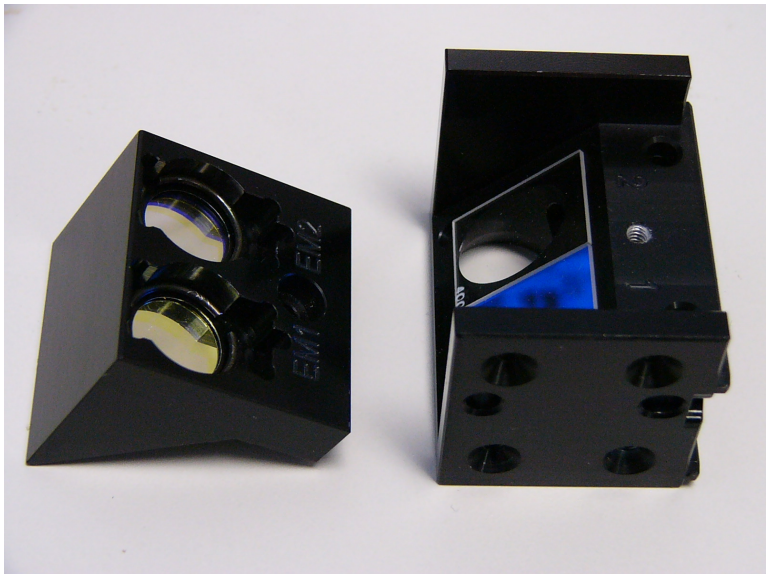
CAUTION **LED cubes and filter cubes.** Wear gloves when changing components to avoid contaminating them.

Important: When removing or replacing a filter or C-clip filter retainer, do not use a sharp instrument. Use several layers of lens paper and your finger to remove and replace

filters and clips. Using a sharp instrument, such as a flat screwdriver, will scratch the filter surface and make it unusable.

The mirrors are seated on a shelf in the bottom of the cube and are not secured in place.

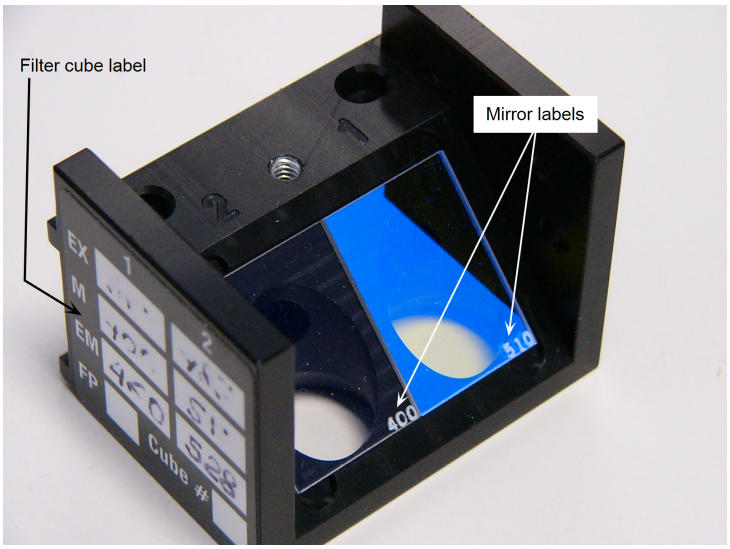
1. To remove an emission filter, prepare a multi-layered "cushion" of lens paper. Using your finger covered with the lens paper, gently push against the filter and its retainer until they pop out.
2. The bottom of the cube contains the excitation filters and mirrors. Remove the mirrors before removing the excitation filters:



Filter cube with top removed

   - Make note of the mirror placement and label orientation.
   - Wearing linen or cloth gloves, carefully grasp the mirror by its edges, lift it out of the cube, and store it properly.
3. To remove an excitation filter, use a cotton swab to gently push against the filter, the aperture, and the C-clip retainer until they pop out.

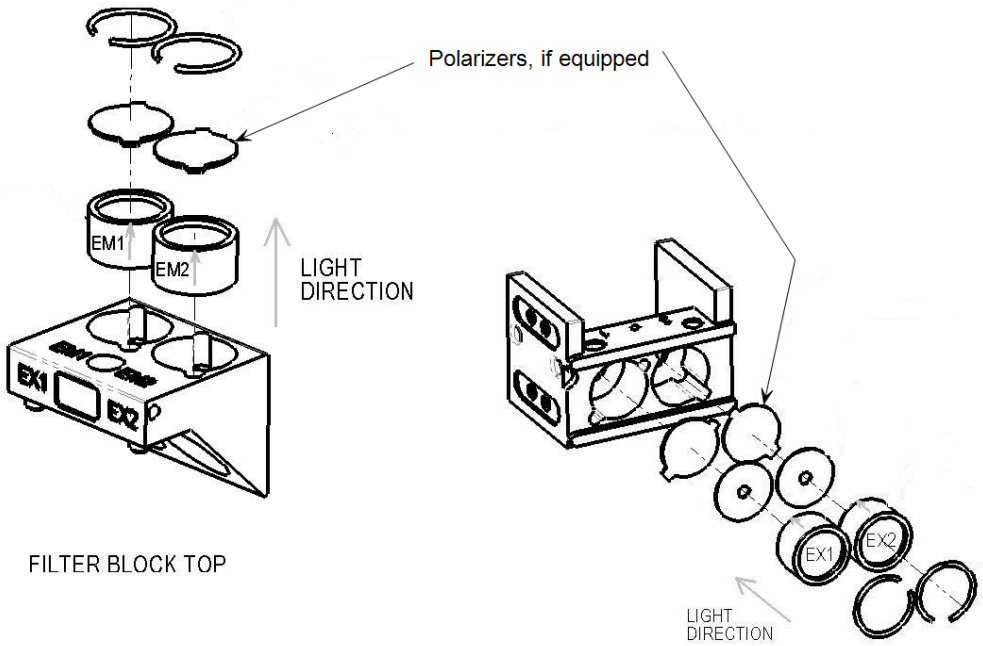
Mirror direction is important. The mirror label should be in the lower-right corner of the mirror and readable. If the mirror is not positioned correctly in the filter cube, your measurement data may be inaccurate.



Mirrors positioned in the filter cube

To replace a filter, plug, or mirror

1. To replace a filter or plug:
    a. Orient it as shown below. Observe the arrow on the filter indicating the light direction, then drop it into the desired location.



Note: Make note of the filter position number (EX1/EX2 or EM1/EM2).

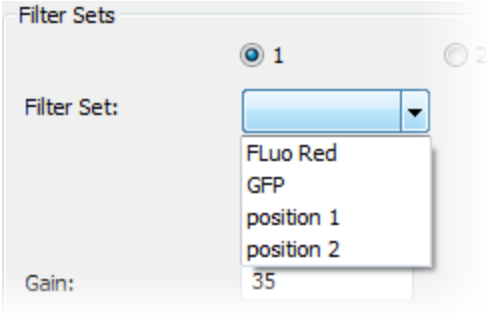
    b. Using your fingers, squeeze the sides of the C-clip retainer, and then insert it into the top of the hole containing the new filter. Cover your finger with several layers of lens paper, and then push down on all sides of the retainer until it sits flush against the filter.
    c. Gently wipe both sides of the filter with lens paper.
2. To replace a mirror, hold the mirror by its edges, turn it so that its label is face-up and readable, and place it on the shelf in the filter cube.
3. Place the filter cube top back into the cube and replace the screw and washer.
4. When finished, install the filter cube in the reader.

Fluorescence Filter Cube Library

If you have more than one filter cube, you can save time and ensure consistency by defining each cube for Gen5 in the Filter Cubes Library.

The Gen5 Filter Cubes Library keeps a record of each of your filter cubes and offers the cubes for selection when you define a read step for the Cytation 5.

The cubes are listed alphabetically. Default cubes are named position 1 and 2 (as shown right in the Gen5 read step window; FluoRed and GFP are custom cubes).



To open the Filter Cubes Library, from the Gen5 main screen, select **System > Optics Library > Filter Cubes**. You can add, modify, and delete filter cube information and select the desired behavior at runtime when a filter cube is installed or not (a Read Plate Prompt Option).

Adding a Filter Cube to the Optics Library

Save time when changing filter cubes by creating a filter cube record in Gen5's Optics Library. Then, you can instantly update the reader's internal software with the current filter cube configuration.

1. Click **System > Optics Library > Filter Cubes**.
2. Click **Add**, and enter a name for the filter cube. You will select this name when defining the protocol/experiment procedure.
3. Enter a name for Filter Set 1.

Note: Fluorescence Polarization Cube: Only Filter Set 1 is available for definition; the filters and mirrors of Filter Set 2 must be identical to Filter Set 1.

4. Define the Excitation and Emission filters:
   - Select **Band Pass**, **Long Pass**, or **Short Pass**, and enter the wavelength and bandwidth.
   - Select **Plug** to indicate the presence of a plug.
   - Select **Hole** to indicate an empty location.
5. Select the mirror type and enter the excitation and emission ranges.

Cut-off (nm)	Excitation Range	Emission Range
50%	200–850	200–850
320	260–305	335–750
365	290–350	380–800
400	320–390	410–800
435	385–425	445–610
455	400–450	460–710
510	440–505	515–640
525	475–520	530–670
545	512–535	555–578
550	415–540	560–850
555	541–550	560–595
570	515–565	575–735
595	540–590	600–770
635	640–780	400–630
660	580–655	665–850

6. Define Filter Set 2, if necessary.
7. Click **OK**.

Imaging LED Cubes and Filter Cubes

Cytation 5 models with imaging capability are equipped with up to four LED cubes and four filter cubes. The LED cubes hold an LED light source; the filter cubes contain excitation and emission filters and a dichroic mirror.

The LED cubes and filter cubes are accessed through a door on the left side of the instrument and are seated on a slide that you can pull out of the instrument, Changing LED Cubes and Imaging Filter Cubes below.

Note: Do not open the door to access the cubes during instrument operation. Doing so may result in invalid data.

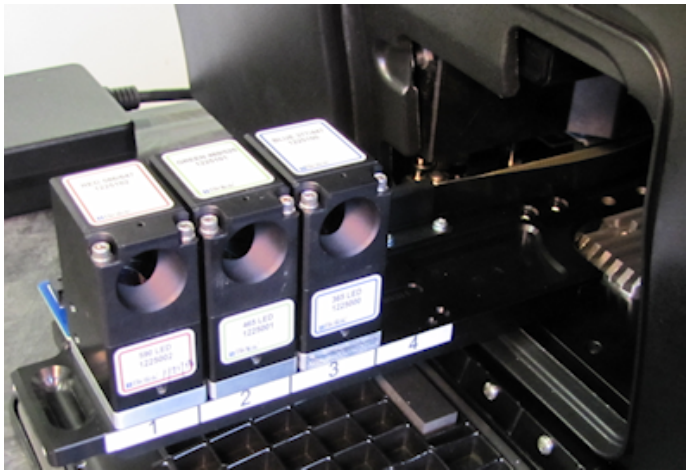
Changing LED Cubes and Imaging Filter Cubes

Important: Perform this procedure in the specified order to prevent damage to the LED cubes.

Before physically changing the LED and Filter cubes, to prevent overpowering the LED cubes, you must first tell the Cytation 5 which LED cube configurations are going to be installed.

Important: Wear gloves when changing components to avoid contaminating them.

Important: If used, the Laser AutoFocus cube (PN 1225010) must be in Position 1.



First, use Gen5 to update the Cytation 5's onboard settings:

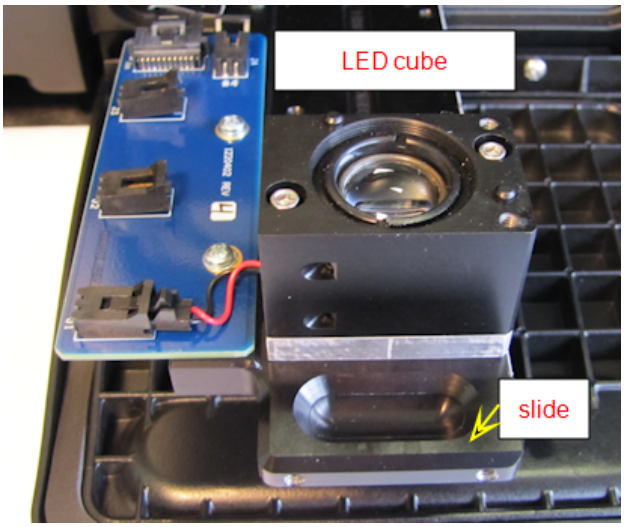
1. In Gen5, select **System>Instrument Configuration**. Double-click the Cytation 5 item and click **Setup**. Click the **Imaging Configuration** tab.
2. Set the LED cube and filter cube configurations for your components.
3. When defined, click **Send Values**. (Keep this Gen5 window open until the rest of the procedure is completed.)
4. Turn off the Cytation 5. But keep the Instrument Configuration tab open in Gen5.
5. Open the side access door, and pull the filter slide out of the instrument.
6. Using a 3/32" hex wrench, remove the two screws holding the imaging filter cube on the LED cube, and lift off the filter cube.
7. Unclip the LED cube's wire from the board, remove the two screws holding the LED cube on the filter slide, and remove the LED cube.
8. Install the new LED cube and imaging filter cube on the slide in the desired position, then push the slide back into the instrument.
9. Power up the instrument.
10. After the self-test is completed, on the Instrument Configuration tab, click **Auto Calibration**.

After the calibration procedure is finished, the instrument is ready to use.

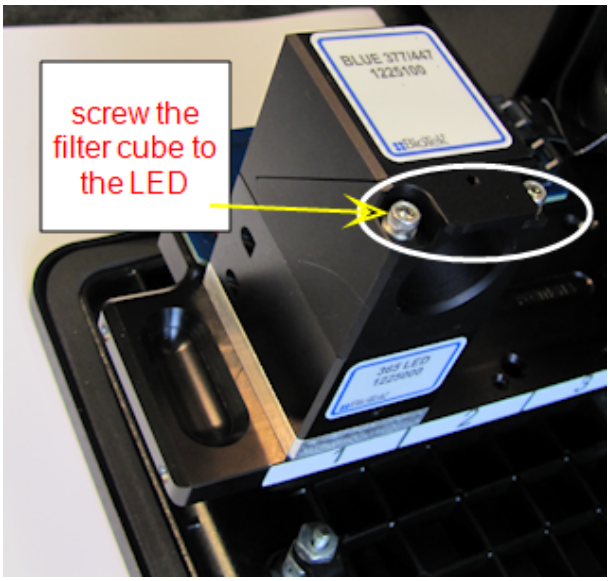
Installing the LED Cubes and Filter Cubes

Note: Do not open the side access door during operation. Doing so may affect measurements.

1. With the instrument powered down, turn the knob on the side access door to release the latch, and open the door.
2. Slide the filter slide out of the instrument.
3. Place the LED cube in its predefined position on the filter slide.
4. Insert the screws into the LED cube, and screw them into the filter slide.



5. Insert the LED cube's wire clip into the socket on the carrier.
6. Place the imaging filter cube on top of the LED cube you installed in the previous step.
7. Using a hex wrench, attach the filter cube to the LED cube.
8. Slide the filter slide back into the instrument.



LED Cube and Imaging Filter Cube Library

System > Optics Library > LED and Filter Cubes

The Gen5 LED Cube and Filter Cube Library contains files describing the LED cubes and filter cubes available for use with the Cytation 5. The cubes are listed by color, filter cube part number, excitation and emission wavelengths, and LED cube part number.

Import LED Cube and Filter Cube Definitions

Gen5 automatically installs definitions for the LED cubes and imaging filter cubes that were available when your Gen5 software version was produced. When you create a protocol or experiment procedure, the defined cubes are available for selection.

If you purchase a newer or custom cube, you must install its definitions from an XML file available from BioTek.

1. Go to https://www.biotek.com/products/software-robotics-software/gen5-software-features-for-imaging-microscopy/software/.

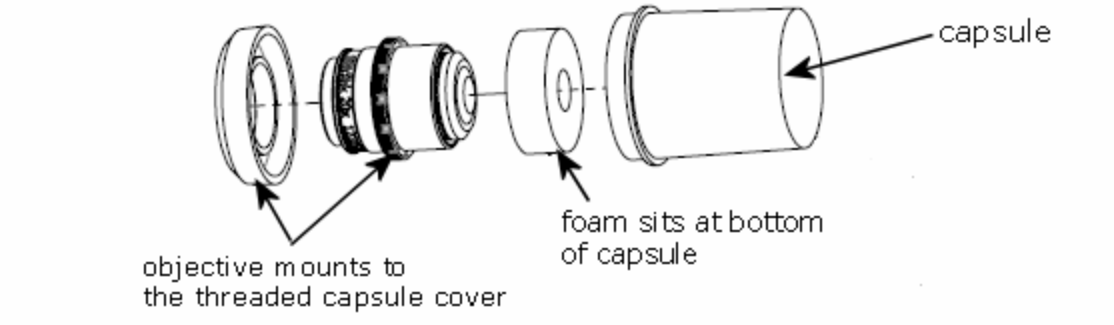
   Note: Contact Technical Support if this link is not accessible.

2. Select the option that matches your cube, and click its download link.
3. After downloading the XML file, "Save As" a copy of the file to your computer.
4. In Gen5, go to **System > Optics Library > LED and Filter Cubes**.
5. Click **Import**, and navigate to the folder containing the XML file.
6. Select the file, and click **Open**. If the XML file represents a new LED cube and filter cube, Gen5 imports its definitions. Otherwise, a message will say the LED Filter Cube list is up-to-date.

Imaging Objectives

Readers with the imaging module support fluorescence and brightfield reads using objectives. objectives can be installed simultaneously.

The objectives are shipped in capsules in the objectives case in the accessories box. Keep the capsules for storing the objectives when not in use.



The objectives are configured in the Imaging Configuration tab in the Reader Setup dialog. Gen5 communicates with the instrument to set and get the currently configured objectives. If you select an objective that is not defined as installed in the instrument, when validating an imaging read step, Gen5 displays an error message.

Import Objective Definitions

Gen5 automatically installs Objective Definitions for the objectives that were available when your Gen5 software version was produced. If you purchase a newer objective, you must install its definitions from an XML file provided by BioTek.

After downloading the XML file to your computer, import its contents:

1. Go to https://www.biotek.com/products/software-robotics-software/gen5-software-features-for-imaging-microscopy/software/.

   Note: Contact Technical Support if the link is not accessible.

2. Select the option that matches your objective, and download its file.
3. After downloading the XML file, save a copy of the file to your computer.
4. In Gen5, go to **System > Optics Library > Objectives**.
5. Click **Import**, and navigate to the folder containing the XML file.
6. Select the file, and click **Open**. If the XML file represents a new objective, Gen5 imports its definitions. Otherwise, a message will say the Objective list is up-to-date.

Good Advice from Olympus® Objective Manufacturers

Cleaning Optical Elements - The first rule in fluorescence microscopy (and all other forms of microscopy) is to keep the optical elements completely free of dust, dirt, oil, solvents, and any other contaminants. The microscope should be kept in a low-vibration smoke-free room that is clean as possible and has minimal disturbance of the circulated air. Use a dust cover on the microscope when not in use and keep all accessories in air-tight containers. Avoid using corrosive solvents to clean any part of the microscope, and use only diluted soapy water to clean non-optical surfaces. Objectives should be kept clean using the following tips:

- Never drag anything across the lens surface with a high degree of pressure, including lens paper, to avoid the possibility of introducing very fine scratches onto the surface.
- Clean the lens with one of the Recommended Cleaning Solvents on page 104. Avoid other solvents because they might react with optical coatings on the glass.
- Dust the lens surface with compressed gas prior to cleaning with a solvent to remove loose particles.
- Soak an optics-grade cotton swab with lens cleaner or ethanol and very gently wipe it over the lens several times, turning the cotton tip before each pass. Blot excess solvent with lens tissue and allow the lens to dry thoroughly. Repeat this procedure.

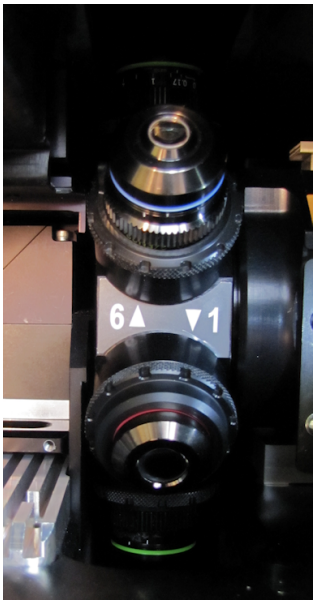
Changing an Objective

Note: Do not open the side access door during operation. Doing so may affect measurements.

Note: Before installing a 20X, 40X, or 60X objective, either phase or standard, set its correction collar to match your plate type. Adjust the Correction Collar on the facing page for more information and instructions.

After defining the objectives and their locations in Gen5 *(as described on page 28)*:

1. Open the side access door.



Objective turret

Note: Most objectives use an adapter to fit into the objective turret. Make sure to remove both the objective and its adapter.

2. To change an installed objective, grasp the objective you want to change, unscrew it from the objective turret, and remove it from the instrument.
3. Screw each new objective into its defined position. Do not overtighten.

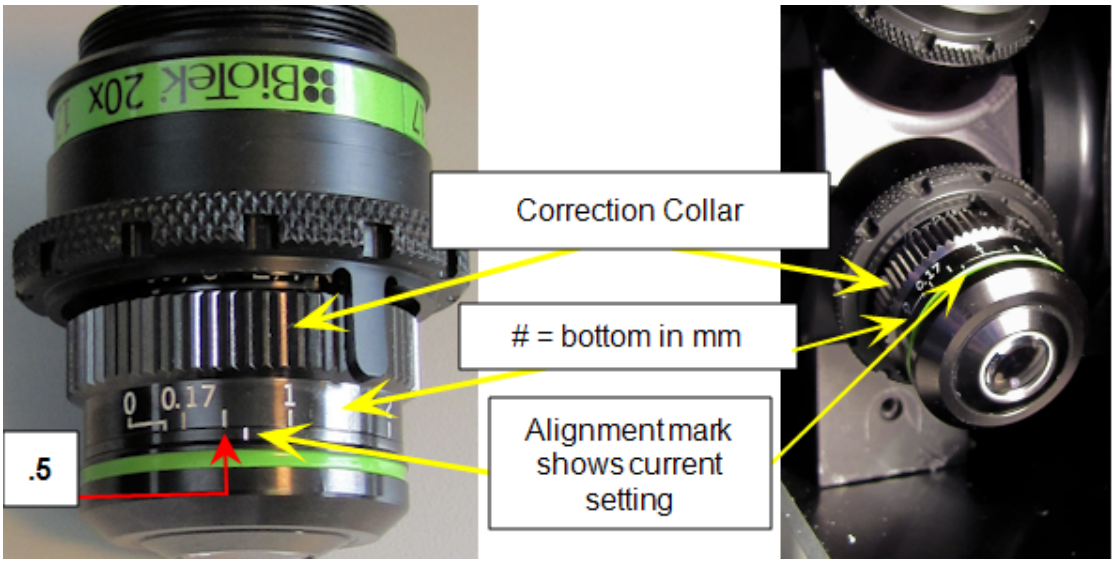
Note: Screw securely: Especially with high-power objectives, make sure the objective's adapter threads properly into its place in the turret. Pay close attention when installing them. If you feel a stop or misalignment, remove the objective and try again. Verify proper installation by making sure there is no gap between the adapter and the turret.

4. Close the door.
5. Run **Auto Calibration** *(as described on page 32)*.

After the calibration procedure is finished, the instrument is ready to use.

Adjust the Correction Collar

Adjust the correction collar on high-power objectives, 20x, 40x, and 60x, to match the bottom thickness of the sample vessel. If the collar is improperly adjusted images will be blurry and autofocus may not perform correctly.



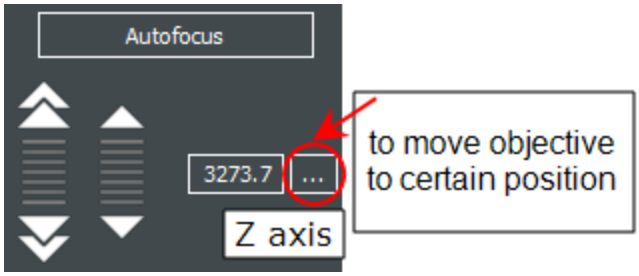
20x Objectives: uninstalled and installed

1. First, set the correction collar to the appropriate value from this table:

Plate Type	Typical Bottom Thickness
Glass-bottom microplate	0.17 mm
Cover slip	0.17 mm
Plastic microplate	0.5 mm
Low-density microplate	up to 1.0 mm

Check your plate specs for the actual bottom thickness.

2. Reinstall the objective in the instrument.
3. In Gen5, click  and try to focus on a sample: Run **Find Image**.
4. If images are still blurry, use the Focus controls to manually focus on the well. Record the current position when you are satisfied with the image. Auto Expose may help find focus.
5. Run **FM Scan** (from the Focus panel) and record Current position at the peak value.



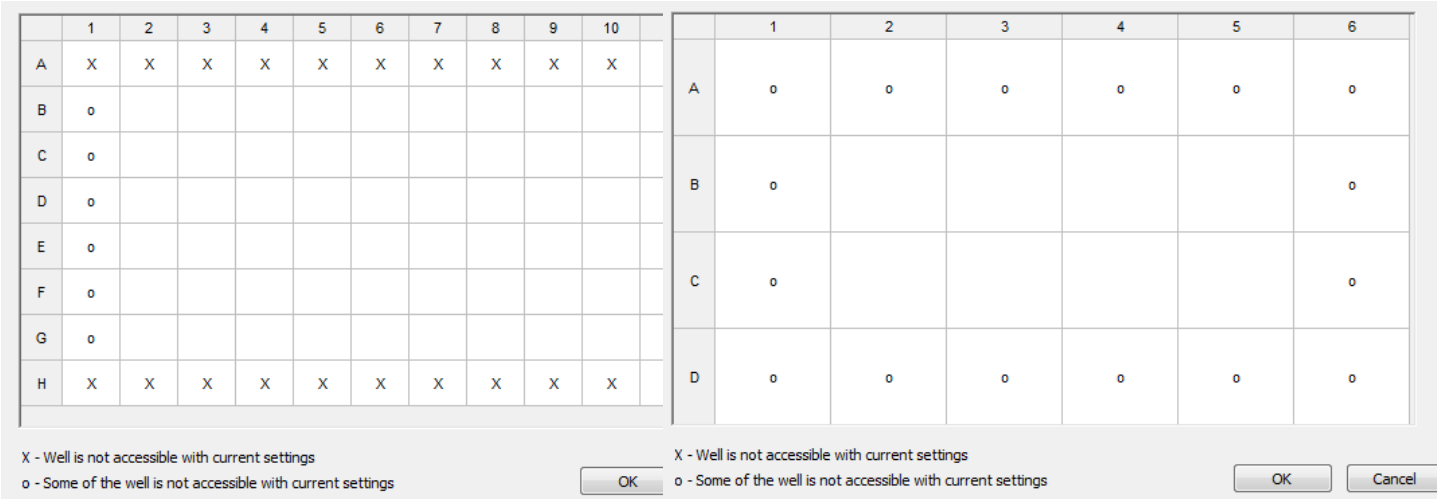
If the FM scan peak value doesn't match the manual focus value (by more than a few microns), the correction collar is not properly set. It may only need a minor adjustment and the optimal setting can be anywhere between the numbers and notches on the collar:

- Turn the collar to a lower number if the FM scan peak is higher than the manual focus value.
- Turn the collar to a higher number if the FM peak is lower than manual focus value.

Redo this step for new or different vessels, as needed.

In manual mode, select a readable well before selecting the high-power objective. And, use the focus controls, rather than Auto Focus, for the best results.

40X, 60X, and 100X Objectives: Limitation



96- and 24-well plate maps when using a high-power objective

Depending on the vessel, high-power objectives may not be able to capture images of all wells or sections of the vessel. Keep this in mind when you are plating your samples. This limitation is visible when selecting the wells to read in a read step (click the Full Plate button). On the contrary, the limitation is not obvious when defining the Plate Layout.

Gen5 identifies this limitation in the plate map:

- X to show when no part of the well can be imaged;
- O to show when only a portion of the well can be imaged.

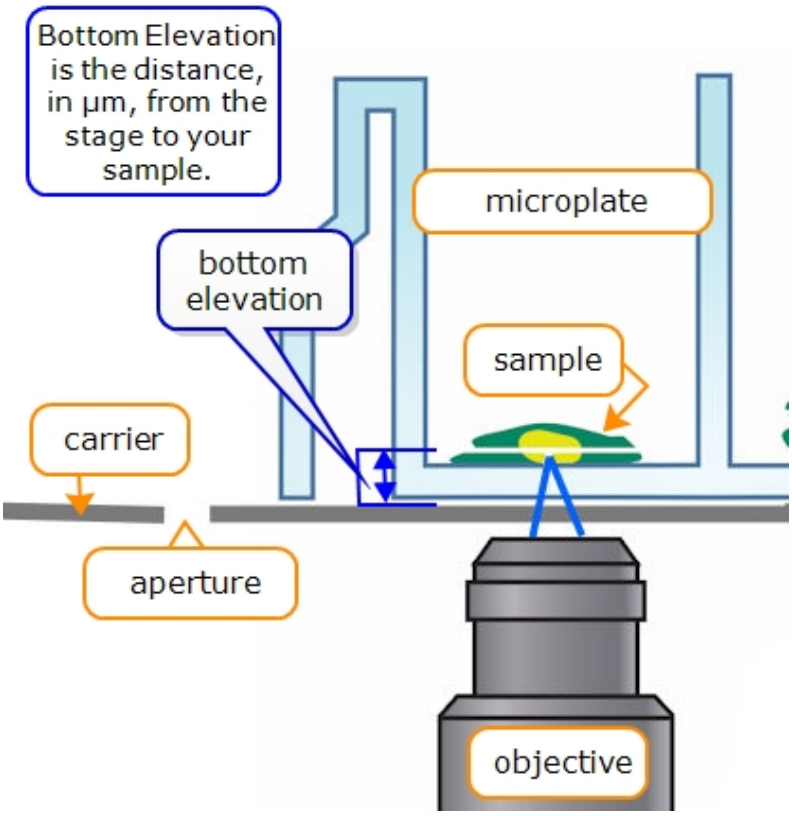
To view the plate map in manual mode, click **Well**.

To view the plate map in experiment mode:

1. Create a Procedure and select the Plate Type.
2. Click **Read**, and set **Image** as the detection method.
3. Set the Objective to 40x, 60x, 100x (phase or standard) and click **Full Plate**.

Define your vessel's Bottom Elevation

Make sure your microplates, slides, and other sample vessels are accurately described in Gen5. Bottom elevation is the distance in microns (µm) from the top of the plate carrier to the surface with samples. For imaging, bottom elevation is the **starting point for auto focus** - an essential parameter.



The carrier surface and calibration aperture are at focus position "0": Bottom Elevation is measured from 0 to the sample surface.

You need: The vessel with fixed, properly stained samples.

When creating a new plate type record, save time by copying a plate type with similar dimensions.

To define Bottom Elevation for your imaging vessel, perform these steps:

1. In Gen5, select **System>Plate Type**.
2. Highlight your plate type in the list and select **View/Modify**. (Or, create a new plate record.)
3. Open the **Imaging Parameters** window and click  - the manual-mode button.
4. Select your magnification and LED-Filter cube or brightfield light source.

   Use the strongest wavelength for your samples, (e.g., Blue (DAPI) or Green (GFP)).
5. Run **Auto Expose** and then **FM Scan**. When you get a defined peak: Click **Set Focus Height to Max FM Ratio**.
6. Click **Save Settings**. Gen5 will update the **Bottom Elevation** value.

Chapter 5

Periodic Maintenance

This chapter provides instructions for maintaining the Cytation 5 and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

Periodic Maintenance Overview

A general maintenance regimen for all Cytation 5 models includes periodically cleaning all exposed surfaces and inspecting/cleaning the objectives, emission and excitation filters, and mirrors (if used).

For models with the external dispense module, additional tasks include flushing/purging the fluid path and cleaning the tip prime trough, priming plate, supply bottles, dispense tubing, and injectors.

Daily Cleaning for the Dispense Module

To ensure accurate performance and a long life for the dispense module and injectors, flush and purge the fluid lines with deionized (DI) water every day or after completing an assay run, whichever is more frequent. Some reagents may crystallize or harden after use and clog the fluid passageways. Take special care when using molecules that are active at very low concentrations (e.g., enzymes, inhibitors). Remove any residual reagent in the dispense lines using a suitable cleaning solution (review the reagent's package insert for specific recommendations).

Flushing the tubing at the end of each day, letting the DI water soak overnight, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. BioTek recommends performing a visual inspection of the dispense accuracy before running an assay protocol that includes dispense steps.

BioTek also recommends flushing the module with DI water before conducting the decontamination procedure.

Note: Accumulated algae, fungi, or mold may require decontamination. See the *As-Needed Maintenance* chapter for complete decontamination instructions.

Recommended Maintenance Schedule

This table recommends periodic maintenance tasks and the frequency with which each task should be performed.

Important: The risk and performance factors associated with your assays may require that some or all of the maintenance procedures be performed more frequently than shown here.

Task	Daily	Quarterly	As Needed
All models:			
Decontamination	before shipment or storage		
PM-1: Clean exposed surfaces			√
Models with filter-based fluorescence capability:			
PM-2: Inspect/clean excitation and emission filters		√	
PM-3: Inspect/clean mirrors			annually
Models with injectors:			
PM-4: Flush/purge the fluid path	√		
PM-5: (Optional) Run Dispense protocol			√
PM-6: Empty/clean tip prime trough	√		
PM-7: Clean priming plate			√
PM-8: Clean dispense tubes and injectors		√	√
Models with imaging:			
PM-9: Clean objectives		√	√

Task	Daily	Quarterly	As Needed
Models with Peltier Cooling Module:			
PM-10: Clean filter			annually

Note: Find Decontamination instructions in the *As-Needed Maintenance* chapter.

Warnings and Precautions

WARNING

Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

WARNING

Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, stop the program and turn off the instrument. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

CAUTION

Liquids. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

CAUTION

Lubricants. Do not apply lubricants to moving parts. Lubricant on components in the carrier compartment will attract dust and other particles, which may cause the instrument to produce an error.

WARNING

Potential Biohazards. Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears.

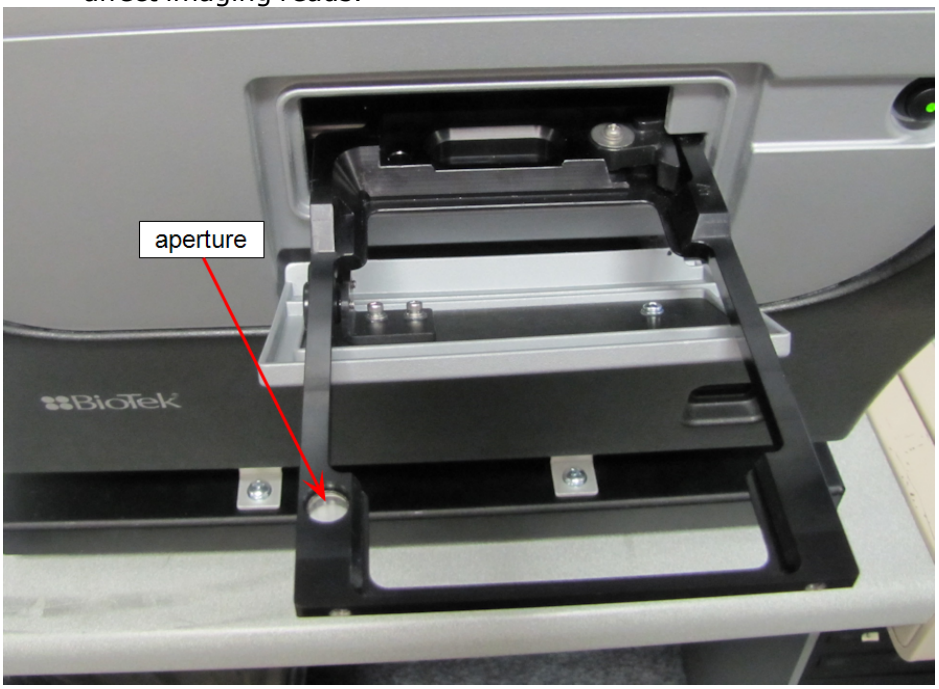
Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.

PM-1: Clean Exposed Surfaces

Exposed surfaces may be cleaned (not decontaminated) with a cloth moistened (not soaked) with water or water and a mild detergent. You'll need:

- Deionized or distilled water
- Clean, lint-free cotton cloths
- Mild detergent (optional)
- Canned air

1. Turn off and unplug the instrument.
2. Moisten a clean cotton cloth with water, or with water and mild detergent. **Do not soak the cloth.**
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. Instruments with imaging capability: Use canned air to blow debris from the aperture on the carrier. Do not wipe with liquid, which can seep inside aperture's glass plates and affect imaging reads.



5. Wipe all exposed surfaces of the dispense module (if used).
6. Wipe all exposed surfaces of the gas controller module (if used).
7. If detergent was used, wipe all surfaces with a cloth moistened with water.
8. Use a clean, dry cloth to dry all wet surfaces.

Important: *Models with a dispenser:* If the tip priming trough or priming plate overflow and spill fluid inside the instrument, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. The internal chamber and probes are not customer-accessible. If overflow is significant, contact Technical Support.

PM-2: Inspect/Clean Excitation and Emission Filters

Applies only to models with filter-based fluorescence capabilities (F models).

Laboratory air is used to cool the flash bulb, and the filter cubes can become dusty as a result. Filters should be inspected and cleaned at least every three months. You'll need:

- Isopropyl, ethyl, or methyl alcohol
- 100% pure cotton balls or high-quality lens-cleaning tissue
- Cloth gloves
- Magnifying glass

CAUTION **LED cubes and filter cubes.** Wear gloves when changing components to avoid contaminating them.

1. Open the access door on the front of the instrument. Slide the filter cube out of its compartment.
2. Inspect the glass filters for speckled surfaces or a "halo" effect. This may indicate deterioration due to moisture exposure over a long period of time.

Note: If you have any concerns about the quality of the filters, contact your BioTek representative.

3. Using cotton balls or lens-cleaning tissue moistened with a small amount of high-quality alcohol, clean each filter by lightly stroking its surface in one direction.
4. Use a magnifying glass to inspect the surface; remove any loose threads left from the cotton ball.
5. Replace the filter cube and close the door.

PM-3: Inspect/Clean Mirrors

Applies only to models with filter-based fluorescence capabilities.

We recommend inspecting (and cleaning, when necessary) the mirrors and polarizing filters (if equipped) annually, especially if the filter cube has been opened or changed.

These optical components are delicate and must be handled with care. The glass and anti-reflective (AR) coated surfaces will be damaged by any contact, especially by abrasive particles. ***In most cases, it is best to leave minor debris on the surface.*** However, if

performance indicators or obvious defects in the mirrors or filters suggest cleaning them, here are some guidelines:

- Use of oil-free dry air or nitrogen under moderate pressure is the best method for removing excessive debris from an optical surface. If the contamination is not dislodged by the flow of gas, please follow the cleaning instructions below.
- The purpose of the cleaning solvent is only to dissolve any adhesive contamination that is holding debris on the surface. The towel needs to absorb both the excessive solvent and entrap the debris so that it can be removed from the surface. Surface coatings on dichroics are typically less hard than the substrate. It is reasonable to expect that any cleaning will degrade the surface at an atomic level. Consideration should be given as to whether the contamination in question is more significant to the application than the damage that may result from cleaning the surface. In many cases, the AR coatings that are provided to give maximum light transmission amplify the appearance of contamination on the surface.

Materials

- 7/64" hex key
- Linen or cloth gloves
- Anhydrous reagent-grade ethanol
- Kimwipes® or comparable lint-free wipes
- Magnifying glass
- 100% pure cotton balls (for the polarizing filters)

Procedure

1. Follow the instructions for Changing the Filters and Mirrors in a Filter Cube on page 76 .
2. Wet absorbent towels such as Kimwipes, ***not*** lens paper, with anhydrous reagent-grade ethanol. Wear gloves or use enough toweling so that solvents do not dissolve oils from your hands that can seep through the toweling onto the coated surface.
3. Drag the trailing edge of the ethanol-soaked Kimwipe across the surface of the mirror, moving in a single direction. A minimal amount of pressure can be applied while wiping. However, too much pressure will damage the mirror.
4. Use the magnifying glass to inspect the surface; if debris is still visible, repeat with a new Kimwipe.
5. To replace the mirror, hold it by its edges, turn it so that its label is face-up and readable, and place it on the shelf in the filter cube.
6. Place the filter cube top back onto the cube and replace the screw and washer.
7. When finished, reinstall the filter cube in the reader.

PM-4: Flush/Purge the Fluid Path

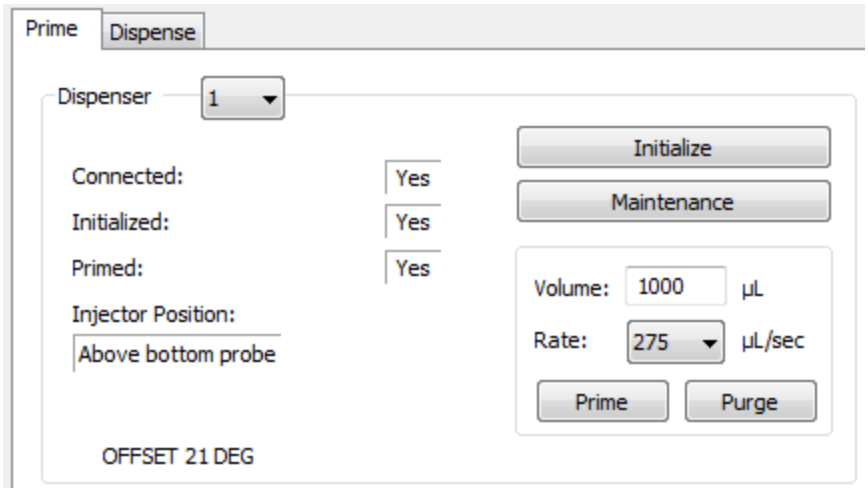
Applies only to instruments used with a dispenser.

At the end of each day when using the dispense module, flush the fluid path using the Gen5 priming utility. Leave the fluid to soak overnight or over a weekend, and then purge the fluid before using the instrument again.

Note: This flushing and purging routine is also recommended before disconnecting the outlet tubes, and before decontamination to remove any assay residue prior to applying isopropyl alcohol or sodium hypochlorite.

To flush the fluid path:

1. Fill two supply bottles with deionized or distilled water. Insert the supply (inlet) tubes into the bottles.
2. Place the priming plate on the carrier.
3. In Gen5, use the **Instrument Control** tab (or select it from the **System** menu).



4. Select 

.
5. Click the **Prime** tab and select **Dispenser 1**.
6. Set the Volume to **5000 µL**. Keep the default prime rate.
7. Click **Prime** to start the process.
8. Repeat the process for **Dispenser 2**. When the process is complete, carefully remove the priming plate from the carrier and empty it.

Leave the water in the system overnight or until the instrument will be used again. Purge the fluid from the system and then prime with the dispense reagent before running an assay.

To purge the fluid from the system:

1. Place the inlet tubes in empty supply bottles or a beaker.
2. Select **Instrument Control**.
3. Click the **Prime** tab and select **Dispenser 1**.
4. Set the Volume to **2000 µL**.
5. Click **Purge** to start the process.
6. When the purge is complete, repeat the process for **Dispenser 2**.

Note: Before reusing the system, run a quick Dispense protocol to visually verify the dispense accuracy or perform the more thorough Dispense Accuracy and Precision Tests (in *Chapter 7, Instrument Qualification*).

PM-5: Run a Dispense Protocol (Optional)

Applies only to instruments with a dispenser.

After flushing/purging the system and before running an assay that requires a dispense step, visually inspect the dispensing accuracy.

1. Create a Dispense protocol in Gen5:
    a. Create a new protocol with the plate type set to match the plate you will use.
    b. Add a Dispense step with the following parameters:
        - Select Dispenser **1**.
        - Set Tip Priming to **Before this dispense step** and Volume to **10 µL**.
        - Set the Dispense Volume to **100 µL** (or an amount to match your assay protocol).
        - Adjust the Rate to support the dispensing volume.
        - Click **OK** to close the dialog and add the Dispense step to the procedure.
    c. Add another Dispense step with the same parameters for Dispenser **2**.
    d. Add a Read step with the following parameters (Gen5 requires a Read step in a Dispense protocol):
        - Select any Detection Method.
        - Set the Read Type to **Endpoint.**
        - Click **Full Plate**, click **Clear All**, then select well **A1**. Click **OK**.
        - Select any wavelength or define one Filter Set.
        - Click **OK** to close the dialog and add the Read step to the procedure.
    e. Click **OK** to close the procedure.
    f. Select **File > Save** and give the protocol an identifying name, such as "Dispense Observation."
2. Fill the reagent bottles with a DI water–Tween solution (e.g., add 1 mL Tween 20 to 1000 mL of deionized water).
3. Create a new experiment using the "Dispense Observation" protocol.
4. Click **Read** and follow the prompts.
5. When the procedure is complete, visually assess the fluid level in the wells for accuracy. If the well volume appears to be unevenly distributed, clean the internal dispense tubes and injectors.

PM-6: Clean the Tip Priming Trough

Applies only to instruments with a dispenser.

The tip priming trough is a removable cup located in a rear pocket of the microplate carrier, used for performing the Tip Prime. The trough holds about 1.5 mL of liquid and must be periodically emptied and cleaned. Gen5 will instruct you to do this at the start of a run that requires tip priming.

1. Extend the microplate carrier and carefully remove the tip priming trough from the carrier.
2. Wash the trough in hot, soapy water. Use a small brush to clean in the corners.
3. Rinse the trough thoroughly and allow it to dry completely.
4. Replace the trough in the microplate carrier.

PM-7: Clean the Priming Plate

Applies only to instruments with a dispenser.

Clean the priming plate regularly to prevent bacteria growth and residue buildup. Wash the plate in hot, soapy water, using a small brush to clean in the corners. Rinse thoroughly and allow it to dry completely.

PM-8: Clean the Dispense Tubes and Injectors

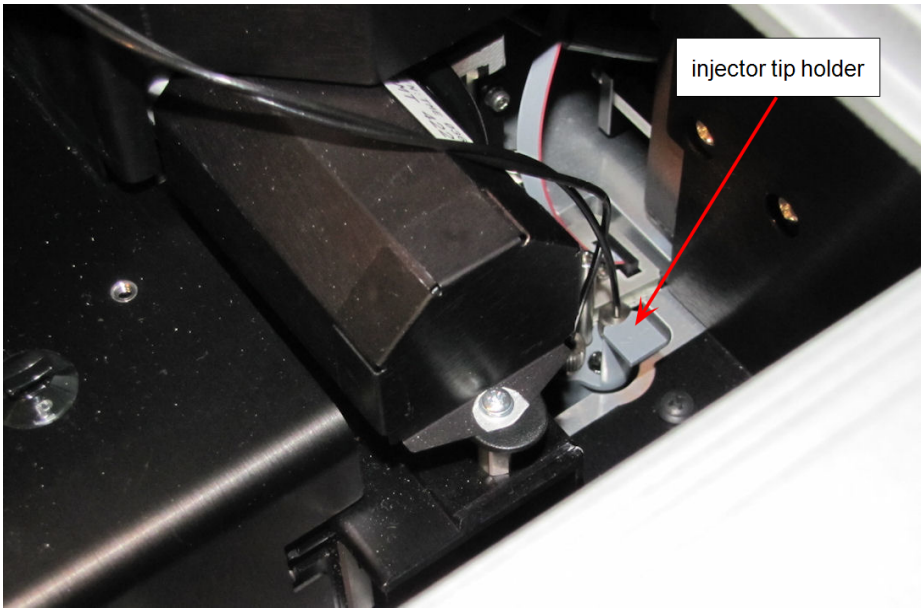
Applies only to instruments with a dispenser.

The Cytation 5's dispense tubes and injectors require routine cleaning, at least quarterly and possibly more frequently depending on the type of fluids dispensed.

Required Materials

- Protective gloves
- Safety glasses
- Mild detergent
- Clean, lint-free cotton cloths
- Deionized or distilled water
- Stylus (stored in a plastic cylinder affixed to the rear of the dispense module or reader) (PN 2872304)

Remove the Dispense Tubes and Injector Holders



1. Open the door on the top of the reader.
2. Grasp the injector tip holder by the tab and pull it up out of its socket.
3. Using your fingers, remove the thumbscrews securing the light shield to the top of the reader and slide the shield up the outlets tubes.
4. Slide the injector tip holder through the hole in the top of the reader.
5. Turn each tube's thumbscrew counterclockwise and gently pull each tube from its injector tip.
6. On the dispense module, turn each outlet tube's thumbscrew counterclockwise to disconnect it from the injector.

Clean the Dispense Tubes and Injectors

Some reagents can crystallize and clog the tubing and injectors. Daily flushing and purging can help to prevent this, but more rigorous cleaning may be necessary if reagent has dried in the tubing or injectors.

To clean the dispense tubes, soak them in hot, soapy water to soften and dissolve any hardened particles. Flush each tube by holding it vertically under a stream of water.

To clean the injectors:

1. Gently insert the stylus into each injector tip to clear any blockages. (The stylus is stored in a plastic cylinder affixed to the rear of the dispense module.)
2. Stream water through the pipe to be sure it is clean. If the water does not stream out, try soaking in hot, soapy water and then reinserting the stylus.

Note: Be careful not to damage the injector tips. A damaged tip might not dispense accurately.

PM-9: Clean the Objectives

Applies only to "V" and "W" models with the imaging module.

Pinch Hazard. Some areas of the instrument and the dispense module can present pinch hazards when the instrument is operating. The objective turret and dispense module are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.

CAUTION

Objectives. Do not use these cleaning solvents: Methyl ethyl ketone (MEK), Dimethyl ketone (acetone)

Clean the Cytation 5's objectives when necessary using optical-grade swabs or lens paper moistened with lens cleaning solution or deionized water. *Do not rub the lens.*

Materials

- Air puffer
- Tweezers
- Magnifying glass
- Lens cleaning tissue
- Optical-grade swabs
- Cleaning solvent

Recommended Cleaning Solvents
Electro-Wash PX from Chemtronics®
Isopropyl alcohol, 70%/30% with deionized water
Methyl alcohol, 70%/30% with deionized water
Ethyl alcohol, 70%/30% with deionized water

1. From the Gen5 main screen, go to **System > Instrument Configuration**, select **Cytation 5**, click **View/Modify > Setup**.
2. In the Objective Configuration area of the Imaging Configuration tab, click **Access** next to the desired objective to rotate the objective turret to the access position.
3. Open the side door of the instrument. Grasp the objective, unscrew it, and remove it from the instrument.
4. Inspect the lens, using a magnifying glass if necessary, to determine if there is dirt or dust present. If so, use a blower or a small paintbrush to remove any dirt and dust.

Note: Any dirt or dust on the surface of the lens can cause extensive damage if dragged across the surface.

Best practices:

- Do not allow the lens to air dry.
- Always use an unused portion of the lens tissue when wiping the lens.
- If smears are still present after performing these steps, repeat the procedure.

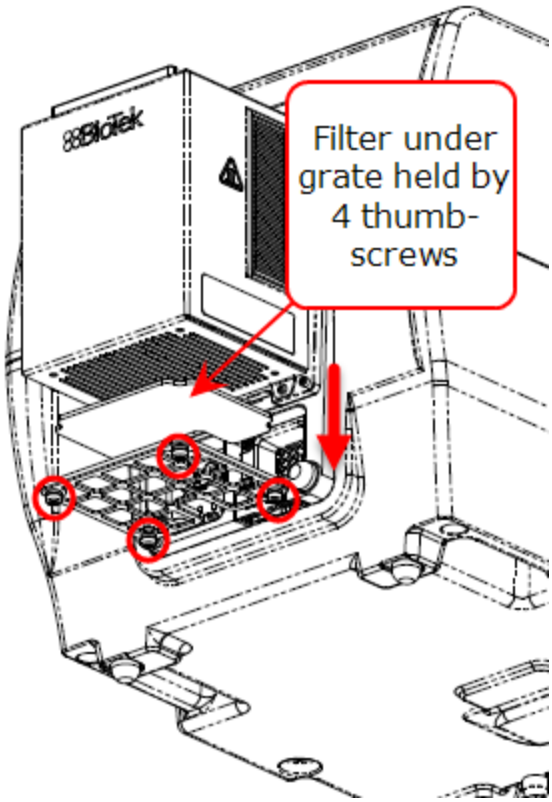
5. Soak either an optical-grade swab or a piece of lens-cleaning tissue wrapped around tweezers in lens-cleaning solvent or deionized water.
6. Hold the swab or tissue-wrapped tweezers still and rotate the objective's lens around it.
7. Dry the lens immediately with a clean lens tissue.
8. Replace the objective in the objective turret, and screw it in to secure it.
9. Repeat these steps to clean the other objectives, if necessary.
10. Return to the Imaging Configuration tab (opened in step 1), click **Auto Calibration** to calibrate the objectives.

When the calibration is finished, the instrument is ready to use.

PM-10: Clean Peltier Cooling Module Filter

Applies only when the Peltier Cooling Module is installed

BioTek recommends removing and cleaning the filter annually or as needed.



Once a year or more frequently if instrument usage or the lab environment are known to require more rigorous maintenance than typical, remove the filter on the bottom of the cooling module and clean it.

1. Release the 4 thumbscrews that hold the grate and filter in place on the underside of the Peltier Cooling Module.
2. Wash the grate and filter in mild soap and water. Let them air dry before reinstalling.

Chapter 6

As-Needed Maintenance

This chapter contains maintenance and component-replacement procedures that need to be performed occasionally.

Decontamination

- The instrument requires decontamination prior to shipping, storage, and disposal.
- Decontamination is required by the U.S. Department of Transportation regulations.
- Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.
- BioTek Instruments, Inc., recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither BioTek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the biohazard(s) they handle.

Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

Liquids. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

Potential Biohazards. Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears.

Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.

Required Materials

For all models:

- Sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as an alternative to bleach)
- Deionized or distilled water
- Safety glasses
- Surgical mask
- Protective gloves

- Lab coat
- Biohazard trash bags
- 125-mL beakers
- Clean, lint-free cotton cloths

Additional materials for models with the dispense module:

- Screwdriver
- Small brush for cleaning the tip priming trough and priming plate
- (Optional) Mild detergent

Procedure for all instruments: Decon External Surfaces

Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

Liquids. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

Important: The sodium hypochlorite (bleach) solution is caustic; wear gloves and eye protection when handling the solution.

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

Note: Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth.**
4. Open the plate carrier door and slide out the plate carrier.
5. Wipe the carrier/stage and all exposed surfaces of the instrument.
6. Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. Reassemble the instrument as necessary.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Procedure for Models with a Dispenser

Note: Perform the Routine Procedure when the Cytation 5 is functioning normally. If you are unable to perform a prime due to a system failure, perform the Alternative Decon Procedure for Dispenser Modules on page 112.

Routine Procedure

Important: If disinfecting with sodium hypochlorite (bleach), be sure to flush repeatedly with deionized water to remove the bleach.

If disinfecting with alcohol, do not immediately prime with deionized water, because the drying effect of the alcohol is an important aspect of its disinfectant properties.

Purge any fluid (PM-4: Flush/Purge the Fluid Path on page 99) and detach the outlet tubes from the instrument. If it is not installed, attach only the dispenser's communication cable to the instrument. Remove the supply bottles and their holders.

Clean Exposed Surfaces

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). Alternatively use 70% isopropyl alcohol.

   Note: Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Open the plate carrier door and slide out the plate carrier.
4. Moisten a cloth with the bleach solution or alcohol. Do not soak the cloth.
5. Wipe the plate carrier and all exposed surfaces of the instrument.
6. Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. If the dispenser is installed, purge any fluid (PM-4: Flush/Purge the Fluid Path on page 99) and detach the outlet tubes from the instrument. If it is not installed, attach only the dispenser's communication cable to the instrument. Remove the supply bottles and their holders.

Decontaminate the Fluid Lines

1. Place a beaker with 20 mL of 0.5% sodium hypochlorite solution or 70% isopropyl alcohol near SYRINGE 1 on the dispenser and put the SYRINGE 1 inlet tube in the beaker.

2. If you have not already done so, detach the dispenser's outlet tubes from the instrument. Place the ends of the outlet tubes in an empty beaker and set the beaker next to the dispenser.
3. In Gen5, use the **Instrument Control** tab (or select it from the **System** menu), and select 

.
4. Select **Dispenser 1**, enter a Volume of **5000 µL**, and keep the default dispense Rate.
5. Place the priming plate on the carrier.
6. Run two prime cycles, for a total of 10,000 µL.
7. Wait at least 20 minutes to allow the solution to disinfect the tubing.
8. Remove the inlet tube from the beaker of disinfectant solution.
9. Change the **Volume** to **1000 µL**.
10. Run one prime cycle, to flush the disinfectant out of the fluid lines.
11. Empty the beaker containing the outlet tubes. Put the tubes back in the empty beaker.
12. If sodium hypochlorite (bleach) was used, perform the **Rinse the Fluid Lines** steps below.

Otherwise (or after performing the Rinse procedure), repeat the process for SYRINGE 2/Dispenser 2.

Rinse the Fluid Lines

Note: Perform this procedure only if decontamination was performed using sodium hypochlorite.

1. Place a beaker containing at least 30 mL of deionized water on the dispenser.
2. Place the SYRINGE inlet tube in the beaker.
3. If you have not already done so, place the outlet tubes in an empty beaker.
4. In Gen5, select the Dispenser and set the Volume to **5000 µL**, and keep the default dispense Rate.
5. Run five prime cycles, for a total of 25,000 µL.
6. Pause for 10 minutes and then run one prime cycle with 5000 µL. This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
7. Empty the beaker containing the outlet tubes.
8. Wipe all surfaces with deionized water.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Clean the Tubing and Injectors

Perform PM-8: Clean the Dispense Tubes and Injectors on page 102.

Decontaminate the Tip Priming Trough and Priming Plate

1. Remove the tip priming trough and priming plate.
2. Wash the tip priming trough and priming plate in hot, soapy water. Use a small brush or cloth to clean the corners of the trough and plate.
3. To decontaminate, soak the trough and plate in a container of 0.5% sodium hypochlorite or 70% isopropyl alcohol for at least 20 minutes.
   - If decontaminating in a bleach solution, thoroughly rinse with deionized water.
   - If decontaminating with alcohol, let the trough and plate air dry.
4. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Alternative Decon Procedure for Dispenser Modules

If you are unable to prime the tubing due to a system failure, decontaminate the instrument and the dispenser as follows:

1. Begin by cleaning the dispenser as described: PM-8: Clean the Dispense Tubes and Injectors on page 102.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). Alternatively, use 70% isopropyl alcohol.

   Note: Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Slide the microplate carrier out of the instrument.
4. Moisten a cloth with the bleach solution or alcohol. Do not soak the cloth.
5. Use the cloth to wipe:
   - All exterior surfaces of the instrument
   - All surfaces of the plate carrier
   - The exposed surfaces of the dispenser, including the syringe valves
6. Remove the tubing and the syringes from the dispenser and soak them in the bleach or alcohol solution. Wait for 20 minutes.
7. Moisten a cloth with DI or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
8. Rinse all tubing and the syringes with DI water.
9. Use a clean, dry cloth to dry all surfaces on the instrument and the dispenser.
10. Reassemble the dispenser as necessary.

11. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Dispenser Syringe Replacement

Refer to the ***Periodic Maintenance*** chapter for cleaning procedures you must perform regularly and especially in the case of poor performance (for example, when Dispense Accuracy and Precision tests fail). If cleaning the dispenser does not eliminate performance problems, or if a syringe is obviously leaking, perform these instructions to replace a faulty syringe. Contact Technical Support to order replacement syringes.

To change a syringe, first use Gen5 to put the syringe in its maintenance position.

Syringe Maintenance Position

Note: Do not change the syringe position or calibrate the dispensers unless instructed to do so as part of installation, upgrade, or maintenance.

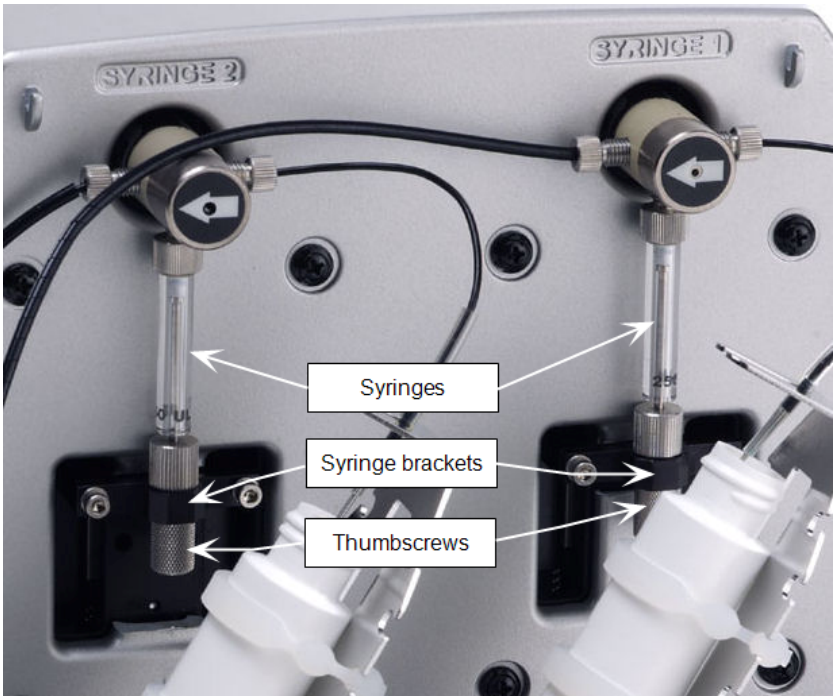
When a syringe needs to be installed or replaced, it must first be moved to its "maintenance position."

1. From the Gen5 main screen, select **System > Instrument Control > Cytation 5** and click the **Prime** tab.
2. Select the Dispenser number (**1** or **2**) associated with the syringe.
3. Click **Maintenance**. The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve.

Replace the Syringe

After using Gen5 to move the syringe into its maintenance position:

1. Using your fingers, unscrew the bottom thumbscrew that secures the syringe, underneath the bracket. Retain this bottom thumbscrew; it is needed for the replacement syringe.
2. Unscrew the top thumbscrew to disengage the syringe from the valve.



3. Remove the new syringe from its protective box. (The syringe should already be assembled in one piece. Install the Dispenser (Optional) on page 21, if it is not.).
4. Hold the syringe vertically with the threaded end at the top. Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
5. Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
6. Pass the thumbscrew (used to hold the old syringe) up through this hole and thread it into the bottom of the syringe. Hold the syringe from rotating while tightening the thumbscrew. Finger-tighten only.
7. From the Gen5 main screen, select **System > Instrument Control > Cytation 5**. Click the **Prime** tab and click **Initialize**.

Chapter 7

Instrument Qualification

This chapter contains procedures for qualifying the initial and ongoing performance of the Cytation 5 and the dispense module (if equipped).

Instrument Qualification Overview

BioTek Instruments recommends performing the following Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) procedures for the Cytation 5.

Every Cytation 5 and dispenser is fully tested at BioTek prior to shipment and should operate properly upon initial setup. If you suspect that a problem occurred during shipment, if you have received the equipment after returning it to the factory for service, and/or if regulatory requirements dictate that you qualify the equipment on a routine basis, perform the procedures outlined in this chapter.

Note: A Product Qualification Package (PN 1320532N) for the Cytation 5 is available for purchase. The package contains complete procedures, Gen5 protocols, checklists, and logbooks for performing Installation Qualification, Operational Qualification, Performance Qualification, and Periodic Maintenance. Contact your local BioTek dealer for more information.

Installation Qualification confirms that the instrument and its components have been supplied as ordered and ensures that they are assembled and configured properly for your lab environment.

- The recommended IQ procedure consists of setting up the instrument and its components as described in *Chapter 2, Installation*, and performing the System Test. For models with injectors, a quick Injector Test is also performed, to ensure that the dispense module is properly installed and there are no leaks.
- The IQ procedure should be performed initially (before the reader is used for the first time).
- The successful completion of the IQ procedure verifies that the instrument is installed correctly. The Operational Qualification procedure should be performed immediately following the successful IQ.

Operational Qualification confirms that the equipment operates according to specification initially and over time.

- The recommended OQ procedure consists of performing the system test, Absorbance Plate Test, luminescence test, a series of fluorescence tests, imaging autofocus and carrier level tests , and, if the external dispense module is used, the Dispense Accuracy and Precision Tests.
- The OQ procedure should be performed initially (before first use) and then routinely; the recommended interval is annually. It should also be performed after any major repair or upgrade to the hardware or software.
- Although out-of-tolerance failures will be detected by the OQ tests, results should be compared with those from the routine Performance Qualification tests and previous OQ tests to monitor for trends.

- The successful completion of the OQ procedure, in combination with results that are comparable to previous PQ and OQ tests, confirms that the equipment is operating according to specification initially and over time.

Performance Qualification confirms that the instrument consistently meets the requirements of the tests performed at your laboratory.

- The recommended PQ procedure consists of performing the system test, Absorbance Plate Test, luminescence test, a series of fluorescence tests, imaging autofocus and carrier level tests, and, if the external dispense module is used, the Dispense Accuracy and Precision Tests.
- Your facility's operating policies may also require that you routinely perform an actual assay, to confirm that the reader will consistently give adequate results for the assays to be run on it.
- These tests should be performed routinely; the recommended interval is monthly or quarterly, depending on the test. This frequency may be adjusted depending on the trends observed over time.
- The successful completion of the PQ procedure confirms that the equipment is performing consistently under normal operating conditions.

Recommended Qualification Schedule

This table defines BioTek-recommended intervals for qualification for an instrument used two to five days a week. The schedule assumes that the Cytation 5 is properly maintained as outlined in the **Periodic Maintenance** section.

Important: The risk and performance factors associated with your assays may require that the Operational and Performance Qualification procedures be performed more or less frequently than shown here.

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
All models:				
Installation, setup, and configuration of the instrument, dispense module (if equipped), gas controller (if equipped), controller/host computer, and Gen5 software.	✓			
System Test	✓	✓	✓	
Plate Shaker Test		✓		
Absorbance capability*:				
Software Wavelengths Table Verification	✓	✓	✓	
Absorbance Plate Test (400-800 nm)		✓	✓	
Absorbance Liquid Test 1 or Liquid Test 2[1]		✓		✓
(Optional) Absorbance Liquid Test 3 or 340 nm Absorbance Plate Test		✓		✓
Fluorescence capability*:				

[1]If you have the Absorbance Test Plate (400-800 nm), PN 7260522, run Liquid Test 1; otherwise, run Liquid Test 2.

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
Corners, Sensitivity, Linearity Tests		✓	✓	
Fluorescence Polarization Test		✓		✓
Time-Resolved Fluorescence Test		✓		✓
Luminescence capability*:				
Luminescence Test		✓	✓	
Dispenser system*:				
Injection System Test	✓			
Dispense Accuracy and Precision Test		✓		✓
Imaging capability*:				
Carrier Level Test		✓		✓
Capture an Image		✓		
Joystick Verification (if used)		✓		
Alpha Laser capability*				
Alpha Detection Test		✓		

* If applicable to your reader model

System Test

Each time the Cytation 5 is turned on, it performs a series of tests on the reader's motors, lamp(s), the PMT(s), and various subsystems. The system test may take a few minutes to complete. If all tests pass, the microplate carrier is presented and the green LED on the carrier switch remains on.

If any test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek, the instrument beeps repeatedly and the red LED on the carrier button flashes. If this occurs, press the carrier button to stop the beeping. If necessary, initiate another system test using Gen5 to try to retrieve an error code from the instrument. Refer to *Appendix B, Error Codes* for troubleshooting tips.

Note: If the power-up system test fails, when you initiate a system test using Gen5, Gen5 displays a message stating that the instrument has a pending system test report. Click **OK** in the message box to review the report; it contains information obtained up to the point of the failure.

Run the test using Gen5:

1. Turn on the instrument and launch Gen5.
2. If your assays use incubation, we recommend turning on the incubator and letting it reach its set point before running the system test. To access this feature, from the Gen5 main screen, select **Incubate** from the **Instrument Control** tab.
3. Select **System > Diagnostics > Run System Test**.

   Note: If the test fails during execution, a message appears. Close the message; the test report contains the error code that was generated by the failure.
4. When the test is complete, a dialog appears, requesting additional information. Enter your user name and other information (if desired) and then click **OK**.
5. The results report appears. It should show "SYSTEM TEST PASS".
6. Turn off the incubator.
7. Print the report, if desired.
   - Gen5 stores the results in a database, so the results can be retrieved at any time.
8. If the test failed, look up the error code to determine its cause. If it is something you can fix, turn off the instrument, fix the problem, and then power on the instrument and retry the test.

If the test continues to fail, or if the cause is not something you can fix, contact Technical Support.

Imaging Test Procedures

Two imaging tests are performed to ensure 1) the carrier is level, and 2) satisfactory images are captured.

Important: Do not perform these tests with the special High Contrast Bright Field (HCBF) aperture installed. Replace the HCBF aperture with the standard brightfield aperture before testing the instrument.

Required Materials

- Imaging qualification plate, PN 1222520
- 4X (recommended), or 2.5X or 10X objective (phase or standard)
- Microscope slide holder (adapter plate) (PN 1220548)

- H&E slide or comparable sample for testing
- Gen5 protocol described on page 122: Cytation 5 <*objective*>_CarrierLevel.prt

Carrier Level Test

This test ensures the carrier is level in relation to the imaging system. You can run the test with any of these objectives: Meiji 2.5X, Zeiss 2.5X, 4X, or 10X (phase or standard) (PL ACH or PL FL). We recommend using the 4X.

Procedure

1. If you have not already done so, create the **Carrier Level** Gen5 protocol as described in Gen5 Imaging Protocol on the next page.
2. Inspect/clean the imaging qualification plate (part number 1222520) prior to use. Inspect the four (4) corner holes. Clean any debris from the holes using clean dry compressed air.
3. Place the imaging qualification plate on the carrier with well A1 on the plate (facing up) adjacent to the etched A1 in the corner of the carrier.
4. Create a new experiment in Gen5 using the *CarrierLevel.prt* protocol for the objective you have chosen for the test.
5. Click **Plate > Read Plate**, save the experiment, then click **OK**.
6. When prompted by Gen5, rotate the plate 180 degrees in the carrier.
7. When the read is finished, analyze the results as described below.

Analyze the Results

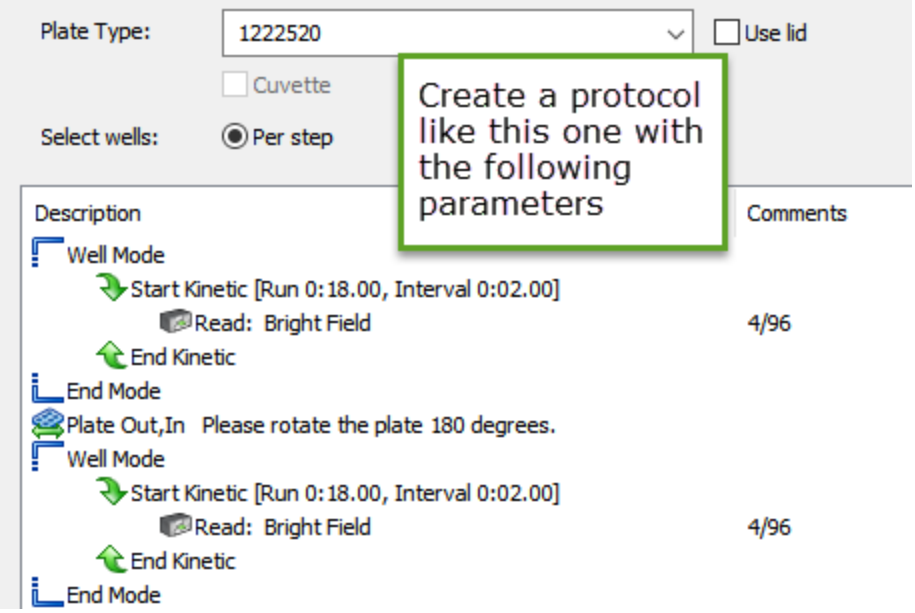
1. Calculate the Mean focus height for each set of ten reads in the "Normal" position in wells A1, A12, H1, and H12.
2. Calculate the Mean focus height for each set of ten reads in the "Turnaround" position in wells A1, A12, H1, and H12.
3. Compare the Mean values for each well in its Normal and Turnaround positions by performing these calculations:
    a. −1 * (A1 Normal Mean − H12 Turnaround Mean)
    b. (H12 Normal Mean − A1 Turnaround Mean)
    c. −1 * (A12 Normal Mean − H1 Turnaround Mean)
    d. (H1 Normal Mean − A12 Turnaround Mean)
4. For A1/H12:
    - Calculate the Mean Delta (µm): (step *a* results + step *b* result) / 2
    - Calculate the Carrier Tilt: Mean Delta / 25400 µm
    - The Carrier Tilt must be less than 0.005" to Pass.
5. For A12/H1:

- Calculate the Mean Delta (µm):(step *c* results + step *d* result) / 2
- Calculate the Carrier Tilt: Mean Delta / 25400 µm
- The Carrier Tilt must be less than 0.005" to Pass.

Gen5 Imaging Protocol

Note: The 4X objective is recommended for the Carrier Level Test. If a 4X objective is not available, use a 2.5X or 10X objective. Phase or standard objectives perform these tests equivalently.

Use the parameters in this table to create the Carrier Level Test protocol. Retain default values for any protocol parameters that are not specified in the tables below.



Carrier Level Test

Cytation 5 <*installed objective*>_CarrierLevel.prt

Parameter	Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520 (imaging qualification plate)

Parameter	Setting
Read Step 1	
Process Mode:	Well Mode
Kinetic Step:	Run Time: 18:00 Interval: 2 seconds Reads: 10
Objective:	2.5X or 4X or 10X
"W" models	De-select (disable) "Use Wide FOV (field of view)"
Read Wells:	A1, A12, H1, H12
Color Channel:	Bright Field
Exposure:	Illumination/LED: 5 Integration time: 50 Gain: 0
Auto Focus Options:	Focus method: Auto focus with optional scan Minimum focus metric ratio: 3 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 75 Offset from bottom of well: 0
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 1
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well:	Selected
Delay after plate movement:	300 msec

Parameter	Setting
After Read Step 1	
Plate Out, In	"Please rotate the plate 180 degrees"
Read Step 2	
Process Mode:	Well Mode
Kinetic Step:	Run Time: 18:00 Interval: 2 seconds Reads: 10
Objective:	2.5X or 4X or 10X
Read Wells:	A1, A12, H1, H12
Color Channel:	Bright Field
Exposure:	Illumination: 5 Integration time: 50 Gain: 0
Auto Focus Options:	Focus method: Auto focus with optional scan Minimum focus metric ratio: 3 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 75 Offset from bottom of well: 0 Search width: 350
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 1
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well:	Selected

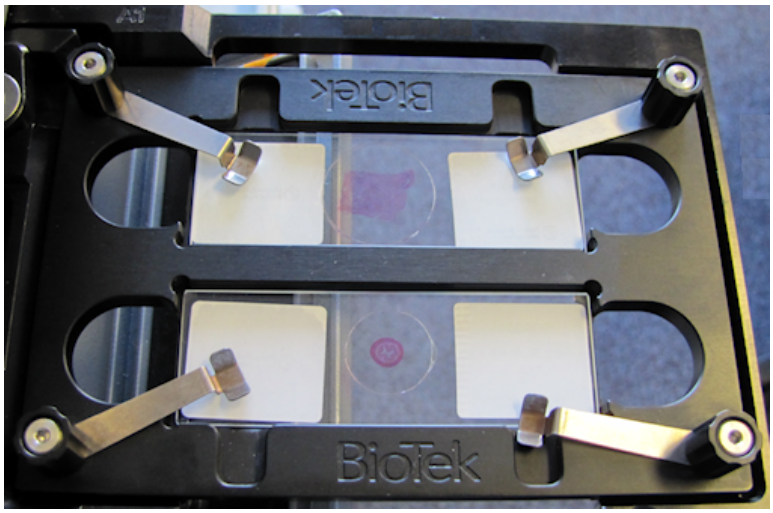
Parameter	Setting
Delay after plate movement:	300 msec

Capture an Image Test

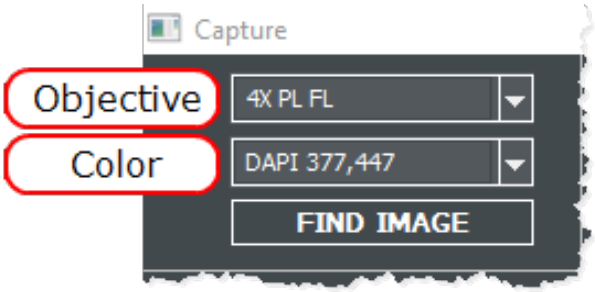
Perform this test to ensure images can be captured in each imaging modality.

Note: BioTek recommends using an H&E slide or comparable sample for this test.

1. Load the H&E slide or comparable sample in the slide holder with its cover slip facing down.

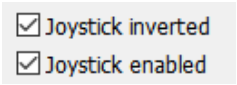


2.  In Gen5, launch manual mode by clicking the microscope button. Choose the **Slide Holder All Mag. (w/A1 mark)** as the Plate Type.
3. Select a "Phase" **Objective**, if available.
4. One at a time, cycle through the options for each **Color** channel capturing an image with available options:
   - Fluorescence: DAPI or GFP
   - Bright Field
   - Phase Contrast
   - Color Bright Field



Note: Apply **Auto Exposure** and **Auto Focus** iteratively as needed in each Color channel.

Joystick Performance Verification



Gen5 recognizes the joystick when it is installed. On the Stage Movement panel, when the joystick is connected, two checkboxes are offered, including **Joystick enabled**. As needed, click the checkbox to use the joystick rather than the computer controls. Choose **Joystick inverted** to reverse the direction settings.

Note: After connecting a joystick to the imager, you must restart the imager to activate the connection.

If the joystick is installed, follow these steps to verify its performance:

1.  In Gen5, launch manual mode. Choose **Slide Holder All Mag. (w/A1 mark)** as the Plate Type and put the slide holder on the stage.

Using the imager

2. Select a 4X or Phase 4X or your lowest objective.
3. Set Color to: DAPI 377, 447.
4. Expand the **Stage Movement** panel and make sure the Joystick enabled checkbox is filled.
5. Use the joystick to center the image vertically and horizontally.
6. Switch to 20X or Phase 20X (or your highest objective).
7. Click **Auto Expose**.
8. Use the joystick focus control to find the image focus.
9. Test all directional positions and focus up/down.

If all works as expected, the test passes. If not, contact Technical Support.

Absorbance Plate Test

Applies only to models with absorbance capabilities.

This test uses BioTek's Absorbance Test Plate (PN 7260522) to confirm the mechanical alignment; optical density accuracy, linearity, and repeatability; and wavelength accuracy of the Cytation 5. The Absorbance Test Plate compares the reader's optical density and wavelength measurements to NIST-traceable values.

Note: An alternate method for confirming accuracy, linearity, and repeatability is Liquid Test 2, described on page 134.

To run this test, you need the BioTek Absorbance Test Plate (PN 7260522), with its accompanying certificate.

- The Absorbance OD Standards section contains NIST-traceable standard OD values for the filters at several different wavelengths. We recommend testing at six wavelengths—those at or close to the wavelengths used in your assays.
- The Wavelength Accuracy Standards section contains Expected Peak wavelength values for the filter in position C6 on the plate. Each value has a valid test range associated with it. For example, an Expected Peak value may be 586 nm with tolerance values of –6/+4 (or a test range of 580 to 590 nm).

Note: The instructions provided below are guidelines. Refer to the Gen5 Help system for more information.

Note: BioTek's Absorbance Test Plate PN 7260551 can be used to confirm optical density accuracy, linearity, and repeatability at 340 nm and is offered as an alternative to conducting Absorbance Liquid Test 3.

Define Absorbance Test Plate Parameters

1. Obtain the certificate that came with the Test Plate.
2. Start Gen5 and from the main screen select **System > Diagnostics > Test Plates > Add/Modify Plates**.
3. Click **Add**. The Absorbance Test Plate dialog appears.
4. Select the appropriate Plate Type and enter the plate’s serial number.
5. Enter the Last Certification and Next Certification dates from the calibration sticker on the Test Plate.
6. If the wavelength values in the top row of the grid in Gen5 are appropriate for your tests, enter the OD values from the data sheet into the grid. Make sure you enter the correct value for each well/wavelength combination.

Note: If you need to change the wavelength values, click **Wavelength List**. Click the Gen5 **Help** button for assistance.

7. Select the number of Peak Wavelength tests to run (up to 4).
8. Enter the Expected Peak value(s) from the certificate and set the Test Range – and + values.
   - If the certificate contains two Spectral Band Pass tables: The Cytation 5 has a band pass wider than 5 nm for wavelengths greater than 285 nm and narrower than 4 nm for 230–285 nm. As a result, we recommend using expected peak values from the 2.4 nm table for peaks that are 285 nm and lower, and from the 5.0 nm table for all other peaks.
9. Review all the values you entered. Click **OK** to save the data.

The information you just entered is available on Gen5 each time the Absorbance Plate Test is performed. It may need to be modified after the annual recertification of your test plate.

Run the Absorbance Plate Test

1. From the Gen5 main screen, select **System > Diagnostics > Test Plates > Run**. If prompted, select the desired Test Plate and click **OK**.
2. When the Absorbance Test Plate Options dialog appears, select **Perform Peak Wavelength Test** if it is not already selected.
3. Highlight the wavelength(s) to be included in this test.

Note: Select only those wavelengths most appropriate for your use of the reader.

4. (Optional) Enter any Comments.
5. Click **Start Test**.
6. Place the Test Plate in the microplate carrier so that well A1 is in the right-rear corner of the carrier.
7. Click **OK** to run the test.
8. When the test is completed, the results report appears. Scroll through the report; every result should show "PASS".

   Gen5 stores the results in a database; they can be retrieved any time. We recommend you print and save the report to document that the test was performed.

Results and Troubleshooting Tips

The Absorbance Test Plate Report contains results for the following:

- *Peak Absorbance:* When the test is performed, the C6 filter is scanned at the test ranges defined by the user in the Absorbance Test Plate dialog. To verify wavelength accuracy, the wavelength of the maximum absorbance is compared with the peak wavelength

value entered in the software, which comes from the certificate supplied with the Absorbance Test Plate. The accuracy of the wavelength should be ± 3 nm (± 2 nm instrument, ± 1 nm filter allowance). If the reader fails this test:

- Make sure the information entered into Gen5 matches the Test Plate's Certificate.
- Verify that the Test Plate has a filter in location C6. (Test Plates with the part numbers 9000547 and 7260551 do not have this filter.)
- Check the C6 filter to make sure it is clean. If needed, clean it with lens paper. Do not remove the filter from the Test Plate, and do not use alcohol or other cleaning agents.
- Verify that the Test Plate is within its calibration certification period. If it is out of date, contact BioTek to schedule a recertification.
- Check the microplate carrier to ensure it is clear of debris.

- *Alignment:* This test measures the alignment of the microplate carrier with the optical path. A reading greater than 0.015 OD represents an out-of-alignment condition. Wells A1, A12, H1, and H12 are the only valid alignment holes for the reader on the PN 7260522 Test Plate.

  If the reader fails this test:

  - Ensure that the Test Plate is correctly seated in the microplate carrier.
  - Check the four alignment holes (A1, A12, H1, H12) to ensure they are clear of debris.
  - Check the microplate carrier to ensure it is clear of debris.

- *Accuracy:* Accuracy is a measure of the optical density of Test Plate wells C1, D4, E2, F5, G3, and H6 as compared with known standard values contained in the Standards Certificate that accompanies each Test Plate.

  If the reader fails this test:

  - Verify that the filter calibration values entered in Gen5 are the same as those on the Test Plate's Standards Certificate.
  - Check the filters on the Test Plate to ensure they are clean. If necessary, clean them with lens paper. Do not remove the filters from the test plate, and do not use alcohol or other cleaning agents.
  - Verify that the Test Plate is within its calibration certification period. If it is out of date, contact BioTek to schedule a recertification.

- *Repeatability:* Repeatability is a measure of the instrument's ability to read the same well with minimum variation between two reads with the well in the same location. If the reader fails this test:

  - Check the filters on the Test Plate to ensure there is no debris that may have shifted between readings and caused changes.
  - Check the microplate carrier to ensure it is clear of debris.

Linearity of the optical density readings is confirmed by default if the optical density readings are accurate. To further verify this, you can perform a regression analysis on the Test Plate OD values in a spreadsheet program such as Microsoft Excel. An R-Squared value of at least 0.9900 is expected.

Absorbance Liquid Tests

Conducting liquid tests confirms the reader's ability to perform to specification with liquid samples. Liquid testing differs from testing with the Absorbance Test Plate in that liquid in the wells has a meniscus, whereas the Test Plate's neutral density glass filters do not. The optics characteristics may differ in these two cases, thus alerting the operator to different types of problems.

Absorbance Liquid Test 1

Absorbance Liquid Test 1 confirms repeatability and alignment of the reader when a solution is used in the microplate. If these tests pass, then the lens placement and optical system cleanliness are proven.

Materials

Note: Manufacturer part numbers are subject to change over time.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended)
- Stock Solution A or B, which may be formulated by diluting a dye solution available from BioTek (Solution A) or from the ingredients listed below (Solution B).

Solution A

- BioTek QC Check Solution No. 1 (PN 7120779, 25 mL; PN 7120782, 125 mL)
- Deionized water
- 5 mL Class A volumetric pipette
- 100 mL volumetric flask

1. Pipette 5 mL BioTek QC Check Solution No. 1 into a 100 mL volumetric flask.
2. Add 95 mL of DI water; cap and shake well. The solution should measure approximately 2.000 OD when using 200 µL in a flat-bottom microwell.

Solution B

- Deionized water
- FD&C Yellow No. 5 dye powder (typically 90% pure)
- Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or BioTek wetting agent (PN 7773002) (a 10% Tween solution)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- Weigh boat
- 1-liter volumetric flask

1. Weigh 0.092 g of FD&C Yellow No. 5 dye powder in a weigh boat.
2. Rinse the contents into a 1-liter volumetric flask.
3. Add 0.5 mL of Tween 20, or 5 mL of BioTek's wetting agent.
4. Fill to 1 liter with DI water; cap and shake well. The solution should measure approximately 2.000 OD when using 200 µL in a flat-bottom microwell.

Prepare the Plate

Note: Be sure to use a new microplate, because fingerprints or scratches may cause variations in readings.

1. Using freshly prepared stock solution (Solution A or B), prepare a 1:2 dilution using deionized water (one part stock, one part deionized water; the resulting solution is a 1:2 dilution).
2. Pipette 200 µL of the concentrated solution (A or B) into the first column of wells in the microplate.
3. Pipette 200 µL of the diluted solution into the second column of wells.

 Shake the plate for four minutes after pipetting and before reading the plate to allow any air bubbles in the solution to settle and the meniscus to stabilize. Alternatively, wait 20 minutes after pipetting the test solution before reading the plate.

Read the Plate

1. Using Gen5, read the microplate *five times* at 405 nm using the Normal read mode, single wavelength, no blanking. Save the data after each read ("Normal" plate position).
2. Without delay, rotate the microplate 180 degrees so that well A1 is in the "H12" position. Read the plate *five more times*, saving the data after each read ("Turnaround" plate position).
3. Print out the ten sets of raw data, or export them to an Excel spreadsheet.

Analyze the Results

1. The plate is read five times in the "Normal" position at 405 nm. Calculate the Mean OD and Standard Deviation of those five reads for each well in columns 1 and 2.
2. For each well in columns 1 and 2, calculate the Allowed Deviation using the repeatability specification for a 96-well plate: ± 1% ± 0.005 OD from 0.000 to 2.000 OD (Mean * 0.010 + 0.005). For each well, its standard deviation should be less than its allowed deviation.

   Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a mean of 0.8004 and a standard deviation of 0.0018. The mean multiplied by 1.0% (0.8004 * 0.010) equals 0.008, and when added to 0.005 equals 0.013; this is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.013, the well meets the test criteria.

3. The plate is read five times in the "Turnaround" position at 405 nm. Calculate the Mean OD of those reads for each well in columns 11 and 12.
4. Perform a mathematical comparison of the Mean values for each microwell in its Normal and Turnaround positions (that is, compare A1 to H12, A2 to H11, B1 to G12,… H2 to A11). To pass the test, the differences in the compared mean values must be within the accuracy specification for a 96-well microplate: ± 1.0% ± 0.010 OD from 0.000 to 2.000 OD.

   Example: If the mean value for well A1 in the Normal position is 1.902 with a specified accuracy of ± 1.0% ± 0.010 OD, then the expected range for the mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD. 1.902 x 0.010 + 0.010 = 0.029; 1.902 - 0.029 = 1.873; 1.902 + 0.029 = 1.931.

Repeatability Specification:

± 1.0% ± 0.005 OD from 0.000 to 2.000 OD

± 3.0% ± 0.005 OD from 2.000 OD to 2.500 OD

Accuracy Specification:

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD

± 3.0% ± 0.010 OD from 2.000 OD to 2.500 OD

Absorbance Liquid Test 2

The recommended method for testing the instrument's alignment, repeatability, and accuracy is to use the Absorbance Plate Test on page 128. If the test plate is not available, this Absorbance Liquid Test 2 can be used instead.

Materials

- A new 96-well, clear, flat-bottom microplate (Corning Costar #3590 is recommended)
- Ten test tubes, numbered consecutively, set up in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Solution A or B (see the instructions for Liquid Test 1)
- A 0.05% solution of deionized water and Tween 20

Prepare the Dilutions

Create a percentage dilution series, beginning with 100% of the original concentrated stock solution (A or B) in the first tube, 90% of the original solution in the second tube, 80% in the third tube, all the way to 10% in the tenth tube. Dilute using the 0.05% solution of deionized water and Tween 20. This solution can also be made by diluting the BioTek wetting agent 200:1.

Test Tube Dilutions for Liquid Test 2

Tube Number	1	2	3	4	5	6	7	8	9	10
Volume of Original Concentrated Solution (mL)	20	18	16	14	12	10	8	6	4	2
Volume of 0.05% Tween Solution (mL)	0	2	4	6	8	10	12	14	16	18
Absorbance expected if original solution is 2.0 at 200 µL	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

Note: The choice of dilutions and the absorbance of the original solution can be varied. Use this table as a model for calculating the expected absorbances of a series of dilutions, given a different absorbance of the original solution.

Prepare the Plate

- Pipette 200 µL of the concentrated solution from Tube 1 into each well of the first column, A1 to H1, of a new flat-bottom microplate.
- Pipette 200 µL from each of the remaining tubes into the wells of the corresponding column of the microplate (Tube 2 into wells A2 to H2, Tube 3 into wells A3 to H3, and so on).

Linearity and Repeatability Tests

1. Using Gen5, read the microplate prepared above five times using Normal mode, dual wavelength at 450/630 nm. Save the data after each read.

Note: Do not discard the plate; you will use it for the Alignment Test.

2. Print out the five sets of Delta OD data, or export them to an Excel spreadsheet.
3. Calculate the results for Linearity:
    - Calculate the mean absorbance for each well, and average the means for each concentration.
    - Perform a regression analysis on the data to determine if there is adequate linearity.

      Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Square value of at least 0.9900 is considered adequate.
4. Calculate the results for Repeatability:
    - Calculate the mean and standard deviation for the five readings taken in Step 1 at each concentration. Only one row of data needs to be analyzed.

- For each mean below 2.000 OD, calculate the allowed deviation using the repeatability specification for a 96-well plate of ± 1.0% ± 0.005 OD. If above 2.000 OD, apply the ± 3.0% ± 0.005 specification.
- The standard deviation for each set of readings should be less than the allowed deviation.

  Example: Absorbance readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a mean of 1.951, and a standard deviation of 0.0026. The mean (1.951) multiplied by 1.0% (1.951 x 0.010) = 0.0195, which, when added to the 0.005 (0.0195 + 0.005) = 0.0245 OD, which is the allowed deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specification:

± 1.0% ± 0.005 OD from 0.000 to 2.000 OD

± 3.0% ± 0.005 OD from 2.000 to 2.500 OD

Alignment Test

1. Using the plate prepared for the Linearity Test on the previous page, conduct a Turnaround test by reading the plate five times with the A1 well in the H12 position. Save the data after each read.

   This test results in values for the four corner wells that can be used to determine alignment.
2. Calculate the means of the wells A1 and H1 in the Normal plate position (data from Linearity Test) and in the Turnaround position (from Step 1).
3. Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the accuracy specification for the instrument.

   Example: If the mean of well A1 in the normal position is 1.902, where the specified accuracy is ± 1.0% ± 0.010 OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. (1.902 x 1.0% = 0.019 + 0.010 = 0.029, which is added to and subtracted from 1.902 for the range.)

   If the four corner wells are within the accuracy range, the reader is in alignment.

Accuracy Specification:

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD

± 3.0% ± 0.010 OD from 2.000 to 2.500 OD

Absorbance Liquid Test 3

Absorbance Liquid Test 3 is provided for sites requiring proof of linearity at 340 nm. This test is optional because the reader has good "front end" linearity throughout its wavelength range.

Note: BioTek Absorbance Test Plate PN 7260551 is offered as an alternative to conducting this test, Absorbance Liquid Test 3.

Materials

Note: Manufacturer part numbers are subject to change.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended)
- Calibrated hand pipette(s)
- Beakers and graduated cylinder
- Precision balance with readability to 0.01 g
- Buffer solution described below

Buffer Solution

- Deionized water
- Phosphate-Buffered Saline (PBS), pH 7.2–7.6, Sigma tablets, #P4417 (or equivalent)
- β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma bulk catalog number N 8129, or preweighed 10-mg vials, Sigma number N6785-10VL (or BioTek PN 98233). Store the powder according to the guidelines on its packaging.

1. Prepare a PBS solution from the Sigma tablets.
2. In a beaker, mix 50 mL of the PBS solution with 10 mg of the β-NADH powder and mix thoroughly. This is the **100% Test Solution**.
3. (Optional) Read a 150-µL sample of the solution at 340 nm; it should be within 0.700 to 1.000 OD. If low, adjust up by adding more powder. Do not adjust if slightly high.

Prepare the Plate

1. Prepare the **75% Test Solution** by mixing 15 mL of the 100% Test Solution with 5 mL of the PBS Solution.
2. Prepare the **50% Test Solution** by mixing 10 mL of the 100% Test Solution with 10 mL of the PBS Solution.
3. Carefully pipette the three solutions into a *new* 96-well microplate:
    - 150 µL of the 100% Test Solution into all wells of columns 1 and 2
    - 150 µL of the 75% Test Solution into all wells of columns 3 and 4
    - 150 µL of the 50% Test Solution into all wells of column 5 and 6

Read the Plate

1. Using Gen5, read the microplate *five times* using Normal mode, single wavelength at 340 nm, no blanking. Save the data after each read.
2. Print out the five sets of raw data, or export them to an Excel spreadsheet.

Analyze the Results

Note: The plate is read five times at 340 nm.

1. For each well, calculate the Mean OD and Standard Deviation of the five readings.
2. For each mean calculated in step 1, calculate the allowed deviation using the repeatability specification for a 96-well plate: ± 1% ± 0.005 OD from 0.000 to 2.000 OD (Mean x 0.010 + 0.005). For each well, its standard deviation should be less than its allowed deviation.

   Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a mean of 0.8004 and a standard deviation of 0.0018. The mean multiplied by 1.0% (0.8004 * 0.010) equals 0.008, and when added to 0.005 equals 0.013; this is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.013, the well meets the test criteria.
3. Calculate the results for Linearity:
   - For each of the three test solutions, calculate the average Mean OD for the wells containing that solution (mean of wells A1 to H2, A3 to H4, and A5 to H6).
   - Perform a regression analysis on the data to determine if there is adequate linearity. The three average Mean OD values are the "Y" values. The solution concentrations are the "X" values (1.00, 0.75, 0.50).

     Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Squared value of at least 0.9900 is considered adequate.

Repeatability Specification:

± 1.0% ± 0.005 OD from 0.000 to 2.000 OD

± 3.0% ± 0.005 OD from 2.000 OD to 2.500 OD

Fluorescence Testing Overview

BioTek provides two options for testing the Cytation 5 fluorescence system. One uses a solid state Fluorescence Test Plate (package PN 1400501[1]). The other uses liquid plates, the materials for which are available for purchase from BioTek (Optional Accessories on page 5).

[1]Fluorescence Test Plate PN 7092092 cannot be used for these tests.

BioTek Fluorescence Plate Tests

The BioTek Fluorescence Test Plate simplifies the process for conducting fluorescence intensity, fluorescence polarization, and time-resolved fluorescence qualification tests. The test plate is solid and, therefore, immune to the pipetting errors, evaporation issues, and costs experienced with conventional liquid tests.

The test-plate package includes Gen5 protocols[1] designed specifically for use with the test plate. The protocols include embedded Microsoft Excel spreadsheets to automatically calculate results and determine pass/fail. The protocols and their spreadsheets were fully validated in accordance with BioTek Instrument's Product Validation policies and procedures.

The package also contains a user guide that describes the test methods, helps you get started with using the plate, and provides important information for cleaning and maintaining the test plate. The guide also provides troubleshooting tips and information on the annual recalibration program.

Requirements

Refer to the ***Fluorescence Test Plate User Manual*** for information on the required materials and prerequisite tasks.

Test Procedure

The **Qualification Tests** section of the ***Fluorescence Test Plate User Manual*** contains a procedure for cleaning the plate and then creating and running experiments based on supplied Gen5 protocols. As described in the user guide, when each experiment is finished, Gen5 exports the measurement data to a prepared Microsoft Excel .xls file. The worksheet (s) in that Excel file calculate results and determine pass or fail.

Identify the reader-specific Gen5 protocols on the USB flash drive that came with the test plate. Use only those protocols that apply to your reader model and your organization's qualification requirements.

Results Analysis

Refer to the ***Fluorescence Test Plate User Manual*** for descriptions of the data reduction calculations for each test, as well as troubleshooting tips. The tests must meet the following criteria to pass:

Fluorescence Intensity (FI) Tests	
Corners	%CV < 3.0
Linearity	R2 >= 0.9500
Sensitivity, filter-based system:	
Top optics, Sodium Fluorescein analogue	Detection Limit <= 10.0 pM

Fluorescence Intensity (FI) Tests	
Top optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Sensitivity, monochromator-based system:	
Top optics, Sodium Fluorescein analogue	Detection Limit <= 20.0 pM
Bottom optics, Sodium Fluorescein analogue	Detection Limit <= 20.0 pM
Top optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Bottom optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Time-Resolved Fluorescence (TRF) Test	Detection Limit <= 250.0 fM
Fluorescence Polarization (FP) Test	Mean PHPR > 340 mP, STD PLPR < 5

[1]Gen5 version 2.07 or higher is required.

Fluorescence Liquid Tests

Perform all the tests that represent the assays performed in your laboratory to confirm the reader is performing to specifications.

Required Materials

Important: Use perfectly clean microplates, free of dust or bottom scratches, e.g., new microplates from sealed packages.

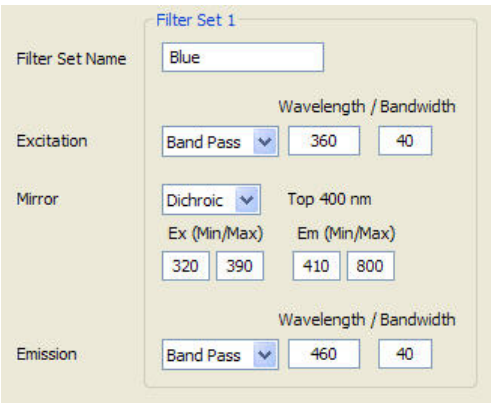
For All Tests:	Test-Specific Materials:
• Deionized or distilled water • Various beakers, graduated cylinders, and pipettes • 95% ethanol (for cleaning clear-bottom plates) • Aluminum foil • (Optional, but recommended) 0.45-micron filter • (Optional) Black polyethylene bag(s) to temporarily store plate(s) • Gen5 protocols listed on page 143.	• Corners-Sensitivity-Linearity Tests Materials on page 152 • Fluorescence Polarization (FP) Test Materials on page 152 • Time-Resolved Fluorescence (TRF) Test Materials on page 153 and • Prepare the Fluorescence Test Solutions on page 153 • Pipette Maps on page 156

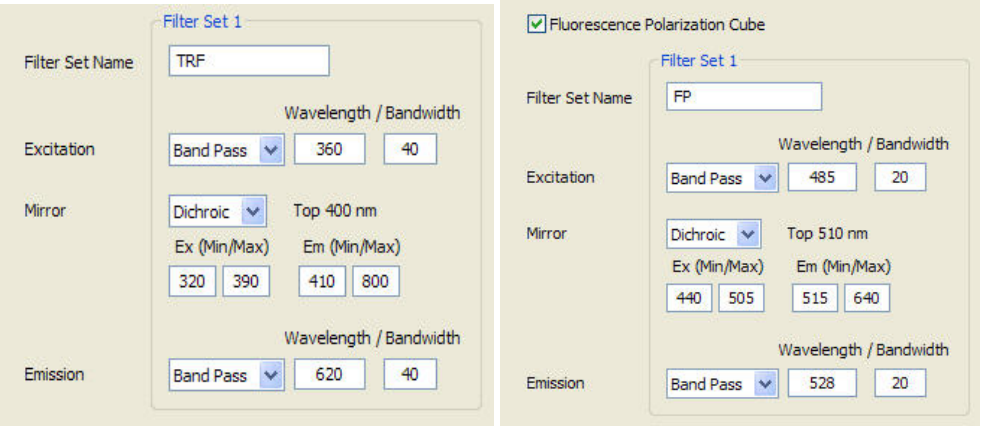
For the Filter-Based Fluorescence System	
Cytation 5_FI_T_SF.prt	Corners, Sensitivity, and Linearity Tests, using the top optics
Cytation 5_FI_T_MUB.prt	Alternative Top Optics Test, using methylumbelliferone
Cytation 5_FP.prt	Fluorescence Polarization Test
Cytation 5_TRF.prt	Time-Resolved Fluorescence Test

For the Monochromator-Based Fluorescence System	
Cytation 5_M_FI_T_SF.prt	Corners, Sensitivity, and Linearity Tests, using the Top optics
Cytation 5_M_FI_B_SF.prt	Corners, Sensitivity, and Linearity Tests, using the Bottom optics
Cytation 5_M_FI_T_ MUB.prt	Alternative Top Optics Test, using methylumbelliferone

Filter Set Setup

Before using the filter-based fluorescence test protocols, create the applicable filter sets shown below in Gen5 ("Green" is used for sodium fluorescein tests, "Blue" for MUB).





Gen5 Fluorescence Liquid Test Protocols

Here are the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type (see *Troubleshooting Tips* on page 161).

Important: Make sure the Plate Type setting in the Gen5 protocol matches the microplate you are using.

Cytation 5_FI_T_SF.prt

This procedure contains two Read steps using filters *(as described on page 142)* to test the top optics: one for the Corners Test and one for the Sensitivity/Linearity Test.

Parameter	Setting
Plate Type:	Costar 96 black opaque (#3915)
Read Step 1	
Detection Method:	Fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:00:45 Interval: 00:00:03 Reads: 16
Step Label:	"Sensitivity Read"

Parameter	Setting
Read Well:	D7
Filter Set:	1 (Green)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm
Gain:	Auto, Scale to High Wells, D7, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 2	
Detection Method:	Fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:01:35 Interval: 00:00:06 Reads: 16
Step Label:	"Sensitivity Read Buffer"
Read Wells:	C9..E9
Filter Set:	1 (Green)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm
Gain:	Auto, Use first filter set gain from FIRST Read Step

Parameter	Setting
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 3	
Detection Method:	Fluorescence
Read Type:	Endpoint
Step Label:	"Corners Read"
Read Wells:	A1..A3, A10..A12, H1..H3, H10..H12
Filter Set:	1 (Green)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm
Gain:	Auto, Scale to High Wells, A3, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 4	
Detection Method:	Fluorescence
Read Type:	Endpoint

Parameter	Setting
Step Label:	"Linearity Read"
Read Wells:	C1..F5
Filter Set:	1 (Green)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm
Gain:	Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point:50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm

Cytation 5_FP.prt

This procedure contains one Read step using filters with Fluorescence Polarization enabled, inside a Plate Mode block.

Parameter	Setting
Detection Method:	Fluorescence polarization
Read Type:	Endpoint
Plate Type:	Costar 96 black opaque (#3915)
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds after shake
Synchronized Mode:	Plate Mode with Timing Control

Parameter	Setting
Read Wells:	A6–H8
Filter Sets:	1 (filter cube)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm
Gain:	Auto, Scale to high Wells, A8, 10000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 60 Lamp Energy: Low
Read Height:	7.00 mm

Cytation 5_TRF.prt

Parameter	Setting
Plate Type:	Costar 96-well white opaque
Delay Step:	3 minutes
Shake Step:	Linear for 30 seconds
Read Step 1	
Detection Method:	Time-resolved fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:00:15 Interval: 00:00:01 Reads: 16
Step Label:	"Sensitivity Read"

Parameter	Setting
Read Well:	A8
Filter Sets:	1 (filter cube)
Filters:	EX 360/40 nm, 620/40 nm
Optics Position:	Top 400 nm
Gain:	Auto, Scale to High Wells, A8, 50000
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low
Time-Resolved FL Options:	Delay before collecting data: 300 µsec Data collection time: 1000 µsec
Read Height:	7.00 mm
Read Step 2	
Detection Method:	Time-resolved fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:00:45 Interval: 00:00:03 Reads: 16
Step Label:	"Sensitivity Read Buffer"
Read Well:	A6–C6
Filter Sets:	1 (filter cube)
Filters:	EX 360/40 nm, 620/40 nm
Optics Position:	Top 400 nm

Parameter	Setting
Gain:	Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low
Time-Resolved FL Options:	Delay before collecting data: 300 µsec Data collection time: 1000 µsec
Read Height:	7.00 mm

Cytation 5_M_FI_T_SF.prt and Cytation 5_M_FI_B_SF.prt

Parameter	Setting
Plate Type:	Top: Costar 96 black opaque Bottom: Greiner SensoPlate
Read Step 1	
Detection Method:	Fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:00:45 Interval: 00:00:3 Reads: 16
Step Label:	"Sensitivity Read"
Read Well:	D7
Wavelength:	1, EX 485/14, EM 528/14 nm
Optics Position:	Top/Bottom
Gain	Auto, Scale to High Wells, D7, 50000

Parameter	Setting
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height (for top optics):	7.00 mm
Read Step 2	
Detection Method:	Fluorescence
Read Type:	Endpoint
Kinetic Loop	Run Time: 00:01:35 Interval: 00:00:6 Reads: 16
Step Label:	"Sensitivity Read Buffer"
Read Well:	C9..E9
Wavelength:	1, EX 485/14, EM 528/14 nm
Optics Position:	Top/Bottom
Gain	Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height (for top optics):	7.00 mm
Read Step 3	

Parameter	Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Step Label:	"Corners Read"
Read Wells:	A1..A3, A10..A12, H1..H3, H10..H12
Wavelength:	1, EX 485/14, EM 528/14 nm
Optics Position:	Top/Bottom
Gain	Auto, Scale to High Wells, A3, 50000
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height (for top optics):	7.00 mm
Read Step 4	
Detection Method:	Fluorescence
Read Type:	Endpoint
Step Label:	"Linearity Read"
Read Wells:	C1..F5
Wavelength:	1, EX 485/14, EM 528/14 nm
Optics Position:	Top/Bottom
Gain	Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal Delay after plate movement: 100 msec

Parameter	Setting
	Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height (for top optics):	7.00 mm

Corners-Sensitivity-Linearity Tests Materials

Note: BioTek offers a liquid test kit (PN 7160010) containing the microplates and solutions needed for all fluorescence liquid tests (SF/MUB/Eu). Kits for each individual procedure are also available: Optional Accessories on page 5. Sodium Fluorescein Test Kit (PN 7160013) contains the buffer and SF already diluted.

Note: Methylumbelliferone can be used as an alternative or supplemental method for performing these tests.

- Buffer:
  - NIST-traceable Sodium Borate Reference Standard (pH 9.18) (e.g., Fisher-Scientific 1 L Sodium Borate Mfr. #159532, or equivalent), or
  - Phosphate-Buffered Saline (PBS), pH 7.2–7.6 (e.g., Sigma tablets, Mfr. #P4417, or equivalent) and pH meter or pH indicator strips with pH range 4 to 10
- Sodium Fluorescein Powder (1-mg vial, BioTek PN 98155)
- *If testing both Top and Bottom optics (mono-based fluorescence only):* A new, clean 96-well glass-bottom Greiner SensoPlate (Mfr. #655892); or a clean Hellma Quartz 96-well titration plate (Mfr. #730.009.QG); or equivalent
- *If testing the Top optics only:* A new, clean 96-well solid black microplate, such as Corning Costar #3915, or equivalent
- Excitation filter 485/20 nm installed
- Emission filter 528/20 nm installed
- 510-nm dichroic mirror installed

Fluorescence Polarization (FP) Test Materials

- A new, clean, 96-well solid black microplate, such as Corning Costar #3915. A Greiner SensoPlate can also be used.

Note: The FP Test can be performed in conjunction with the **top** Corners/Sensitivity/Linearity Tests, in the same microplate.

- The recommended test solutions are available from Invitrogen Corporation in their "FP One-Step Reference Kit" (PN P3088) or from BioTek (PN 7160014). This kit includes:
    - (Green) Polarization Reference Buffer, 15 mL
    - Green Low Polarization Reference, 4 mL
    - Green High Polarization Reference, 4 mL

Note: The Invitrogen kit also includes two red polarization solutions that are not used.

- Excitation filter 485/20 nm installed
- Emission filter 528/20 nm installed
- 510-nm dichroic mirror and polarizers installed

Time-Resolved Fluorescence (TRF) Test Materials

 BioTek offers a preconfigured qualification TRF filter cube (PN 8040555) for purchase.

- 15-mL conical-bottom, polypropylene sample tube
- Excitation filter 360/40 nm installed
- Emission filter 620/40 nm installed
- 400-nm dichroic mirror installed
- A new, clean 96-well solid white microplate, such as Corning Costar #3912
- The recommended test solution (FluoSpheres carboxylate-modified microspheres, 0.2 µm europium luminescent, 2 µL) is available from Invitrogen Corporation (PN F20881) or from BioTek (PN 7160011)

Prepare the Fluorescence Test Solutions

Corners/Sensitivity/Linearity Tests

Important: If using BioTek's sodium fluorescein powder (PN 98155), be sure to hold the vial upright and open it carefully; the material may be concentrated at the top. If a centrifuge is available, spin down the tube before opening. When diluting the sodium fluorescein powder in buffer, it takes time for the powder to completely dissolve. Allow the solution to dissolve for five minutes, with intermittent vortexing, before preparing the titration dyes. Wrap the vial containing the stock solution in foil to prevent exposure to light. Discard unused solution after seven days. Discard any open, unused buffer solution after seven days.

1. The Sodium Borate solution does not require further preparation; proceed to step 2. If you are using PBS, prepare the solution:
   - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
   - Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
   - Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
   - Check the pH; it should be between 7.2 and 7.6 at 25°C.
2. Prepare the sodium fluorescein stock solution:
   - Add 2.0 mL of the buffer solution to the 1 mg Sodium Fluorescein (SF) vial. This yields a 1.3288 mM stock solution.
   - Make sure the dye has completely dissolved and is well mixed.
3. Carefully prepare the dilutions. Label each with "SF" and the concentration:

Mix This SF Solution:	With Buffer:	To Make:	
0.53 mL of 1.3288 mM stock solution	13.47 mL	50.2 µM	
110 µL of 50.2 µM SF	13.89 mL	400 nM	
3.5 mL of 400 nM SF	10.5 mL	100 nM	
0.46 mL of 100 nM SF	13.54 mL	3.3 nM	Corners Test
4.24 mL of 3.3 nM SF	9.76 mL	1 nM	Sensitivity & Linearity Tests

Fluorescence Polarization (FP) Test

As described in Fluorescence Polarization (FP) Test Materials on page 152, the recommended test solutions are available from Invitrogen Corporation or from BioTek. They do not require additional preparation.

Time-Resolved Fluorescence (TRF) Test

As described in Time-Resolved Fluorescence (TRF) Test Materials on the previous page, the recommended test solutions are available from Invitrogen Corporation or from BioTek.

- Shake the FluoSpheres container vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.

- Mix 10 µL of FluoSpheres with 10 mL of deionized water, in a 15 mL conical-bottom, polypropylene sample tube. This yields a 20 nM equivalent suspension.
- Shake the vial vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.
- Mix 10 µL of 20 nM suspension with 10 mL of deionized water, in a 15 mL conical-bottom, polypropylene sample tube. This yields a 20 pM equivalent suspension.
- Refrigerate any unused portions of the FluoSpheres. The temperature must be between +2°C to +6°C.

Note: The prepared TRF plate can be kept for a maximum of seven days, if covered and stored in the dark between +2°C to +6°C.

Note: Allow the plate to sit at room temperature for approximately 15 minutes prior to use.

Fluorescence Test Procedure

1. If you have not already done so, create the Gen5 protocols as described starting on page 143.
2. If you have not already done so, prepare the solutions for the tests you plan to perform. Required Materials on page 141.

   Note: Refer to the pipette maps starting on page 156 for the remaining steps.

3. Perform the Corners/Sensitivity/Linearity tests using the Top optics of the filter-based fluorescence system:
   - Pipette the test solutions into a clean 96-well microplate.
   - Create an experiment based on **Cytation 5_FI_T_SF.prt**.
   - Read the plate, and then save the experiment.
4. Perform the Corners/Sensitivity/Linearity tests for the monochromator-based fluorescence system:
   - Create experiments based on **Cytation 5_M_FI_T_SF.prt** (top optics) and **Cytation 5_M_FI_B_SF.prt** (bottom optics).
   - Read the plate, and then save the experiment
5. To test Fluorescence Polarization capability:
   - Pipette the solutions for the "FP" test into the same plate as used in step 3.
   - Create an experiment based on **Cytation 5_FP.prt**.
   - Read the plate, and then save the experiment.
6. To test the Time-Resolved Fluorescence capability:

- Pipette the solutions for the "TRF" test into a new 96-well solid white plate.
- Create an experiment based on **Cytation 5_TRF.prt**.
- Read the plate and then save the experiment.

7. Calculate and evaluate results as described under **Results Analysis**.

Pipette Maps

Important: Seal the plates with foil or store them in black polyethylene bags until use. When using a clear-bottom plate, if the base of the plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster.

Perform these steps carefully, referring to the plate maps provided.

Corners, Sensitivity, Linearity Test

For the **Corners** test (light gray wells):

- Pipette 200 µL of the *3.3 nM SF* solution into wells A1–A3, A10–A12, H1–H3, and H10–H12.
- *If using a Hellma plate:* Pipette 200 µL of buffer into the wells surrounding the 3.3 nM wells ("CBUF" in the grid).

For the **Sensitivity** test (dark gray wells):

- Pipette 200 µL of the *1 nM SF* solution into well D7.
- Pipette 200 µL of the buffer solution into wells C9, D9, and E9.

For the **Linearity** test (wells C1–F5):

- Use a multichannel pipette with just four tips installed.
- Pipette 150 µL of buffer solution into wells C2–F5. Discard the tips.
- Pipette 150 µL of the *1 nM SF* solution into wells C1–F1.
- Pipette 150 µL of the 1 nM SF solution into wells C2–F2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C2–F2, and dispense into wells C3–F3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C3–F3, and dispense into wells C4–F4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C4–F4, and dispense into wells C5–F5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C5–F5, and discard the tips.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**3.3 nM**	**3.3 nM**	**3.3 nM**	*CBUF*					*CBUF*	**3.3 nM**	**3.3 nM**	**3.3 nM**
B	*CBUF**	*CBUF*	*CBUF*	*CBUF*					*CBUF*	*CBUF*	*CBUF*	*CBUF*
C	150µL: 1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF			
D	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM		200µL: 1.0 nM		BUF			
E	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF			
F	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM							
G	*CBUF*	*CBUF*	*CBUF*	*CBUF*					*CBUF*	*CBUF*	*CBUF*	*CBUF*
H	**3.3 nM**	**3.3 nM**	**3.3 nM**	*CBUF*					*CBUF*	**3.3 nM**	**3.3 nM**	**3.3 nM**

**CBUF applies only to the Hellma Quartz plate.*

Fluorescence Polarization (FP) Test

- Pipette 200 µL of the (green) polarization buffer (BUF) into wells A6–H6.
- Pipette 200 µL of the green high polarization reference (HPR) into wells A7–B7.
- Pipette 200 µL of the green low polarization reference (LPR) into wells A8–H8.

	1	2	3	4	5	6	7	8	9	10	11	12
A						BUF	HPR	LPR				
B						BUF	HPR	LPR				
C						BUF		LPR				
D						BUF		LPR				
E						BUF		LPR				
F						BUF		LPR				
G						BUF		LPR				
H						BUF		LPR				

Time-Resolved Fluorescence (TRF) Test

- Pipette 200 µL of deionized water into wells A6–C6.
- If you have not already done so, shake the vial of 20 pM europium suspension vigorously

for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the vial.

- Pipette 200 µL of the 20 pM europium suspension (Eu) into well A8.

	1	2	3	4	5	6	7	8	9	10	11	12
A						DI		Eu				
B						DI						
C						DI						
D												
E												
F												
G												
H												

Results Analysis

Corners Test

1. Calculate the Mean of the 12 wells containing the 3.3 nM SF test solution (A1–A3, A10–A12, H1–H3, and H10–H12).
2. Calculate the Standard Deviation for the same 12 wells.
3. Calculate the %CV: `(Standard Deviation / Mean) * 100`

The %CV must be < *3.0* to pass.

Sensitivity Test

1. Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (C9, D9, E9).
2. Among the three buffer wells, find the Median Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the SF Concentration well (D7).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean SF Concentration, Buffer Median STD with its corresponding Buffer Mean:
   `(SF Mean – Buffer Mean)/(3 * Buffer STD)`
5. Calculate the Detection Limit, in pM, using the known concentration value of SF and the Calculated `SNR: 1000/SNR`

Filter-Based Fluorescence System	
Optic Probe	**To pass, the Detection Limit must be less than or equal to:**
Top	10 pM

Monochromator-Based Fluorescence System	
Optic Probe	**To pass, the Detection Limit must be less than or equal to:**
EX 485 nm, EM 528 nm	Top/Bottom: 20 pM

Linearity Test

1. Calculate the Mean of the four wells for each concentration in columns 1–5.
2. Perform linear regression using these values as inputs:

Filter- and Monochromator-Based Fluorescence System	
x	**y**
1000	Mean of the 1000 pM wells
500	Mean of the 500 pM wells
250	Mean of the 250 pM wells
125	Mean of the 125 pM wells
62.5	Mean of the 62.5 pM wells

3. Calculate the R-Square value; it must be >= *0.9500* to pass.

Fluorescence Polarization (FP) Test

1. Using the raw data from the Parallel read:
   - Calculate the Mean Blank (wells A6–H6).
   - Calculate the Signal for each HPR well: Subtract the Mean Blank from its measurement value.
   - Calculate the Signal for each LPR well: Subtract the Mean Blank from its measurement value.
2. Using the raw data from the Perpendicular red:
   - Calculate the Mean Blank (wells A6–H6)
   - Calculate the Signal for each HPR well: Subtract the Mean Blank from its measurement value.
   - Calculate the Signal for each LPR well: Subtract the Mean Blank from its measurement value.
3. Calculate the G-Factor for each LPR well:

```
(Parallel LPR Sign * (1–0.02)) / (Perpendicular LPR Signal *
(1+0.02))
```

4. Calculate the Mean G-Factor.
5. Calculate the Polarization value in mP for each HPR well ("PLPR"):

$$\frac{\text{Parallel HPR Signal} - \text{Mean G-Factor} * \text{Perpendicular HPR Signal} * 1000}{\text{Parallel HPR Signal} + \text{Mean G-Factor} * \text{Perpendicular HPR Signal}}$$

6. Calculate the Mean PHPR, in mP.

Optic Probe	To pass, the Mean PHPR must be greater than:
Top, with 510 nm dichroic mirror	340 mP

7. Calculate the Polarization value in mP for each LPR well ("PLPR"):

$$\frac{\text{Parallel LPR Signal} - \text{Mean G-Factor} * \text{Perpendicular LPR Signal} * 1000}{\text{Parallel LPR Signal} + \text{Mean G-Factor} * \text{Perpendicular LPR Signal}}$$

8. Calculate the Standard Deviation of the "PLPR," in mP.

Optic Probe	To pass, the Standard Deviation of the PLPR must be less than:
Top, with 510 nm dichroic mirror	5

Time-Resolved Fluorescence (TRF) Test

1. Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (A6, B6, C6).
2. Among the three buffer wells, find the Median Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the Eu Concentration well (A8).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean Eu Concentration and Buffer Median STD with its corresponding Buffer Mean:
   `(Eu Mean – Buffer Mean)/(3 * Buffer STD)`
5. Calculate the Detection Limit, in fM:
   `20000 / ((Mean Eu – Mean DI water) / (3 * Standard Deviation DI water))`

Optic Probe	To pass, the Detection Limit must be less than or equal to:
Top, with 400 nm dichroic mirror	250 fM

Troubleshooting Fluorescence Liquid Tests

If any tests fail, please try the following suggestions. **If the test(s) continue to fail, print the results and contact Technical Support.**

- Are the solutions fresh? Discard the plate and any open, unused test solutions after seven days.
- Are the excitation/emission filters clean? Are they in the proper locations and in the proper orientation in the filter cube?
- If the Corners Test continues to fail, the hardware may be misaligned.
- Are you using new/clean plates? If the base of a clear-bottom plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster. If the test fails again, the optical probe(s) may need to be cleaned.
- Review the pipetting instructions to verify the plate was correctly prepared: Pipette Maps on page 156.
- Does the Plate Type setting in the Gen5 protocol match the plate you used?
- For injector models, spilled fluid inside the reader may be fluorescing, which can corrupt your test results.
- When testing Fluorescence Polarization capability using a solid black plastic microplate, if the standard deviation for the buffer wells is too high, try moving the buffer wells to another column. With some black plastic plates, the wells in the center of the plate may be slightly distorted due to the plate molding process, and this can affect the standard deviation.
- The Read steps in the protocols use the Gen5 Automatic Gain Adjustment feature to determine optimum sensitivity values for the plate. If an Auto Gain Result value is outside the range of 30–200, this may indicate a problem.

  If the value is less than 30:

  - The stock solution/dilution concentrations may be too high. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
  - If all of the tests are passing but the Gain value is low, a PMT in your reader may just be very sensitive.

  If the value is greater than 200:

  - The stock solution/dilution concentrations may be too low. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
  - For injector models, spilled fluid inside the reader may be fluorescing, which can corrupt your test results.
  - The PMTs or optical path(s) may be deteriorating, or the optics or other hardware may be misaligned.

Fluorescence Test Procedure (Methylumbelliferone)

As an alternative to using Sodium Fluorescein, Methylumbelliferone ("MUB") can be used to test the top optics.

Required Materials

Note: BioTek offers a liquid test kit (PN 7160012) containing the microplates and solutions used in the MUB fluorescence liquid test.

Important: Use perfectly clean microplates, free of dust or bottom scratches, e.g., new microplates from sealed packages.

Note: Manufacturer part numbers are subject to change over time.

- Methylumbelliferone ("MUB") (10-mg vial, BioTek PN 98156)
- Carbonate-Bicarbonate buffer ("CBB") capsules (BioTek PN 98158)
- 100% methanol (BioTek PN 98161)
- A new, clean 96-well solid black plate microplate, such as Corning Costar #3915 or equivalent.
- Excitation filter 360/40 nm installed
- Emission filter 460/40 nm installed
- 400 nm dichroic mirror installed
- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- Aluminum foil
- (Optional, but recommended) 0.45-micron filter
- Gen5 protocols *(as described on page 165)*:
    - *Cytation 5_FI_T_MUB.prt* tests filter-based fluorescence performance.
    - *Cytation 5_M_FI_T_MUB.prt* tests monochromator-based fluorescence performance.

Test Solutions

1. Prepare the buffer (CBB) solution:
    - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
    - Open and dissolve the contents of two CBB capsules (do not dissolve the outer gelatin capsule) into 200 mL of the water.

- Stir the solution (preferably using a stir table) until the CBB is completely dissolved.

2. Prepare the MUB stock solution:
   - Add 1 mL of 100% methanol to the 10 mg vial of MUB.
   - Make sure all of the dye has completely dissolved and is well mixed. This yields a *10 mg/mL* stock solution.
   - Wrap the solution in aluminum foil to prevent exposure to light.
3. Prepare the dilutions. Label each with "MUB" and the concentration.

Mix This MUB Solution:	With:	To Make:
0.5 mL of 10 mg/mL stock solution	4.5 mL of 100% methanol	1 mg/mL
0.88 mL of 1 mg/mL solution	4.12 mL of CBB	176 µg/mL
0.1 mL of 176 µg /mL solution	9.9 mL of CBB	1.76 µg /mL
0.5 mL of 1.76 µg /mL solution	4.5 mL of CBB	176 ng/mL
1 mL of 176 ng/mL solution	9 mL of CBB	17.6 ng/mL (100 nM)

Procedure

1. If you have not already done so, create the Gen5 protocols on page 165.
2. If you have not already done so, prepare the test solutions.
3. Perform the Sensitivity/Linearity tests using the *Top* filter-based optics:
   - Refer to the pipette map in the next section and pipette the solutions into a clean, 96-well solid black plate.
   - Create a Gen5 experiment based on *Cytation 5_FI_T_MUB.prt*.
   - Read the plate, and then save the experiment.
4. Perform the Sensitivity/Linearity tests for the *Top* monochromator-based fluorescence system:
   - Create a Gen5 experiment based on *Cytation 5_M_FI_T_MUB.prt*.
   - Read the plate, and then save the experiment.
5. Calculate and evaluate the results as described under Results Analysis, starting on page 163.

Results Analysis

Sensitivity Test

1. Calculate the Mean and Standard Deviation for the 16 reads for each of the buffer wells (C9, D9, E9).

2. Among the three buffer wells, find the Median Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the MUB concentration well (D7).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean MUB Concentration and Buffer Median STD with its corresponding Buffer Mean:
   `(Mean MUB – Buffer Mean)/(3 * Buffer STD)`
5. Calculate the Detection Limit, in ng/mL, using the known concentration value of MUB and the calculated SNR: 17.6/SNR

Filter-Based Fluorescence System	
Optic Probe	**To pass, the Detection Limit must be less than or equal to:**
Top, with 400 nm dichroic mirror	0.16 ng/mL (0.91 nM)

Monochromator-Based Fluorescence System	
Optic Probe	**To pass, the Detection Limit must be less than or equal to:**
Top	0.16 ng/mL (0.91 nM)

Linearity Test

1. Calculate the Mean of the four wells for each concentration in columns 1–5.
2. Perform linear regression using these values as inputs:

Filter- and Monochromator-Based Fluorescence Systems	
x	**y**
100	Mean of the 100 nM wells
50	Mean of the 50 nM wells
25	Mean of the 25 nM wells
12.5	Mean of the 12.5 nM wells
6.25	Mean of the 6.25 nM wells

3. Calculate the R-Square value; it must be ≥ *0.9500* to pass.

Pipette Map

Perform these steps carefully and refer to the grid below:

For the Sensitivity test (dark gray wells):

- Pipette 200 µL of CBB buffer into wells C9, D9, and E9.
- Pipette 200 µL of the 17.6 ng/mL (100 nM) MUB solution into well D7.

For the Linearity test (wells C1–F5):

- Use a multi-channel pipette with just four tips installed.
- Pipette 150 µL of buffer into wells C2–F5. Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into wells C1–F1. Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into wells C2–F2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C2–F2 and dispense into wells C3–F3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C3–F3 and dispense into C4–F4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C4–F4 and dispense into wells C5–F5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from wells C5–F5. Discard the tips.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	100 nM	50 nM	25 nM	12.5 nM	6.25 nM				BUF			
D	100 nM	50 nM	25 nM	12.5 nM	6.25 nM		MUB 100 nM		BUF			
E	100 nM	50 nM	25 nM	12.5 nM	6.25 nM				BUF			
F	100 nM	50 nM	25 nM	12.5 nM	6.25 nM							
G												
H												

Gen5 Protocol Reading Parameters

The following tables list the recommended reading parameters. Your tests may require modifications to some of these parameters, such as the Plate Type or Sensitivity value. (Troubleshooting Fluorescence Liquid Tests on page 161).

Important: Make sure the Plate Type setting in the Gen5 protocol matches the microplate you are using.

Cytation 5_FI_T_MUB.prt

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96-well black opaque
Read Step 1	
Kinetic Loop:	Run Time: 00:00:45 Interval: 00:00:03 Reads: 16
Step Label:	"Sensitivity Read"
Read Well:	D7
Filter Sets:	1 (Blue)
Filters:	EX 360/40 nm, EM 460/40 nm
Optics Position:	Top 400 nm
Gain:	Auto, Scale to High Wells, D7, 80000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 2	
Kinetic Loop:	Run Time: 00:01:35 Interval: 00:00:06 Reads: 16

Parameter	Default Setting
Step Label:	"Sensitivity Read Buffer"
Read Well:	C9..E9
Filter Sets:	1 (Blue)
Filters:	EX 360/40 nm, EM 460/40 nm
Optics Position:	Top 400 nm
Gain:	Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 3	
Step Label:	"Linearity Read"
Read Well:	C1..F5
Filter Sets:	1 (Blue)
Filters:	EX 360/40 nm, EM 460/40 nm
Optics Position:	Top 400 nm
Gain:	Auto, Scale to High Wells, C1, 80000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm

Cytation 5_M_FI_T_MUB.prt

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96-well black opaque
Read Step 1	
Kinetic Loop:	Run Time: 00:00:45 Interval: 00:00:03 Reads: 16
Step Label:	"Sensitivity Read"
Read Well:	D7
Wavelengths:	EX 360/14 nm, EM 460/14 nm
Optics Position:	Top
Gain:	Auto, Scale to High Wells, D7, 80000
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 2	
Kinetic Loop:	Run Time: 00:01:35 Interval: 00:00:06 Reads: 16
Step Label:	"Sensitivity Read Buffer"

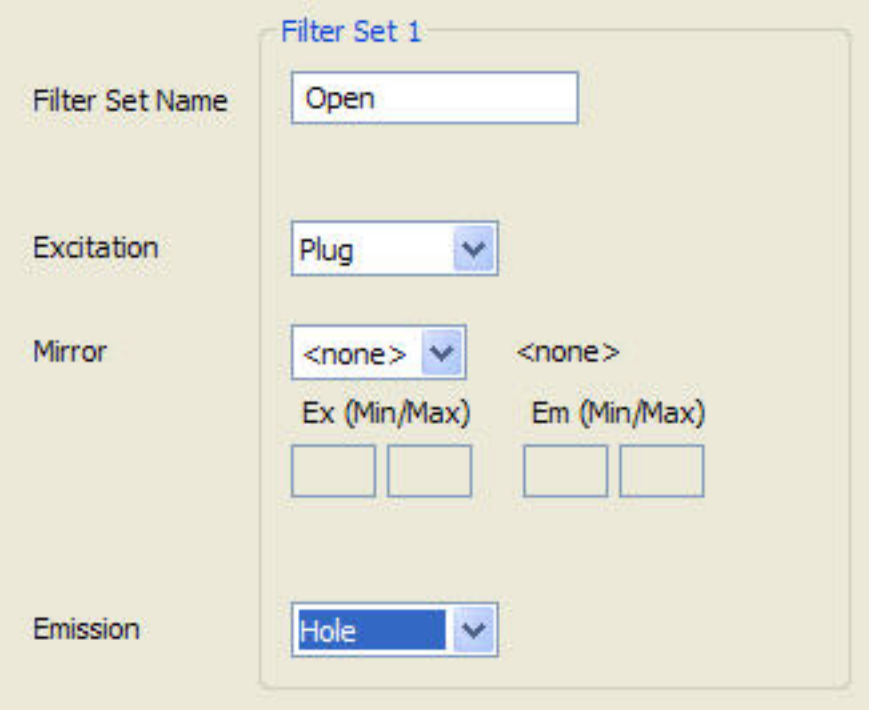
Parameter	Default Setting
Read Well:	C9..E9
Filter Sets:	1 (Blue)
Wavelengths	EX 360/14 nm, EM 460/14 nm
Optics Position:	Top 400 nm
Gain:	Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm
Read Step 3	
Step Label:	"Linearity Read"
Read Well:	C1..F5
Filter Sets:	1 (Blue)
Wavelengths:	EX 360/14 nm, EM 460/14 nm
Optics Position:	Top 400 nm
Gain:	Auto, Scale to High Wells, C1, 80000
Read Speed:	Normal Delay after plate movement: 100 msec Measurements per data point: 50 Lamp Energy: Low Dynamic Range: Standard
Read Height:	7.00 mm

Luminescence Test

For models with luminescence capability, BioTek uses the Harta Luminometer Reference Microplate to test the luminescence system. The test plate is LED-based and NIST-traceable. Contact BioTek to purchase a plate (PN 8030015; includes microplate carrier adapters).

Prerequisite

Before using the *Cytation 5 F-LumTest_Harta.prt* protocol described in this section, create the filter set shown below:



Harta Plate Test

Materials

- Harta Luminometer Reference Microplate, PN 8030015
- Harta Plate Adapter, PN 1222205
- Filter cube PN 8040553, LUM Filter Block *or* a filter cube with a plug in EX position 1 or 2 and a hole in the corresponding EM position
- Default filter cube PN 8040583, LUM-Green Filter Block *or* a filter cube with a plug in EX position 1 or 2 and a hole in the corresponding EM position
- Gen5 Luminescence Test Protocol Parameters on page 173

Procedure

1. Turn on the Harta reference plate using the I/O switch on the back of the plate.
2. Check the plate's battery by pressing the test button on the back of the plate and ensuring that the test light turns on.

Note: The test light may be difficult to see in bright light. Change your angle of view or move to a darker environment if you cannot see it.

3. Place the Harta plate adapter on the reader's carrier, then place the test plate on top of the adapter.
4. Create an experiment based on the *Cytation 5 F-LumTest_Harta.prt* or *Cytation 5 M-LumTest_Harta.prt* protocol and read the plate.

Note: The read starts with a three-minute delay.

5. Calculate and evaluate results as described under *Results Analysis* below.

Note: Be sure to turn off the Harta plate when you are finished with the test to preserve battery life.

Plate Layout

Cytation 5 F-LumTest_Harta.prt

	1	2	3	4	5	6	7	8	9	10	11	12
A		A2 meas					battery check	battery check				
B												
C												
D	BUF	BUF	BUF	BUF								
E	BUF	BUF	BUF	BUF								
F	BUF	BUF	BUF	BUF								
G	BUF	BUF	BUF	BUF								
H												

Cytation 5 M-LumTest_Harta.prt

	1	2	3	4	5	6	7	8	9	10	11	12
A		A2 meas					battery check	battery check				
B												
C												
D												
E												
F	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
G	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
H												

Results Analysis

Important: Through a manual correlation process, it was found that the system requires approximately 35 photons per attomole of ATP, thus a conversion factor of 0.02884 attomole/photon was applied to determine ATP concentration from the NIST data in photons/s.

1. On the Harta plate's Calibration Certificate, locate the NIST measurement for the A2 position and convert it to attomoles:
   `(A2 NIST measurement * 0.02884)`
2. Determine if the plate's battery is still functioning properly:
   - If A8 > (0.2 * A7), the battery is good. Otherwise, it requires replacement.

   Note: A replacement battery is included with each Harta plate. A new spare battery will be supplied when the plate is recertified.
3. Calculate the signal-to-noise ratio:
   `(A2 – Mean of the buffer cells)/(3 * Standard deviation of buffer cells)`
4. Calculate the detection limit:
   `A2 NIST measurement in attomoles/signal-to-noise ratio`
   - If the reader is equipped with the low-noise PMT, the detection limit must be <= *75 amol* to pass.
   - If the reader is equipped with the red-shifted PMT, the detection limit must be <= *500 amol* to pass.

To determine which PMT is installed, in Gen5, select **System>Instrument Configuration>View/Modify>Instrument Settings** in the Reader Settings window.

Gen5 Luminescence Test Protocol Parameters

Protocol reading parameters for luminescence testing:

Cytation 5 F-LumTest_Harta.prt

Parameter	Setting
Plate Type:	8030015 Harta - w/o 8032028 adapter
Delay Step:	3 minutes
Read Step 1	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Reference well A2"
Read Wells:	A2
Filter Sets:	1 (open)
Excitation:	Plug
Emission:	Hole
Gain:	135
Integration Time:	0:10.00 MM:SS.ss
Delay after plate movement:	350 msec
Dynamic Range:	Standard
Read Height:	7.00 mm

Parameter	Setting
Read Step 2	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Background"
Read Wells:	D1..G4
Filter Sets:	1 (Open)
Excitation:	Plug
Emission:	Hole
Gain:	135
Integration Time:	0:10.00 MM:SS.ss
Delay after plate movement:	350 msec
Dynamic Range:	Standard
Read Height:	7.00 mm
Read Step 3	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Battery Check"
Read Wells:	A7–A8
Filter Sets:	1 (Open)
Excitation:	Plug

Parameter	Setting
Emission:	Hole
Gain:	60
Integration Time:	0:01.00 MM:SS.ss
Delay after plate movement:	350 msec
Dynamic Range:	Extended
Read Height:	10. 00 mm

Cytation 5 M-LumTest_Harta.prt

Parameter	Default Setting
Plate Type:	8030015 Harta - w/o 8032028 adapter
Delay Step:	3 minutes
Read Step 1	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Reference well A2"
Read Well:	A2
Wavelength	1
Gain:	150
Integration Time:	0:10.00 MM:SS.ss
Delay after plate movement:	350 msec
Dynamic Range:	Standard

Parameter	Default Setting
Read Height:	1.00 mm
Read Step 2	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Background"
Read Wells:	F1..G12
Wavelength	1
Gain:	150
Integration Time:	0:10.00 MM:SS.ss
Delay after plate movement:	350 msec
Dynamic Range:	Standard
Read Height:	1.00 mm
Read Step 3	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	"Battery Check"
Read Wells:	A7–A8
Wavelength	1
Gain:	80
Integration Time:	0:01.00 MM:SS.ss

Parameter	Default Setting
Delay after plate movement:	350 msec
Dynamic Range:	Extended
Read Height:	1.00 mm

Luminescence Troubleshooting

If the test fails, try the following suggestions. If the test continues to fail, print the results and contact Technical Support.

- Ensure that the reading is performed through a hole in the filter cube, not through a glass filter.
- Verify that the filter cube settings in Gen5 match the physical cube.
- Make sure the Harta test plate is present and positioned correctly on the carrier.
- Make sure the test plate adapter is present.
- If the test continues to fail, the optical probe(s) may need to be cleaned. Contact Technical Support for instructions.

Alpha Detection Test

This section applies only to models with the alpha module.

The alpha laser has been factory-calibrated to meet specification. BioTek Instruments, Inc., has developed a test protocol that can be used with AlphaScreen™ TruHits™ Kit, available from PerkinElmer (Mfg. #6760627), to verify the functionality of the alpha laser system. Because the detector for the alpha system is functionally and optically identical to the luminescence system, the luminescence test may be used to verify detector functionality.

The *Crosstalk* test is a measure of how well the optical system can distinguish the signals emitted from the well being read from those of any adjacent well. This test also determines the signal-to-noise ratio (SNR) of the test plate and verifies that the signal is at an acceptable level for the sample material used. The test is designed for use with AlphaScreen TruHits, and it is assumed that 96-well plates are used with 100 µL well volumes.

 After pipetting the solutions into the microplate, allow the plate to sit at room temperature for 15 minutes before starting the experiment. Cover the plate to prevent any light from hitting the beads.

Required Materials

Note: Manufacturer part numbers are subject to change over time.

- Recommended test solution, AlphaScreen TruHits Kit, available from PerkinElmer (Mfg. #6760627)
- Buffer: Phosphate-Buffered Saline (PBS), pH 7.2–7.6 (e.g., Sigma tablets, Mfg. #P4417 or equivalent)
- Clean 96-well solid white microplate
- 1.5-mL conical-bottom, polypropylene sample tube
- Alpha filter cube
- Gen5 protocol on page 181

Test Solutions

Note: AlphaScreen beads are light sensitive. All tests should be performed under subdued laboratory lighting of less than 100 lux.

1. Prepare the PBS buffer solution:
    a. (Optional, but recommended) Use a 0.45-micron filter to filter 200 mL of deionized or distilled water.
    b. Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
    c. Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
    d. Check the pH; it should be between 7.2 and 7.6 at 25°C.
2. Prepare the TruHits bead suspension:
    a. Shake the container of TruHits bead suspension vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.
    b. Pipette the Donor and Acceptor beads into the bottom of a 1.5 mL conical-bottom, polypropylene sample tube
        - Donor beads 8 µL
        - Acceptor beads 8 µL
    c. Replace the tube cap and cover the tube with foil.
    d. Incubate for 15 minutes at room temperature.
    e. Add diluent to a final volume of 1 mL.
        - PBS 984 µL
    f. Replace the tube cap and cover the tube with foil.
    g. Incubate for 15 minutes at room temperature.
    h. This yields a TruHits bead suspension that is 20 µg/mL with respect to the Acceptor beads.
    i. Discard the remaining bead solution.
    j. If extra beads have been reacted, they can be stored for later use. For storage, refrigerate any unused portions of the TruHits bead suspension. The temperature must be between +2°C and +6°C.

Note: Do **NOT** use sodium azide as a preservative as it will affect the AlphaScreen signal. Procline™ 300 at 0.03% is suggested.

Pipette Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
B	BUF	20 µg/mL OMB	BUF	BUF	20 µg/mL OMB	BUF	BUF	20 µg/mL OMB	BUF	BUF	20 µg/mL OMB	BUF
C	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
D												
E												
F												
G	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
H	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF

Procedure

Crosstalk Test

1. Pipette 100 µL of PBS in wells A1–A12, B1, B3–B4, B6–B7, B9–B10, B12, C1–C12, and G1–H12 (see pipette map, "BUF" wells).
2. Pipette 100 µL of 20 µg/mL AlphaScreen beads suspension into wells B2, B5, B8, and B11 (see pipette map, "20 µg/mL OMB" wells).

Note: Allow the plate to sit at room temperature for approximately 15 minutes prior to use.

3. Create an experiment based on the *Cytation 5 AlphaTest_Crosstalk.prt* protocol. Read the plate, and then save the experiment.
4. Open the Plate menu and export the data to the embedded Power Export spreadsheet. The spreadsheet reports pass or fail for the test performed. See *Results Analysis*, next, for descriptions of the calculations and troubleshooting tips.
5. Print the spreadsheets and store them with your test records.

Results Analysis

1. Calculate the crosstalk for each of the four wells of AlphaScreen beads solution by dividing the background-subtracted Mean value of the surrounding adjacent wells by the background-subtracted AlphaScreen beads suspension well.

2. Average the % crosstalk of the four test wells to determine level of crosstalk (`Crosstalk Mean`).
3. Verify that the % crosstalk is less than or equal to 0.1%
4. Calculate the signal-to-noise ratio (SNR) by using the following equation:
   `SNR = (signal mean – background mean)/(SQRT(signal STD² + background STD²))`
5. Verify that SNR is greater than or equal to *10*.

Troubleshooting Alpha Tests

If the test fails, please try the following suggestions. If the test(s) continue to fail, print the results and contact Technical Support.

- Are the solutions fresh?
- Have the solutions been stored properly (between +2°C and +6°C)?
- Has the kit been exposed to excessive light (in excess of 100 lux)?
- If the Crosstalk test continues to fail, the laser may not be firing. Contact Technical Support.

Gen5 Protocol Reading Parameters

The information in the following table represents the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as Plate Type or Gain value (see *Troubleshooting Tips* above).

Note: The Plate Type setting in the Gen5 protocol should match the plate you are actually using.

Cytation 5 AlphaTest_Crosstalk.prt

This procedure contains one read step and calculates crosstalk based on the full plate data.

Parameter	Default Setting
Plate Type	Costar 96-well white opaque
Detection Method	Alpha
Read Type	Endpoint
Read Wells	Full plate
Gain	120

Parameter	Default Setting
Delay after plate movement	0 msec
Excitation time	100 msec
Delay after excitation	120 msec
Integration time	100 msec
Read height	7.00 mm

Dispense Module Qualification Tests

This section applies only to instruments with the dispenser.

BioTek provides qualification tests to ensure that the dispense module performs to specification initially and over time. We recommend performing these tests before first use, and then every three months.

- The **Accuracy Test** is a measure of the mean volume per well for multiple dispenses. The actual weight of the dispensed fluid is compared to the expected weight and must be within a certain percentage to pass. Pass/Fail criteria depend on the per-well volume dispensed: 2.0% for 80 µL, 5.0% for 20 µL, and 20.0% for 5 µL. It is assumed that one gram is equal to one milliliter.

  The test uses a single green dye test solution and a 96-well microplate (per injector) to test the three different volumes. The balance is tared with the empty plate, and then the 80 µL dispense is performed for columns 1–4. The fluid is weighed and the balance is tared again (with the plate on the balance). This process is repeated for the 20 µL and 5 µL dispenses. It is assumed that the solutions used are at room temperature. A precision balance (three-place) is used to weigh the plate.

- The **Precision Test** is a measure of the variation among volumes dispensed to multiple wells. For each volume dispensed (80 µL, 20 µL, and 5 µL) to four columns, the %CV (coefficient of variation) of 32 absorbance readings is calculated. Pass/Fail criteria depend on the per-well volume dispensed: 2.0% for 80 µL, 7.0% for 20 µL, and 10.0% for 5 µL. The plate is read in an absorbance reader at 405/750 nm for columns 1–4 and at 630/750 nm for columns 5–12.

The two tests are performed simultaneously and use the same plate.

Required Materials

Note: Manufacturer part numbers are subject to change over time.

- Absorbance reader with capability of reading at 405, 630, and 750 nm. The reader must have an accuracy specification of ± 1.0% ± 0.010 OD or better and a repeatability specification of ± 1.0% ± 0.005 OD or better.

  Note: The Cytation 5 may be used if it is equipped with Absorbance capabilities and has passed the Absorbance Plate Test or Absorbance Liquid Test 1.

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200 µL hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250-mL beaker

- New 96-well, clear, flat-bottom microplates
- BioTek's Green Test Dye Solution (PN 7773003) undiluted, *or* one of the alternate test solutions listed in the next section
- 100-mL graduated cylinder and 10-mL pipettes (if not using BioTek's Green Test Dye Solution)
- Gen5 software installed on the host PC
- Gen5 protocols as defined by the procedure beginning on page 189

Alternate Test Solutions

Note: 80 µL of test solution with 150 µL of deionized water should read between 1.300 and 1.700 OD at 405/750 nm. The solutions should be at room temperature.

If you do not have BioTek's Green Test Dye Solution (PN 7773003), prepare a dye solution using one of the following methods:

Using BioTek's Blue and Yellow Concentrate Dye Solutions:

Ingredient	Quantity
Concentrate Blue Dye Solution (PN 7773001, 125 mL)	4.0 mL
QC (Yellow) Solution (PN 7120782, 125 mL)	5.0 mL
Deionized water	90.0 mL

Using FD&C Blue and Yellow Dye Powder:

Ingredient	Quantity
FD&C Blue No. 1	0.200 grams
FD&C Yellow No. 5	0.092 grams
Tween 20	1.0 mL
Sodium Azide N_3Na	0.100 gram
Deionized water	Make to 1 liter

Procedure for Models with Absorbance Capabilities

Note: If you have not already done so, create Gen5 protocols *Cytation 5 Disp 1 Test.prt* and *Cytation 5 Disp 2 Test.prt*. Instructions begin on page 189.

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. Purge both dispensers with the Volume set to 2000 µL. This prevents the water from diluting the dye. Remove the inlet tubes from the supply bottles.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. In Gen5, create an experiment based on *Cytation 5 Disp 1 Test.prt*.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

   Important: Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

   Note: When each dispense step is finished, you will weigh the plate, record the weight, tare the balance with the plate on it, and then place the plate back on the carrier for the next step.

7. Select **Plate > Read** and click **READ**. Gen5 prompts you to empty the tip priming trough.
8. When ready, click **OK** at the Load Plate dialog to begin the experiment. The sequence is as follows:
   a. Dispense 80 µL/well to columns 1–4.
   b. Remove the plate and weigh it. Record the weight and tare the balance.
   c. Place the plate on the carrier and dispense 20 µL/well to columns 5–8.
   d. Remove the plate and weigh it. Record the weight and tare the balance.
   e. Place the plate on the carrier and dispense 5 µL/well to columns 9–12.
   f. Remove the plate and weigh it. Record the weight.
   g. Manually pipette 150 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.
   h. Place the plate on the carrier for a 15-second shake, the "80 µL" read at 405/750 nm, and the "20 and 5 µL" read at 630/750 nm.
9. When processing is complete, select **File > Save As**. Enter an identifying file name and click **Save**.
10. Remove the plate from the carrier and set it aside.
11. Repeat steps 4–9 using *Cytation 5 Disp 2 Test.prt*.

12. See instructions for analyzing the results on page 186.

Important: Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

Note: When all tests are complete, prime both dispensers with at least 5000 µL of deionized water to flush out the green dye solution.

Results Analysis

Note: For your convenience, worksheets are included at the end of this chapter for recording the dispense weights, Delta OD values, calculations, and pass/fail.

The pass/fail criteria for each set of 32 wells with the same dispense volume is based on the calculated coefficient of variation (% CV) and Accuracy % Error.

For each volume dispensed (80 µL, 20 µL, 5 µL), for each dispenser (1, 2):

- Calculate the Standard Deviation of the 32 wells
- Calculate the Mean of the 32 wells
- Calculate the %CV: `(Standard Deviation / Mean) x 100`
- Calculate the Accuracy % Error:
  `((Actual Weight – Expected Weight)/Expected Weight)* 100`

Note: Expected Weights for 32 wells: 80 µL (2.560 g), 20 µL (0.640 g), 5 µL (0.160 g). It is assumed that one gram is equal to one milliliter.

Dispense Volume	To pass, %CV must be	To pass, Accuracy % Error must be:
80 µL	≤ 2.0%	≤ 2.0%
20 µL	≤ 7.0%	≤ 5.0%
5 µL	≤ 10.0%	≤ 20.0%

Procedure for Models without Absorbance Capabilities

The Product Qualification package contains Gen5 protocols for use with this test. When running this procedure, you'll create experiments based on these protocols.

Cytation 5 Disp 1 Test No Read.prt and **Cytation 5 Disp 2 Test No Read.prt**

Each protocol contains three Dispense steps for dispensing three different volumes of the green dye test solution into a microplate.

Note: Despite the "No Read" text in the protocol names, the last step in both protocols is actually a brief Read step. This step is necessary because Gen5 requires a Read step with any Dispense procedure; however, *the resulting measurement value is not used*.

To avoid errors when running the experiments, examine the following default protocol parameters. If your Cytation 5 model does not support some of the parameters, change the Read step in both protocols so that it will work with your model. The Read step is defined as follows:

- Read well *A1* only
- Detection Method: *Luminescence (filter-based)*
- Filter Set: *Open*
- Read Height: *7.00 mm*
- Gain: *135*

Cytation 5 Disp 1 Test Other Reader.prt and **Cytation 5 Disp 2 Test Other Reader.prt**

These protocols contain the absorbance Read steps necessary for testing the dispense module, and they also contain Microsoft Excel spreadsheets for performing the results analysis.

These two protocols can be used only if your absorbance reader is a BioTek reader and is supported by Gen5. If you cannot use these protocols, prepare your reader to perform two reads with the following characteristics:

	80 µL Read	**20 & 5 µL Read**
Primary Wavelength	405 nm	630 nm
Reference Wavelength	750 nm	750 nm
Plate Columns	1-4	5-12

1. If you have not already done so, create the necessary Gen5 protocols; instructions begin on page 189.
2. Prime both dispensers with 4000 µL of deionized or distilled water.
3. Purge both dispensers with the Volume set to 2000 µL. This prevents the water from diluting the dye. Remove the inlet tubes from the supply bottles.
4. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
5. In Gen5, create an experiment based on **Cytation 5 Disp 1 Test No Read.prt**.
6. Place a new 96-well microplate on the balance and tare the balance.
7. Place the plate on the microplate carrier.

Important: Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

Note: Gen5 provides instructions for processing the plates; follow the steps carefully. When each dispense step is finished, you will weigh the plate, record the weight, tare the balance with the plate on it, and then place the plate back on the carrier for the next step.

8. Select **Plate > Read** and click **READ**. Gen5 prompts you to empty the tip priming trough.
9. When ready, click **OK** at the Load Plate dialog to begin the experiment. The sequence is as follows:
    a. Dispense 80 µL/well to columns 1–4.
    b. Remove the plate and weigh it. Record the weight and tare the balance.
    c. Place the plate on the carrier and dispense 20 µL/well to columns 5–8.
    d. Remove the plate and weigh it. Record the weight and tare the balance.
    e. Place the plate on the carrier and dispense 5 µL/well to columns 9–12.
    f. Remove the plate and weigh it. Record the weight.
    g. Manually pipette 150 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.
10. Close the experiment without saving it.

Note: If you are *not* using a BioTek reader for taking absorbance measurements, read the plate using the wavelengths shown in the table earlier, and then perform the Results Analysis as described on page 186.

Also, be sure to shake the plate after each dispense step to ensure the dye and water is mixed adequately.

11. If you are using a BioTek absorbance reader, configure Gen5 to communicate with the reader.
12. Create a Gen5 experiment based on the **Cytation 5 Disp 1 Test Other Reader** protocol.
13. Initiate a plate read. Place the plate on the carrier, and click **OK** at the Load Plate dialog. The protocol instructs the absorbance reader to:
    a. Perform the "80 µL" read at 405/750 nm.
    b. Perform the "20 and 5 µL" read at 630/750 nm.
14. When processing is complete, click **Plate > Export** to export the data to the embedded PowerExport spreadsheet.
15. Enter the 80 µL, 20 µL, and 5 µL dispense weights recorded. Results are calculated based on the dispense weights; each dispense volume should show "PASS" for Accuracy % Error and for %CV. See the calculation descriptions below and *Section 6, System Description* for troubleshooting tips.

16. Save the changes, and then print the spreadsheet. Sign the sheet and store it with your test records.
17. Close Excel, and then close the experiment.
18. Remove the plate from the carrier, and set it aside.
19. Repeat steps 5-17 using **Cytation 5 Disp 2 Test No Read.prt** and **Cytation 5 Disp 2 Test Other Reader**.
20. See instructions on analyzing the results on page 186.

Note: When all tests are complete, prime both dispensers with at least 5000 µL of deionized water to flush out the green dye solution.

Gen5 Test Protocols for Models with Absorbance Capabilities

1. Select **System > Instrument Configuration**, and add/configure the Cytation 5 (if it is not already there).
2. Create a new protocol.
3. Perform the steps in the following three sections to define the Procedure, customize the Plate Layout, and add Data Reduction steps, to test Dispenser #1.
4. When finished, select **File > Save As** and save the file as *Cytation 5 Disp 1 Test.prt*.
5. Repeat steps 2–4 above to create *Cytation 5 Disp 2 Test.prt* to test Dispenser 2.

Define the Procedure

In brief, the protocol's procedure follows the sequence below. After each Dispense step, the plate is ejected to allow the operator to weigh it and then tare the balance.

- Dispense 80 µL dye to columns 1–4
- Dispense 20 µL dye to columns 5–8
- Dispense 5 µL dye to columns 9–12
- Shake the plate for 15 seconds
- Read columns 1–4 at 405/750 nm and calculate the Delta OD
- Read columns 5–12 at 630/750 nm and calculate the Delta OD

The detailed procedure is described on the next page. To add a step to the procedure, click the appropriate button on the left side of the Procedure dialog and define the required parameters.

Note: The comments suggested for use with the Plate Out/In steps are optional, but they may be useful for the person running the test. When the Plate Out/In step is executed, Gen5 displays its comment in a message box.

Gen5 Procedure Steps		
#	**Step Type**	**Details**
1	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
2	Plate Out,In	Suggested comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
3	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A5..H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at rate 250 µL/sec
4	Plate Out,In	Suggested comment: Weigh the plate (20 uL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click OK to continue.
5	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A9..H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at rate 225 µL/sec
6	Plate Out,In	Suggested comment: Weigh the plate (5 uL test). RECORD the weight. PIPETTE 150 uL/well of DI water into all 12 columns. Place the plate back on the carrier. Click OK to perform the Read steps.
7	Shake	Linear at 567 cpm (3 mm) for 15 seconds
8	Read	Step label: "80 ul Read_Disp 1" (or _Disp 2)

Gen5 Procedure Steps		
#	**Step Type**	**Details**
		Wells: A1..H4 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 405 and 750 nm
9	Read	Step label: "20 and 5 ul Read_Disp 1" (or _Disp 2) Wells: A5..H12 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 630 and 750 nm

Customize the Plate Layout (Optional)

The results analysis worksheet at the end of this chapter requires the calculation of the Standard Deviation, Mean, and % CV of the ODs read for each dispense volume in each plate (six sets of calculations). By identifying the wells by their dispense volumes in the Plate Layout, Gen5 will calculate these values for you.

1. In the protocol, open the Plate Layout dialog.
2. Set the Type set to **Assay Control**, and define three control types: Disp_80, Disp_20, and Disp_5.
3. In the Plate Layout, select **Disp_80** and highlight wells **A1 to H4**.
4. Select **Disp_20** and highlight wells **A5 to H8**.
5. Select **Disp_5** and highlight wells **A9 to H12**.
6. Click **OK** to save the changes and close the dialog.

Note: After running the experiment, view the Statistics for each Delta OD Data Set to view the calculations

Add Data Reduction Steps

Each Read step is performed using two wavelengths, so you will create two data reduction steps to calculate the Delta OD values.

1. In the protocol, open the Data Reduction dialog and click **Custom**.
2. Click **Select multiple data sets** and then select **DS2**.
3. Set the Data In for DS1 to the 80 µL Read step at **405** nm.
4. Set the Data In for DS2 to the 80 µL Read step at **750** nm.
5. Click **OK** to return to the Transformation dialog.
6. In the New Data Set Name field, type an identifying name such as *Delta OD 80 ul_Disp 1*.
7. Clear **Use single formula for all wells**.
8. In the Current Formula field, type **DS1–DS2** and then highlight wells **A1 to H4** to assign the formula.
9. Click **OK** to add the transformation to the Data Reduction list.
10. Create another Transformation similar to the above, with these characteristics:
    - DS1 set to the 20 and 5 µL Read step at **630** nm
    - DS2 set to the 20 and 5 µL Read step at **750** nm
    - New Data Set Name resembling *Delta OD 20 and 5 uL_Disp 1*
    - Remember to clear **Use single formula for all wells**
    - Formula DS1–DS2 applied to wells A5 to H12
11. Click **OK** to close the Data Reduction dialog. When you are finished, the Data Reduction Steps list shows two Delta OD transformations.

Gen5 Test Protocols for Models without Absorbance Capabilities

The test procedure must dispense three volumes of fluid to a microplate and then read the plate on an absorbance reader. The procedure is performed twice, once for each dispenser. You will create two Gen5 protocols to perform the dispense steps. If you will use a BioTek absorbance reader that is supported by Gen5, you will create one additional protocol to perform the Read step.

Create the Dispense Protocols

Note: Perform these steps to create a protocol to test Dispense 1. Then, open a copy of the protocol and change the relevant Procedure parameters to Dispenser 2.

1. In Gen5, create a new protocol.
2. Define the Procedure with the steps and settings as described in the following table:

#	Step Type	Details
1	Dispense	Dispenser <select 1 or 2, depending on the

#	Step Type	Details
		protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
2	Plate Out,In	Suggested comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
3	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A5..H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at rate 250 µL/sec
4	Plate Out,In	Suggested comment: Weigh the plate (20 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click OK to continue.
5	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A9..H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at rate 225 µL/sec
6	Plate Out,In	Suggested comment: Weigh the plate (5 uL test). RECORD the weight. Set the plate aside and click OK.
7	Read	Wells: A1 Detection Method: <select any valid method> Read Type: Endpoint Read Speed: Normal

#	Step Type	Details
		Wavelength: <select any valid wavelength (s)>
The Read step is necessary because Gen5 requires a Read step within any Dispense procedure. When the test is run, the measurement value is not used.		

3. Save the protocols as *Cytation 5 Disp 1 Test.prt* and *Cytation 5 Disp 2 Test.prt*.

Create the Read Protocol (if needed)

1. In Gen5, create a new protocol for the BioTek reader.
2. Define the Procedure with the steps and settings as described in the following table:

#	Step Type	Details
1	Read	Step Label: "80 ul Read" Wells: A1..H4 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 405 nm and 750 nm
2	Read	Step Label: "20 and 5 ul Read" Wells: A5..H12 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 630 nm and 750 nm

3. Create Data Reduction steps to calculate Delta OD values:
   - Select **Protocol > Data Reduction** and select *Custom*.
   - Within this dialog, click **Select Multiple Data Sets** and then click **DS2**.
     - Set the Data In for DS1 to the 80 ul Read step at **405** nm.
     - Set the Data In for DS2 to the 80 ul Read step at **750** nm.
   - Click **OK** to return to the dialog.
   - In the New Data Set Name field, type an identifying name such as "**Delta OD 80 ul_Disp 1**."
   - Clear **Use single formula for all wells**.

- In the Current Formula field, type **DS1-DS2** and then assign the formula to wells **A1 to H4**.
- Click **OK** to add the transformation to the Data Reduction list.
- Create another Transforming similar to above, with these characteristics:
  - DS1 set to the 20 and 5 ul Read step at **630** nm
  - DS2 set to the 20 and 5 ul Read step at **750** nm
  - New Data Set Name resembling "**Delta OD 20 and 5 ul_Dispense (#)**"
  - Formula **DS1-DS2** applied to wells **A5 to H12**

4. The results analysis worksheet at the end of this chapter requires the calculation of the Standard Deviation, Mean, and % CV of the ODs read for each dispense volume in each plate (six sets of calculations). By identifying the wells by their dispense volumes in the Plate Layout, Gen5 will calculate these values for you.
   - Open the Plate Layout dialog.
   - Set the Type set to **Assay Control**, and define three control types: Disp_80, Disp_20, and Disp_5.
   - In the Plate Layout, select **Disp_80** and highlight wells **A1 to H4**.
   - Select **Disp_20** and highlight wells **A5 to H8**.
   - Select **Disp_5** and highlight wells **A9 to H12**.
   - Save the protocol as *Cytation 5 Disp Test Other Reader.prt*.

Note: After running the experiment, view the Statistics for each Delta OD Data Set to view the calculations.

Dispense Accuracy & Precision Tests — Dispenser #____

	80 µL Dispense Delta ODs @405/750 nm			
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

80 µL weight: _____ g

Expected weight: 2.5600 g

Accuracy % Error: _____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 2.0% ☐ P ☐ F

	20 µL Dispense Delta ODs @630/750 nm			
	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: _____ g

Expected weight: 0.6400 g

Accuracy % Error: _____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 7.0% ☐ P ☐ F

	5 µL Dispense Delta ODs @630/750 nm				
	9	10	11	12	
A					A
B					B
C					C
D					D
E					E
F					F
G					G
H					H

5 µL weight: _____ g

Expected weight: 0.1600 g

Accuracy % Error: _____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/
Approved By: _____

Signature: _____

Dispense Accuracy & Precision Tests — Dispenser #____

	80 µL Dispense Delta ODs @405/750 nm			
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

80 µL weight: _____ g

Expected weight: 2.5600 g

Accuracy % Error: _____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 2.0% ☐ P ☐ F

	20 µL Dispense Delta ODs @630/750 nm			
	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: _____ g

Expected weight: 0.6400 g

Accuracy % Error: _____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 7.0% ☐ P ☐ F

	5 µL Dispense Delta ODs @630/750 nm				
	9	10	11	12	
A					A
B					B
C					C
D					D
E					E
F					F
G					G
H					H

5 µL weight: _____ g

Expected weight: 0.1600 g

Accuracy % Error: _____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/
Approved By: _____

Signature: _____

Appendix A

Specifications

This appendix contains BioTek's published specifications for the Cytation 5. Note: Unless otherwise stated, all specifications are based on normal plate-carrier speed, not "slower carrier speed."

General Specifications

Microplates/Labware
The Cytation 5 accommodates standard 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with 128 x 86 mm geometry, (1536-well plates for imaging only), Take3 and Take3 Trio Micro-Volume Plates, microplate slides, T25 cell culture flasks (with adapter), and 35 mm, 60 mm, and 100 mm Petri dishes (with adapter). Maximum Plate Height: 25.4 mm (1.0").

Hardware and Environmental	
Light Source	
Absorbance, Fluorescence (monochromator)	Xenon flash light source, 20W maximum average power
Fluorescence (FI/FP), filter-based:	Xenon flash light source, 5W maximum average power
TRF (filter-based):	Xenon flash light source, 5W maximum average power
Dimensions:	20.25" D x 15.50" W x 17.5" H (51.4 cm D x 39.4 cm W x 44.5 cm H)
Weight:	With all modules installed, without power supply or dispense module, < 80 lbs. (36.3 kg)
Environment:	Operational temperature 18ºC to 30ºC (64.4ºF to 86ºF) *Note:* Performance measurements, including detection limits, were verified up to 25ºC (77ºF). Storage temperature -25ºC to 50ºC (-13ºF to 122ºF)
Humidity:	Operational: 10% to 85% relative humidity (non-condensing) Storage: 10% to 80% relative humidity (non-condensing)
Power:	The instrument is powered from an external 250W (minimum), 24VDC power supply compatible with 100-240 volts AC @50-60Hz.

Hardware and Environmental	
Incubation:	Temperature control ranges from 4ºC over ambient to 65ºC Temperature variation ± 0.5ºC across the plate @ 37ºC, tested with Innovation Instruments, Inc. temperature test plate. Top and bottom incubation controlled via software-adjustable gradient. *For models with the alpha laser module:* Alpha laser module operation is disabled above an internal instrument temperature of 35º C.

Dispense/Read Specifications

Dispense/Read, for models with the dual-reagent dispense module	
Plate Type	Injectors dispense to standard height 6-, 12-, 24-, 48-, 96-, and 384-well microplates.
Detection Method	Absorbance, Fluorescence (FI, FP, TRF), Luminescence, Imaging (well mode only)
Volume Range	5–1000 µL with a 5–20 µL tip prime
Reagent Dead Volume	< 1100 µL, with dead volume recovery function (purge)
Injection Speeds	225, 250, 275, 300 µL/second
Accuracy	± 1 µL or 2.0%, whichever is greater
Precision	≤ 2.0% for volumes of 50–200 µL ≤ 4.0% for volumes of 25–49 µL ≤ 7.0% for volumes of 10–24 µL ≤ 10.0% for volumes of 5–9 µL

Maximum Delay between End of Dispense and Beginning of Read 96/384-well plates, default probe heights (using normal plate-carrier speed)	
Bottom Mono Fluorescence	T ≤ 0.5 second
Luminescence	T ≤ 0.5 second
Top Filter Fluorescence	T ≤ 1.0 second
Top Mono Fluorescence	T ≤ 1.0 second
Absorbance	T ≤ 1.0 second

Absorbance Specifications

Note: For the performance specifications described in this section, the gain on the optics test should be <= 8.

Optics	
Wavelength Range	230 to 999 nm
Wavelength Bandpass	< 4 nm (230–285 nm), < 8 nm (> 285 nm)
Measurement Range	0.000 to 4.000 OD
Resolution	0.0001 OD
Increment	1 nm
Wavelength Accuracy	± 2 nm
Wavelength Precision	± 0.2 nm
Minimum kinetic interval (450 nm)	≤ 20 seconds, sweep mode, 96-well microplate

Plate In/Plate Out Speed
≤ 35 seconds, 450 nm, sweep mode, 96-well microplate

Accuracy, Linearity, Repeatability
Specifications apply from 250–999 nm, 200 µL (96-well microplates)
Accuracy (tested with certified neutral density glass)
96-well plate, normal read speed 0–2.0 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms 2.0–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 100 ms
384-well plate, normal read speed 0–2 OD: +/-2% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1.0 OD: +/-1% +/-0.010 OD
Linearity (by liquid dilution)
96-well plate, normal read speed

Accuracy, Linearity, Repeatability
0–2.0 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms 2.0–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 100 ms
384-well plate, normal read speed 0–2.0 OD: +/-2% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1.0 OD: +/-1% +/-0.010 OD
Repeatability (tested with certified neutral density glass/measured by one standard deviation: 8 measurements per data point)
96-well and 384-well plate, normal read speed 0–2.0 OD: +/-1% +/-0.005 OD, delay after plate movement = 100 ms 2.0–2.5 OD: +/-3% +/-0.005 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1.0 OD: +/-2% +/-0.010 OD

Take3 Plate
260 nm dsDNA Detection Limit: 5 ng/µL

Fluorescence Specifications (Mono-Based)

The Cytation 5 measures fluorescence with monochromators from the top and bottom of 6- to 384-well plates.

Monochromator-Based Fluorescence	
Excitation range	250–700 with low-noise PMT 250–900 nm with red-shifted PMT
Emission range	250–700 nm with low-noise PMT 300–700 nm for emission scans with low-noise PMT 250–900 nm with red-shifted PMT 300–900 nm for emission scans with red-shifted PMT
Selectable increment	1 nm
Bandpass	Variable from 9 nm to 50 nm in 1 nm increments (both excitation and emission)
Minimum kinetic interval	< 20 seconds, sweep mode, 96-well microplate

Plate In/Plate Out Speed
≤ 35 seconds, sweep mode, 96-well microplate

Sensitivity
Sodium Fluorescein in phosphate buffered saline (PBS) DL ≤ 20 pM top or bottom read, 5 pM typical Excitation 485 nm, Emission 528 nm
Methylumbelliferone (MUB) in carbonate-bicarbonate buffer (CBB) DL ≤ 0.16 ng/mL (0.91 nM) top read Excitation 360 nm, Emission 460 nm
Propidium Iodide (PI) in PBS DL ≤ 62.5 ng/mL bottom read

Sensitivity
Excitation 485 nm, Emission 645 nm

Fluorescence Specifications (Filter-Based)

The Cytation 5 measures fluorescence with filters from the top of 6- to 384-well plates.

Plate In/Plate Out Speed
≤ 35 seconds for filter set, sweep mode, 96-well microplate

Fluorescence Intensity
DL ≤ 10 pM solution of Sodium Fluorescein in PBS Excitation 485/20, Emission 528/20, 510 nm mirror
DL ≤ 0.16 ng/mL (0.91 nM) solution of Methylumbelliferone in CBB, Excitation 360/40, Emission 460/40, 400 nm mirror

Time-Resolved Fluorescence	
DL Europium ≤ 250 fM Excitation 360/40 nm, Emission 620/40 nm, 400 nm mirror	
Integration time	20 to 2000 µs
Delay	0 to 2000 µs
Granularity	1-µs step

Fluorescence Polarization
5 mP standard deviation at 1 nM Sodium Fluorescein Excitation 485/20 nm, Emission 528/20 nm, 510 nm mirror Excitation range: 400 to 700 nm Emission range: 400 to 700 nm

Luminescence Specifications

The Cytation 5 measures luminescence from the top of 6- to 384-well plates. The following requirements apply to 96-well plates with 200 µL/well, at room temperature. Production testing is performed using a Harta plate.

Luminescence	
DL	≤ 75 amol/well, 30 amol typical with low-noise PMT ≤ 500 amol/well with red-shifted PMT
Integration Time	10 seconds
Gain	150
Blank Wells	16

Imaging Specifications

Imaging specifications based on using an NIH 3T3 plate: 10,000 cells per well, GFP stain, 1 image per well, 96-well Costar 3603 black-sided, plastic-bottom plate.

Read Speed	
At 20X using standard Autofocus	< 10 minutes with normal plate-carrier speed; < 15 minutes with “slower carrier speed”

Appendix B

Error Codes

This appendix lists and describes Cytation 5 error codes that may appear in Gen5.

Overview

When a problem occurs during operation with the Cytation 5, an error code appears in Gen5. Error codes typically contain four characters, such as "5A01," and in most cases are accompanied by descriptive text, such as "**Plate carrier hit obstruction and lost steps**." With many errors, the instrument will beep repeatedly; press the carrier button to stop this alarm.

Some problems can be solved easily, such as "2B0A: Priming plate not detected" (place a priming plate on the carrier). Other problems can be solved only by BioTek service personnel. This appendix lists the most common and easily resolved error codes that you may encounter.

Note: Error codes beginning with "A" (e.g., A100) indicate conditions that require immediate attention. If this type of code appears, turn the instrument off and on. If the System Test is not successful, record the error code and contact Technical Support.

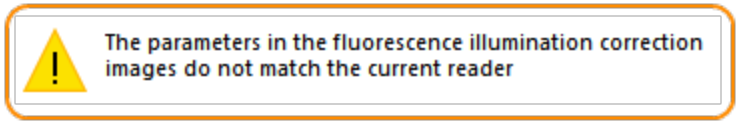
If an error code appears in Gen5, you may want to run a System Test for diagnostic purposes. In Gen5, select **System > Diagnostics > Run System Test**.

Note: If an error message appears while an experiment is in process and after having received measurement data, it is your responsibility to determine if the data is valid.

Use this appendix to diagnose problems and solve them if possible. If you need further assistance, contact Technical Support. See Contact Information on page viii.

Note: For errors that are displayed during operation of the Cytation 5 with the BioStack, refer to the BioStack User manual.

Cytation 5 "W" Models: FL Illumination Correction



Objective Setup Plate (PN 1852501) is required

"W" models with their WFOV cameras require "fluorescent illumination correction" files that are created and stored on the controller or host computer during initial instrument calibration. If another computer is used to control the reader, the correction files must be recaptured:

1. In Gen5, select **System>Instrument Configuration>Setup>Imaging Configuration**.

2. Click **FL Illumination Correction** and follow prompts to put the objective setup plate on the carrier.

You do not need to rerun Auto Calibration unless you are also changing objectives or LED-Filter cubes.

Error Codes

This table lists the most common and easily resolved error codes that you may encounter. If an error code appears in Gen5, look for it here. If you find the code, follow the suggestions provided for solving the problem. If you cannot find the code or if you are unable to solve the problem, please contact Technical Support. The Gen5 Help system also provides troubleshooting tips.

Code	Description and Possible Remedy
2353	*Filter block not found on filter/mirror slide* Verify that the filter block is correctly installed and that it matches the Gen5 optics library.
2B0x	*Dispenser syringe 1 or 2 (respectively) did not home* x=1–3 Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. (Refer to the *Installation* chapter for instructions.) Restart the instrument.
2B0A	**Priming plate not detected** Place priming plate on the carrier.
2B04	Dispenser syringe 1 or 2 (respectively) failed position verify. Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. Restart the reader. (Refer to the *Installation* chapter for instructions.)
2C02-03	Dispense tips mismatch: dimensions of defined dispense tips do not match the installed tips. Check the "Instrument Configuration" to ensure the correct tips were selected.
2D4E	Gas is not on. Check the connections and status of the gas controller. Make sure it is turned on and the tubing is properly connected.
2D0A	Tip priming cup is full. Empty the priming cup.
2D4F	Environmental cover is open, and must be closed for the current operation. Close the cover and do not open it until the session is ended.
2D50	Incompatible stage insert. Only the "Humidity" or environmental-chamber stage insert supports gas recharging. If attempting to incubate, install the top cover.

Code	Description and Possible Remedy
2D51	Gas recharge in progress. Wait until the gas recharge step is complete.
2D52	Fan test failure. Contact Technical Support.
37x0/47x0 38x0/48x0 39xy/49xy	*Noise Test Errors* *Offset Test Errors* *Dark Range Errors* x=0, 1; y=0–6 This series of System Test errors may indicate too much light inside the chamber. Make sure the plate carrier door and the top hinged door are properly closed. For models with the dispense module, if the dispense tubes are not connected to the reader, re-install the light shield that shipped with the instrument (or cover the hole with black tape). Restart the reader.
4xxx	*PMT overload well error at <well #xxx>* This error typically means that the fluid in a well has oversaturated the PMT (i.e., the well is too bright). Try lowering the sensitivity value in the read step. *To identify the well:* Wells are counted starting at A1, moving left-to-right, row-by-row. The row and column of the well can be extracted from the well number code by applying the following formula (example uses 8 x 12 geometry, 96-well plate): 1. Convert the ASCII hex string to a decimal equivalent. Ex: "057" indicates 57 hex, yielding a well code of 87 decimal. 2. Row = (well code) / (columns in plate), rounded up to a whole number. Ex: 87/12 = 7.25, indicating row 8 (or H). 3. Column = (well code) - ((row-1) * (columns in plate)). Ex: 87 - ((8 - 1) * 12) = column 3. NOTE: If this code is returned during an area scan, it indicates the scan point corresponding to the row/column equivalent in the currently defined scan map, *not* the actual well where the error occurred.
4Exy	*Detector saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).* x=0, 1; y=0–6 This error can indicate one of several scenarios. It is possibly due to incorrect chemistry, e.g., the fluorescence standards dispensed to the plate exceed expectations. Try lowering the gain in your Read step(s). For models with the dispense module, the internal chamber may require cleaning (contact Technical Support). If a *4E18* error is detected during monochromator-based fluorescence, the

Code	Description and Possible Remedy
	luminescence probe may be picking up stray light. Try installing a plug in the filter cube. Restart the reader.
4Fxy	**Fluorescence signal out of range** x=0,1; y=0–6 Verify that the Gen5 Fluor/Lum wavelengths table matches the actual filter installed in the filter cube. Verify that there is no filter wavelength overlap between the emission/excitation positions. Verify that the microplate door is fully closing and the instrument cover is properly installed and sealed. Try lowing the gain in your Read step(s). The reading chamber may be contaminated by a spill that is fluorescing; see the Periodic Maintenance chapter.
5003 5103	**Filter cube did not home** Generally, this error indicates the filter cube is not seated properly in the reader. Remove it, ensure each filter or plug is properly positioned, and reinstall it securely. Restart the reader.
5403	**Filter cube failed positional verify** Generally, this error indicates the filter cube is not seated properly in the reader. Remove it, ensure each filter or plug is properly positioned, and reinstall it securely. Restart the reader.
55xy	**<Motor> not homed successfully** xy=axis This error indicates that an axis failed a previous verify function and now needs to be homed. Verify that the shipping brackets have been removed. Check for any obstructions that may prevent the carrier, syringes, or filter cube and objective turret from moving normally. Restart the instrument.
570x	**Axis obstruction error** This error indicates that a moving part is being obstructed. Verify that: • the tip priming trough, microplate, plate lid, or other object has not become dislodged in the reading chamber. • the Plate Type selection in the Gen5 procedure is correct for the plate in use, and the Plate Height measurement is correct. • the filter cube is correctly installed. • nothing is preventing the dispenser syringes from moving. For some plate type and read probe combinations, it might not be possible to define the entire area scan matrix offered by Gen5 for some perimeter wells due to the physical limitations of carrier travel. Redefine the area scan to

Code	Description and Possible Remedy
	include a small matrix, or select wells in a different row or column.
500C 520C 550C	**Imager LED/Filter Slide error** Verify that the filter slide shipping bracket is removed and the slide moves freely when the instrument is powered off. An object may be obstructing the path.
500D 520D 540D 550D 570D	**Imager Objective Changer error** Verify that the objective turret shipping bracket is removed and the turret rotates freely when the instrument is powered off. An object may be obstructing the path. Ensure that the installed objective matches the objective configuration. If the objective installed is too tall, it may contact the bottom of the incubator. Verify that the z-axis motor is moving and that the objective is not binding on the incubator.
500E 520E 540E 550E	**Z-Axis error** Verify that the carrier shipping bracket is removed. Axis did not home successfully.
5A0**x**[1]	**Plate carrier hit obstruction and lost steps** x=0, 1 Verify that the microplate is properly and securely seated in the carrier and nothing is obstructing carrier movement inside the reading chamber. Make sure the Plate Type defined in the Gen5 Protocol matches the plate you are using. This error can also occur if the plate type is correct but the lid was left on the plate. If you wish to read the plate with a lid on it, update the plate type record in Gen5 with the correct lid dimensions and Plate Height.
5B00	*Plate carrier needs to be ejected from the reading chamber* The carrier is inside the read chamber and the probe needs to move down for the requested operation. Press the carrier eject button. This may occur if the read was aborted and "home all axes" was not performed. This error can also occur if the carrier is inside and the newly defined plate height is different from the most recently specified plate height. To resolve the error, eject the carrier prior to running the experiment.
7A0C	**Side/Access door interlock signal deactivated while LED is on or during an attempt to turn on the LED**

[1]The last 2 digits in the error code series refer to these components: 00 = carrier X axis, 01 = carrier Y axis, 02 = camera Z axis (focus), 03 = LED-Filter slider, 04 = objective turret, 05 = Brightfield paddle.

Code	Description and Possible Remedy
	Verify the access/side door is closed. Verify the magnet has not become dislodged from the door.
7A0D	**LED intensity at reference diode measures more than 5% below the specified target** Run a System Test to see if the error is generated again. Check to see if the small aperture hole on the top of the imaging filter cube is blocked. Ensure the LED cube and imaging filter cube are present, plugged in, or not defective. Verify the imaging filters and are clean and match the configuration defined in Gen5.
7A0E	**LED intensity at reference diode measures more than 5% above the specified target** Run a System Test to see if the error is generated again. Ensure that the LED cube and imaging filter cube are not defective. Verify that the imaging filters are not delaminating or do not match the configuration defined in Gen5.
7A0F	**Current DAC adjusted to more than double the starting value within the first 20 control cycles** This error can be generated if a System Test is not run after sending new values to the instrument for imager configuration. Run a System Test to clear the error. Verify that the imaging filter cube and fluorescence filter cube configuration is correct. Ensure that the LED cube and imaging filter cube are not defective.
7A10	**With current DAC adjusted to maximum output, LED intensity at reference diode measures more than 5% below the specified target** Run a System Test to see if the error is generated again. Check to see if the small aperture hole on the top of the imaging filter cube is blocked. Ensure the LED cubes and imaging filter cubes are present, plugged in, or not defective. Verify the imaging filters are clean and match the configuration defined in Gen5.
7A85	**LED gain calibration signal is out of range** This error occurs only during the System Test. Ensure the imaging filter cubes and fluorescence filter cube are present and **clean**. Dust or finger prints can scatter light in unexpected ways and cause this error.

Code	Description and Possible Remedy
	Check the wire clip between the LED cube and the instrument's PCB to ensure it is not loose or defective. Verify that the light aperture on the top of the imaging filter cube is not blocked. Ensure that the LED cube and imaging filter cube are not defective.
7A86	**LED control timeout** The LED output setpoint was not reached within 20 msec after turn on. Verify the LED cube and imaging filter cube settings. The XML file must match the LED cube and imaging filter cube contents. Run a System Test to reset LED gain calibrations.
7A87	**LED-on current feedback is out of range (low)** Ensure the LED cube is present, plugged in, or not defective. Run a System Test to see if the error is generated again.
7A88	**LED-on current feedback is out of range (high)** Run a System Test to see if the error is generated again.
7A89	**User-initiated positional calibration needed** Run imager objective calibration in Gen5 setup.
7A8A	**Carrier calibration needed** Run imager objective calibration in Gen5 setup.
7A85	**LED calibration signal is out of range** Ensure the imaging filter cubes and fluorescence filter cube are present and **clean**. Dust or finger prints can scatter light in unexpected ways and cause this error. Verify that the light aperture on the top of the imaging filter cube is not blocked.

Appendix C

Safety Information

Veiligheidsmededelingen
Avis de sécurité
Sicherheitshinweise
Avvisi di sicurezza
Avisos de seguridad

This appendix contains safety information for the Cytation 5, translated into Dutch, French, German, Italian, and Spanish.

Safety Notices

Veiligheidsmededelingen

Avis de sécurité

Sicherheitshinweise

Avvisi di sicurezza

Avisos de seguridad

Pay special attention to the following safety notices in all product documentation.

Let vooral op de volgende veiligheidsmededelingen in alle productdocumentatie.

Portez une attention particulière aux avis de sécurité suivants dans l'ensemble de la documentation du produit.

Achten Sie besonders auf die folgenden Sicherheitshinweise in allen Produktdokumentationen.

Prestare particolare attenzione agli avvisi di sicurezza presenti in tutta la documentazione del prodotto.

Preste especial atención a los siguientes avisos de seguridad en toda la documentación del producto.

WARNING A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.
De aanduiding WAARSCHUWING duidt op een gevaar. Deze vestigt de aandacht op een bedieningsprocedure, praktijk of iets dergelijks die, indien niet correct uitgevoerd of nageleefd, persoonlijk letsel of de dood tot gevolg kan hebben. Ga niet verder bij een aanduiding WAARSCHUWING voordat de aangegeven voorwaarden volledig begrepen zijn en eraan voldaan is.
Un AVERTISSEMENT signale un danger. Il attire l'attention sur une procédure d'utilisation, une pratique ou autre qui, si elle n'est pas correctement exécutée ou respectée, peut entraîner des dommages corporels, voire un décès. Ne passez pas outre l'AVERTISSEMENT uniquement si les conditions indiquées sont entièrement comprises et remplies.
Ein WARNHINWEIS weist auf eine Gefahr hin. Er weist auf ein Betriebsverfahren, eine Vorgehensweise oder ähnliches hin, deren falsche Ausführung oder Nichtbeachtung zu Verletzungen oder zum Tod führen können. Fahren Sie bei einem WARNHINWEIS erst dann mit Ihrer Arbeit fort, wenn die angegebenen Bedingungen vollständig verstanden und erfüllt sind.
Un avviso di AVVERTENZA indica un pericolo. Richiama l'attenzione su procedure operative, pratiche o azioni simili che, se non rispettate o eseguite correttamente, potrebbero causare lesioni personali o decesso.

Non procedere ignorando un avviso di AVVERTENZA fino a quando le condizioni indicate non sono state completamente comprese e soddisfatte.
Un aviso de ADVERTENCIA indica un peligro. Destaca la importancia de un procedimiento operativo, una práctica o un proceso similar que, si no se realiza o se sigue correctamente, podría provocar lesiones o la muerte. No siga adelante sin antes comprender y cumplir plenamente los requisitos indicados en el aviso de ADVERTENCIA.

CAUTION

A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.
De aanduiding VOORZICHTIG duidt op een gevaar. Deze vestigt de aandacht op een bedieningsprocedure, praktijk of iets dergelijks die, indien niet correct uitgevoerd of nageleefd, schade aan het product of verlies van belangrijke gegevens tot gevolg kan hebben. Ga niet verder bij een aanduiding VOORZICHTIG voordat de aangegeven voorwaarden volledig begrepen zijn en eraan voldaan is.
Une MISE EN GARDE signale un danger. Elle attire l'attention sur une procédure d'utilisation, une pratique ou autre qui, si elle n'est pas correctement exécutée ou respectée, peut endommager le produit ou entraîner la perte de données importantes. Ne passez pas outre la MISE EN GARDE uniquement si les conditions indiquées sont entièrement comprises et remplies.
Ein VORSICHTSHINWEIS weist auf eine Gefahr hin. Er weist auf ein Betriebsverfahren, eine Vorgehensweise oder ähnliches hin, deren falsche Ausführung oder Nichtbeachtung zu einer Beschädigung des Produkts oder zum Verlust wichtiger Daten führen kann. Fahren Sie bei einem VORSICHTSHINWEIS erst dann mit Ihrer Arbeit fort, wenn die angegebenen Bedingungen vollständig verstanden und erfüllt sind.
Un avviso di ATTENZIONE indica un pericolo. Richiama l'attenzione su procedure operative, pratiche o azioni simili che, se non rispettate o eseguite correttamente, potrebbero causare danni al prodotto o perdita di dati importanti. Non procedere ignorando un avviso di ATTENZIONE fino a quando le condizioni indicate non sono state completamente comprese e soddisfatte.
Un aviso de PRECAUCIÓN indica un peligro. Destaca la importancia de un procedimiento operativo, una práctica o un proceso similar que, si no se realiza o no se sigue correctamente, podrían provocar daños en el producto o la pérdida de datos importantes. No siga adelante sin antes comprender y cumplir plenamente los requisitos indicados en el aviso de PRECAUCIÓN.

Warnings and Precautions

Electrical Hazards

Elektrische gevaren

Risques électriques

Elektrische Gefahren

Rischi elettrici

Peligros eléctricos

WARNING **Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.
Interne spanning. Zet altijd de stroomschakelaar uit en haal de stekker uit het stopcontact voordat de buitenkant van het instrument wordt gereinigd.
Tension interne. Désactivez toujours l'interrupteur d'alimentation électrique et débranchez l'alimentation avant de nettoyer la surface extérieure de l'instrument.
Spannung im Geräteinneren. Vor dem Reinigen der Außenfläche des Geräts grundsätzlich den Stromschalter ausschalten und das Stromkabel aus der Steckdose ziehen.
Tensione interna. Spegnere sempre l'interruttore dell'alimentazione e scollegare l'alimentazione prima di pulire le superfici esterne dello strumento.
Tensión interna. Siempre apague el interruptor y desconecte la fuente de alimentación antes de limpiar la superficie exterior del instrumento.

WARNING **Power Rating.** The instrument’s power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.
Vermogensklasse. De voeding of het netsnoer van het instrument moet worden aangesloten op een stopcontact dat spanning en stroom levert binnen de gespecificeerde nominale waarden voor het systeem. Gebruik van een niet-compatibel stopcontact kan leiden tot elektrische schokken en brandgevaar.
Puissance électrique nominale. L'alimentation ou le cordon d'alimentation de l'instrument doit être raccordé(e) à une prise de courant qui fournit la tension et le courant correspondants à la puissance spécifiée du système. L'emploi d'une prise de courant incompatible peut entraîner un choc électrique et un risque d'incendie.
Leistungsbemessung. Die Stromversorgung des Geräts bzw. das Anschlusskabel muss mit einer Steckdose verbunden werden, deren Spannungs- und Stromwerte innerhalb der für das System vorgeschriebenen Nennwerte liegen. Die Verwendung einer nicht kompatiblen Steckdose kann zu einem elektrischen Schlag und

Brandgefahr führen.
Potenza nominale. L'alimentazione o il cavo di alimentazione dello strumento devono essere collegati a una presa di corrente che fornisca tensione e corrente comprese entro il valore nominale previsto per il sistema. L'uso di una presa di alimentazione non compatibile può causare scosse elettriche e rischi di incendio.
Potencia nominal. La fuente de alimentación o el cable de alimentación del instrumento tienen que conectarse a un receptáculo que suministre tensión y corriente dentro de la potencia especificada para el sistema. El uso de un receptáculo incompatible puede producir descargas eléctricas y riesgo de incendio.

WARNING

Electrical Grounding. Never use a plug adapter to connect primary power to the external power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.
Elektrische aarding. Gebruik nooit een stekkeradapter om de primaire stroom aan te sluiten op de externe voeding. Het gebruik van een adapter verbreekt de verbinding met de aarding van het elektriciteitsnet, waardoor een ernstige schok kan ontstaan. Sluit het netsnoer altijd rechtstreeks aan op een geschikt stopcontact met werkende aarding.
Mise à la terre électrique. N'utilisez jamais d'adaptateur de prise pour raccorder l'alimentation principale à l'alimentation électrique extérieure. L'utilisation d'un adaptateur déconnecte la terre du secteur, créant un risque important de choc. Raccordez toujours le cordon d'alimentation directement à une prise appropriée dotée d'une mise à la terre fonctionnelle.
Elektrische Erdung. Verwenden Sie niemals einen Steckeradapter zum Anschließen der Primärstromversorgung an die externe Stromversorgung. Bei Verwendung eines Adapters wird die Verbindung zur Gebäudeerde unterbrochen, sodass ein erhebliches Stromschlagrisiko besteht. Das Stromkabel ist immer direkt an eine geeignete Steckdose mit Funktionserdung anzuschließen.
Messa a terra elettrica. Non usare mai un adattatore per collegare l'alimentazione principale all'alimentazione esterna. Se si usa un adattatore, si scollega la messa a terra della rete elettrica creando un grave pericolo di scosse elettriche. Collegare sempre il cavo di alimentazione direttamente a una presa idonea dotata di messa a terra funzionale.
Conexión a tierra. Nunca use un adaptador de enchufe para conectar la corriente principal a la fuente de alimentación externa. El uso de un adaptador desconecta la tierra del servicio y crea un riesgo de descarga grave. Conecte siempre el cable de alimentación directamente a un receptáculo adecuado con una toma de tierra funcional.

WARNING

Service. Only qualified technical personnel should perform service procedures on internal components.
Service. Alleen gekwalificeerd technisch personeel mag

serviceprocedures aan interne onderdelen uitvoeren.
Entretien. L'exécution des procédures d'entretien des composants internes doit être réservée au personnel technique qualifié.
Wartung. Wartungsarbeiten an Komponenten im Geräteinneren sollten nur von qualifizierten Servicetechnikern durchgeführt werden.
Manutenzione. Le procedure di manutenzione sui componenti interni devono essere eseguite esclusivamente da personale tecnico qualificato.
Revisión. Solo puede realizar procedimientos de revisión de los componentes internos el personal técnico cualificado.

CAUTION

Power Supply. Use only the power supply shipped with the instrument, and operate it within the range of line voltages listed on it.
Voeding. Gebruik alleen de voeding die bij het instrument is geleverd en gebruik deze binnen het bereik van de netspanningen die op de voeding staan vermeld.
Alimentation électrique. Utilisez exclusivement l'alimentation électrique fournie avec l'instrument dans la plage de tension de ligne indiquée dessus.
Stromversorgung. Verwenden Sie nur die im Lieferumfang des Geräts enthaltene Stromversorgung und betreiben Sie diese innerhalb des darauf angegebenen Netzspannungsbereichs.
Alimentazione. Usare esclusivamente l'alimentatore fornito con lo strumento, utilizzando quest'ultimo entro l'intervallo delle tensioni di linea indicato sull'unità.
Fuente de alimentación. Use únicamente la fuente de alimentación incluida con el instrumento y úsela en el rango de tensiones de línea indicado en ella.

Chemical/Environmental

Chemisch/Milieu

Substances chimiques/Environnement

Chemie/Umwelt

Rischi chimici/ambientali

Riesgos químicos y medioambientales

WARNING

Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemical-resistant rubber gloves and apron.

Potentiële biologische gevaren. Sommige tests of specimens kunnen een biologisch gevaar inhouden. Er moeten adequate veiligheidsmaatregelen worden getroffen zoals aangegeven in de bijsluiter van de test. Draag altijd een veiligheidsbril en geschikte beschermingsmiddelen, zoals chemicaliënbestendige rubberen handschoenen en een schort.

Risques biologiques potentiels. Certains tests ou échantillons peuvent présenter un risque biologique. Des précautions de sécurité adéquates doivent être prises, comme indiqué dans la notice de l'emballage du test. Portez toujours des lunettes de sécurité et un équipement de protection approprié, comme des gants en caoutchouc résistant aux substances chimiques et un tablier.

Potenzielle Biogefahren. Manche Assays oder Proben stellen eine Biogefahr dar. Es sollten angemessene Sicherheitsvorkehrungen entsprechend der Packungsbeilage des Assays ergriffen werden. Tragen Sie immer eine Schutzbrille und eine geeignete Schutzausrüstung, wie chemikalienbeständige Gummihandschuhe und Schürze.

Potenziali rischi biologici. Alcuni test o campioni potrebbero comportare un rischio biologico. Implementare misure di sicurezza adeguate secondo quanto delineato nel foglietto della confezione del test. Indossare sempre occhiali di sicurezza e dispositivi di protezione appropriati, ad esempio guanti e grembiule in gomma resistenti alle sostanze chimiche.

Riesgos biológicos potenciales. Algunos ensayos y especímenes pueden constituir un riesgo biológico. Se han de tomar precauciones de seguridad suficientes tal como se indica en el folleto del paquete del ensayo. Use siempre gafas de seguridad y equipos protectores adecuados, como guantes de caucho resistentes a productos químicos y un delantal.

WARNING

Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, stop the program and turn off the instrument. Wipe up all spills immediately. Do not

operate the instrument if internal components have been exposed to fluid.

Vloeistoffen. Voorkom dat vloeistoffen op het instrument worden gemorst; het doorsijpelen van vloeistoffen in interne onderdelen kan leiden tot schokgevaar of beschadiging van het instrument. Als een lekkage optreedt terwijl een programma loopt, stopt u het programma en schakelt u het instrument uit. Veeg alle gemorste vloeistof onmiddellijk op. Gebruik het instrument niet als interne onderdelen aan vloeistof zijn blootgesteld.

Liquides. Évitez de renverser des liquides sur l'instrument ; les infiltrations de liquide dans les composants internes créent un risque potentiel de choc ou de détérioration de l'instrument. En cas de déversement de liquide alors qu'un programme est en cours d'exécution, arrêtez le programme et mettez l'instrument hors tension. Essuyez immédiatement tout liquide renversé. N'utilisez pas l'instrument si les composants internes ont été exposés à du liquide.

Flüssigkeiten. Keine Flüssigkeiten auf dem Gerät verschütten! In die Bauteile im Geräteinneren bilden einsickernde Flüssigkeiten ein Potenzial für die Gefahr von Stromschlägen oder Schäden am Gerät. Bei Verschütten von Flüssigkeiten während ein Programm läuft, ist dieses zu stoppen und das Gerät auszuschalten. Verschüttete Flüssigkeiten sind unverzüglich abzuwischen. Das Gerät darf nicht betrieben werden, wenn Komponenten im Geräteinneren Flüssigkeiten ausgesetzt waren.

Liquidi. Evitare di versare liquidi sullo strumento; l'infiltrazione di fluidi nei componenti interni crea rischi di scosse elettriche o danni allo strumento. Se si verifica un versamento durante l'esecuzione di un programma, arrestare il programma e spegnere lo strumento. Ripulire immediatamente tutti i versamenti. Non utilizzare lo strumento se i componenti interni sono stati esposti a fluidi.

Líquidos. Procure no derramar líquidos sobre el instrumento, ya que si se filtran fluidos en los componentes internos se puede producir un riesgo de descarga o de deterioro del instrumento. Si se produce un derramamiento mientras se está ejecutando un programa, detenga el programa y apague el instrumento. Limpie el derrame inmediatamente. No utilice el instrumento si los componentes internos han estado expuestos a fluidos.

CAUTION

Liquids. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

Vloeistoffen. Dompel het instrument niet onder, bespuit het niet met vloeistof en gebruik er geen druipnatte doek op. Zorg ervoor dat er geen water of andere schoonmaakmiddelen in het inwendige van het instrument terechtkomen. Als dit gebeurt, neem dan contact op met de afdeling Technische Ondersteuning.

Liquides. N'immergez pas l'instrument, ne le vaporisez pas de liquide et n'utilisez pas de chiffon non essoré dessus. Ne laissez pas d'eau ou autre solution de nettoyage pénétrer à l'intérieur de l'instrument. Le cas

échéant, contactez l'assistance technique.
Flüssigkeiten. Das Gerät nicht in Flüssigkeit eintauchen oder damit einsprühen und keine tropfnassen Tücher verwenden. Kein Wasser oder andere Reinigungslösung in das Geräteinnere eindringen lassen. Sollte dies vorkommen, setzen Sie sich mit dem technischen Kundendienst in Verbindung.
Liquidi. Non immergere lo strumento, nebulizzarlo con liquidi né usare un panno che non sia stato strizzato bene. Evitare che acqua o soluzioni detergenti penetrino all'interno dello strumento. Se si verifica un'infiltrazione, contattare il supporto tecnico.
Líquidos. No sumerja el instrumento, no lo pulverice con líquidos y no use un paño mojado que gotee sobre él. No permita que entre agua ni otra solución de limpieza en el interior del instrumento. Si esto sucediera, póngase en contacto con el servicio de soporte técnico.

CAUTION

Environmental Conditions. Do not expose the instrument to temperature extremes. For proper operation, temperature near the instrument should remain within the range in the ***Specifications*** section of this document. Performance may be adversely affected if temperatures fluctuate above or below this range.
Omgevingsvoorwaarden. Stel het instrument niet bloot aan extreme temperaturen. Voor een goede werking moet de temperatuur in de buurt van het instrument binnen het bereik blijven zoals aangegeven in het gedeelte Specificaties van dit document. De prestaties kunnen nadelig worden beïnvloed als de temperatuur boven of onder dit bereik schommelt.
Conditions environnementales. N'exposez pas l'instrument à des températures extrêmes. Pour assurer un bon fonctionnement, la température à proximité de l'instrument doit demeurer dans la plage indiquée sous la rubrique Spécifications du présent document. La performance peut être affectée négativement si les températures fluctuent au-dessus ou au-dessous de cette plage.
Umgebungsbedingungen. Das Gerät darf keinen Extremtemperaturen ausgesetzt werden. Für den ordnungsgemäßen Betrieb müssen die Temperaturen in Gerätenähe in den im Abschnitt Spezifikationen dieses Dokuments angegebenen Grenzen bleiben. Temperaturschwankungen über diese Grenzwerte hinaus können die Geräteleistung beeinträchtigen.
Condizioni ambientali. Non esporre lo strumento a temperature estreme. Per il corretto funzionamento, la temperatura nei pressi dello strumento deve restare nell'intervallo indicato nella sezione Specifiche di questo documento. Fluttuazioni delle temperature al di sopra o al di sotto di questo intervallo possono compromettere le prestazioni dello strumento.
Condiciones ambientales. No exponga el instrumento a temperaturas extremas. Para su correcto funcionamiento, la temperatura que rodee al instrumento deberá estar dentro del rango indicado en la sección Especificaciones de este documento. Si las temperaturas fluctúan por encima o por debajo de este rango, el

rendimiento puede verse afectado negativamente.

CAUTION

Sodium Hypochlorite. Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Natriumhypochloriet. Stel geen enkel deel van het instrument langer dan 20 minuten bloot aan de aanbevolen verdunde natriumhypochlorietoplossing. Langdurig contact kan de oppervlakken van het instrument beschadigen. Zorg ervoor dat alle oppervlakken goed worden afgespoeld en schoongeveegd.

Hypochlorite de sodium. N'exposez aucune pièce de l'instrument à la solution d'hypochlorite de sodium diluée comme recommandé pendant plus de 20 minutes. Un contact prolongé peut endommager les surfaces de l'instrument. Veillez à rincer et essuyer soigneusement toutes les surfaces.

Natriumhypochlorit. Kein Teil des Geräts darf der empfohlenen verdünnten Natriumhypochloritlösung länger als 20 Minuten lang ausgesetzt werden. Bei längerem Kontakt drohen Beschädigungen an den Geräteoberflächen. Alle Oberflächen unbedingt abspülen und gründlich abwischen.

Ipoclorito di sodio. Non esporre nessun componente dello strumento alla soluzione di ipoclorito di sodio diluita raccomandata per più di 20 minuti. Un contatto prolungato potrebbe danneggiare le superfici dello strumento. Accertarsi di sciacquare e ripulire accuratamente tutte le superfici.

Hipoclorito sódico. No exponga ninguna parte del instrumento a la solución de hipoclorito sódico diluido recomendada durante más de 20 minutos. Un contacto demasiado prolongado puede dañar las superficies del instrumento. Asegúrese de aclarar y secar concienzudamente todas las superficies.

CAUTION

DMSO Concentration. Dimethyl sulfoxide (DMSO) vapor can coat optical surfaces, which can trigger instrument self-test errors. Using DMSO assay concentrations of 2% or below is recommended. Limit long exposure in kinetic assays or incubated assays when possible.

DMSO-concentratie. Dimethylsulfoxide- (DMSO-) damp kan optische oppervlakken bedekken, wat zelftestfouten van het instrument kan veroorzaken. Het wordt aanbevolen om DMSO-testconcentraties van 2% of minder te gebruiken. Beperk lange blootstelling in kinetische tests of geïncubeerde tests indien mogelijk.

Concentration de DMSO. La vapeur de diméthylsulfoxyde (DMSO) peut recouvrir les surfaces optiques, ce qui peut déclencher des erreurs d'autotest de l'instrument. Il est recommandé d'utiliser des concentrations de test DMSO de 2 % ou moins. Limitez autant que possible les expositions prolongées dans les tests cinétiques ou les tests en incubation.

DMSO-Konzentration. Dimethylsulfoxid(DMSO)-Dampf kann optische Oberflächen beschichten, was Fehler beim Geräteselbsttest auslösen kann. Es wird empfohlen, DMSO-Assay-Konzentrationen von 2 % oder

weniger zu verwenden. Begrenzen Sie wenn möglich die Langzeitexposition in kinetischen Assays oder inkubierten Assays.
Concentrazione di DMSO. Il vapore di dimetilsolfossido (DMSO) può rivestire le superfici ottiche, provocando errori di autoverifica dello strumento. Si raccomanda l'uso di concentrazioni di test di DMSO non superiori al 2%. Se possibile, limitare l'esposizione prolungata nei test cinetici o nei test incubati.
Concentración de DMSO. Los vapores del dimetilsulfóxido (DMSO) pueden recubrir las superficies ópticas, lo cual puede provocar errores en la autoverificación del instrumento. Se recomienda utilizar concentraciones de ensayo de DMSO del 2 % o menos. Limite la exposición prolongada en ensayos cinéticos o ensayos incubados siempre que sea posible.

CAUTION

Lubricants. Do not apply lubricants to moving parts. Lubricant on components in the carrier compartment will attract dust and other particles, which may cause the instrument to produce an error.
Smeermiddelen. Breng geen smeermiddelen aan op bewegende delen. Smeermiddel op onderdelen in het draagcompartiment zal stof en andere deeltjes aantrekken, waardoor het instrument een fout kan produceren.
Lubrifiants. N'appliquez pas de lubrifiants sur les pièces mobiles. La présence de lubrifiant sur les composants dans le compartiment du portoir attire la poussière et autres particules, ce qui peut provoquer une erreur de l'instrument.
Schmierstoffe. Keine Schmierstoffe auf bewegliche Teile auftragen. Schmierstoffe auf Komponenten im Trägerfach ziehen Staub und andere Teilchen an, die zu einem Gerätefehler führen können.
Lubrificanti. Non applicare lubrificanti alle parti in movimento. La presenza di lubrificante sui componenti del vano portapiastra attira polvere e altre particelle che potrebbero causare errori dello strumento.
Lubricantes. No aplique lubricantes en las piezas móviles. El lubricante en los componentes del compartimento del portador atraerá polvo y otras partículas que pueden hacer que el instrumento muestre un error.

Components

Onderdelen

Composants

Komponenten

Componenti

Componentes

Class 1 Laser Product. "A" models. "V" and "W" models when the optional Laser Autofocus Cube is installed.
Klasse 1 laserproduct. "A"-modellen. "V"- en "W"-modellen wanneer de optionele Laser Autofocus Cube is geïnstalleerd.
Produit laser de classe 1. Modèles "A". Modèles « V » et « W » lorsque le cube laser autofocus en option est installé.
Laserprodukt der Klasse 1. „A"-Modelle. „V"- und „W"-Modelle, wenn der optionale Laser Autofokus Cube installiert ist.
Prodotto laser di classe 1. Modelli "A". Modelli "V" e "W" quando è installato il Laser Autofocus Cube opzionale.
Producto láser de clase 1. Modelos "A". Modelos "V" y "W" cuando está instalado el cubo de enfoque automático láser opcional.

Pinch Hazard. Some areas of the instrument and the dispense module can present pinch hazards when the instrument is operating. The objective turret and dispense module are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.
Beknellingsgevaar. Sommige delen van het instrument en de doseermodule kunnen beknellingsgevaar opleveren wanneer het instrument in werking is. De objectiefkoepel en de doseermodule zijn gemarkeerd met het hier getoonde symbool. Houd handen/vingers uit de buurt van deze gebieden wanneer het instrument in werking is.
Risque de pincement. Certaines zones de l'instrument et du module de distribution peuvent présenter des risques de pincement lorsque l'instrument fonctionne. La tourelle d'objectif et le module de distribution sont marqués du symbole illustré ici. Gardez vos mains/doigts à l'écart de ces zones lorsque l'instrument fonctionne.
Quetschgefahr. Einige Bereiche des Instruments und des Dosiermoduls können beim Betrieb des Instruments Quetschgefahren darstellen. Der Objektivrevolver und das Dosiermodul sind mit dem hier abgebildeten Symbol gekennzeichnet. Halten Sie Hände/Finger von diesen Bereichen fern, wenn das Instrument in Betrieb ist.

Rischio di pizzicamento. Alcune aree dello strumento e del modulo di erogazione possono presentare rischi di schiacciamento quando lo strumento è in funzione. La torretta obiettivo e il modulo di erogazione sono contrassegnati dal simbolo mostrato qui. Tenere le mani/dita lontane da queste aree quando lo strumento è in funzione.
Peligro de atrapamiento. Algunas áreas del instrumento y el módulo de dispensación pueden presentar riesgos de pellizcos cuando el instrumento está en funcionamiento. La torreta de objetivos y el módulo dispensador están marcados con el símbolo que se muestra aquí. Mantenga las manos / dedos alejados de estas áreas cuando el instrumento esté en funcionamiento.

Two-person lift. The instrument should be lifted by two people. The instrument with all available modules weighs up to 36.3 kg (45.3 kg with the Peltier Cooling Module installed).
Tillen door twee personen. Het instrument moet door twee personen worden opgetild. Het instrument met alle beschikbare modules weegt maximaal 36,3 kg (45,3 kg met de Peltier-koelmodule geïnstalleerd).
Charge à soulever par deux personnes. L'instrument doit être soulevé par deux personnes. L'instrument avec tous les modules disponibles pèse jusqu'à 36,3 kg (45,3 kg avec le module de refroidissement Peltier installé).
Anheben durch zwei Personen. Das Gerät sollte von zwei Personen angehoben werden. Das Gerät mit allen verfügbaren Modulen wiegt bis zu 36,3 kg (45,3 kg mit installiertem Peltier-Kühlmodul).
Due persone per il sollevamento. Lo strumento deve essere sollevato da due persone. Lo strumento con tutti i moduli disponibili pesa fino a 36,3 kg (45,3 kg con il modulo di raffreddamento Peltier installato).
Levantamiento por dos personas. Es necesario que dos personas levanten el instrumento. El instrumento con todos los módulos disponibles puede pesar hasta 36,3 kg (45,3 kg con el módulo de enfriamiento Peltier instalado).

WARNING

Accessories. Only accessories that meet the manufacturer's specifications shall be used with the instrument.
Accessoires. Bij het instrument mogen alleen accessoires worden gebruikt die voldoen aan de specificaties van de fabrikant.
Accessoires. L'instrument doit être utilisé exclusivement avec des accessoires correspondant aux spécifications du fabricant.
Zubehör. In Verbindung mit dem Gerät dürfen nur Zubehörkomponenten verwendet werden, die den Spezifikationen des Herstellers entsprechen.
Accessori. Utilizzare esclusivamente accessori dello

strumento che rispettano le specifiche del fabbricante.
Accesorios. Solamente aquellos accesorios que cumplan las especificaciones del fabricante deberán usarse con el instrumento.

CAUTION

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.
Verzendingshardware. Alle verzendingshardware moet worden verwijderd voordat het instrument wordt gebruikt en opnieuw worden geïnstalleerd voordat het instrument opnieuw wordt verpakt voor verzending.
Matériel d'expédition. Tout le matériel d'expédition doit être retiré avant d'utiliser l'instrument et réinstallé avant de remballer l'équipement pour expédition.
Festes Versandmaterial. Alle festen Versandmaterialien müssen vor der Inbetriebnahme des Geräts entfernt und vor der Wiederverpackung des Geräts zum Versand neu angebracht werden.
Minuteria di spedizione. Prima di utilizzare lo strumento, rimuovere tutta la minuteria di spedizione, che dovrà essere reinstallata prima di reimballare lo strumento per la spedizione.
Equipo de envío. Antes de utilizar el instrumento es necesario retirar todo el equipo de envío y, del mismo modo, habrá que volver a colocárselo cuando el instrumento se vaya a enviar.

CAUTION

Spare Parts. Only approved spare parts should be used for maintenance. The use of unapproved spare parts and accessories may result in a loss of warranty and potentially impair instrument performance or cause damage to the instrument.
Reserveonderdelen. Voor onderhoud mogen alleen goedgekeurde reserveonderdelen worden gebruikt. Het gebruik van niet-goedgekeurde onderdelen en accessoires kan tot gevolg hebben dat de garantie vervalt en mogelijk de prestaties van het instrument nadelig beïnvloeden of het instrument beschadigen.
Pièces de rechange. Utilisez exclusivement des pièces de rechange approuvées pour l'entretien. L'utilisation de pièces de rechange et accessoires non approuvés peut entraîner l'annulation de la garantie et potentiellement nuire à la performance de l'instrument ou l'endommager.
Ersatzteile. Für die Wartung sollten nur genehmigte Ersatzteile verwendet werden. Die Verwendung nicht genehmigter Ersatzteile und Zubehörkomponenten kann zum Verlust der Garantie führen und möglicherweise die Geräteleistung beeinträchtigen oder Schäden am Gerät verursachen.

Parti di ricambio. Per la manutenzione, usare esclusivamente parti di ricambio approvate. L'uso di parti di ricambio e accessori non approvati potrebbe dare luogo all'annullamento della garanzia e ripercuotersi negativamente sulle prestazioni o causare danni allo strumento.
Repuestos. Durante el mantenimiento, solo deben emplearse repuestos originales. El uso de repuestos y accesorios no autorizados puede producir la pérdida de la garantía y reducir el funcionamiento del instrumento o provocar daños en él.

CAUTION

Service. Only qualified technical personnel should perform service procedures on internal components.
Service. Alleen gekwalificeerd technisch personeel mag serviceprocedures aan interne onderdelen uitvoeren.
Entretien. L'exécution des procédures d'entretien des composants internes doit être réservée au personnel technique qualifié.
Wartung. Wartungsarbeiten an Komponenten im Geräteinneren sollten nur von qualifizierten Servicetechnikern durchgeführt werden.
Manutenzione. Le procedure di manutenzione sui componenti interni devono essere eseguite esclusivamente da personale tecnico qualificato.
Revisión. Solo puede realizar procedimientos de revisión de los componentes internos el personal técnico cualificado.

CAUTION

Instrument Configuration. When you install or change objectives, LED cubes, imaging filter cubes, or fluorescence filter cubes you must update the instrument configuration values in Gen5 and download the information to the instrument.
Configuratie instrument. Wanneer u objectieven, led-kubussen, filterkubussen voor beeldvorming of fluorescentiefilterkubussen installeert of vervangt, moet u de configuratiewaarden van het instrument in Gen5 bijwerken en de informatie naar het instrument downloaden.
Configuration de l'instrument. Lorsque vous installez ou modifiez des objectifs, des cubes LED, des cubes de filtres d'imagerie ou des cubes de filtres à fluorescence, vous devez mettre à jour les valeurs de configuration de l'instrument dans Gen5 et télécharger les informations sur l'instrument.
Gerätekonfiguration. Wenn Sie Objektive, LED-Würfel, Bildfilterwürfel oder Fluoreszenzfilterwürfel installieren oder wechseln, müssen Sie die Gerätekonfigurationswerte in Gen5 aktualisieren und die Informationen auf das Gerät herunterladen.
Configurazione dello strumento. Quando si installano o sostituiscono obiettivi, cubi LED, filtri a cubo per immagini o filtri a cubo fluorescenti, è necessario aggiornare i valori della configurazione dello strumento in Gen5 e scaricare le

informazioni sullo strumento.
Cuando instale o cambie objetivos, cubos de LED, cubos de filtros de imágenes o cubos de filtros de fluorescencia, debe actualizar los valores de configuración del instrumento en Gen5 y descargar la información al instrumento.

CAUTION

LED cubes and filter cubes. Wear gloves when changing components to avoid contaminating them.
Led-kubussen en filterkubussen. Draag handschoenen bij het vervangen van onderdelen om te voorkomen dat ze besmet worden.
Cubes LED et cubes de filtres. Portez des gants lors du changement de composants pour éviter de les contaminer.
LED-Würfel und Filterwürfel. Tragen Sie beim Austauschen von Komponenten Handschuhe, um eine Kontamination zu vermeiden.
Cubi LED e filtri a cubo. Indossare dei guanti quando si sostituiscono i componenti per evitare di contaminarli.
Cubos de LED y cubos de filtro. Use guantes al cambiar los componentes para evitar contaminarlos.

CAUTION

Objectives. Do not use these cleaning solvents: Methyl ethyl ketone (MEK), Dimethyl ketone (acetone).
Objectieven. Gebruik deze schoonmaakmiddelen niet: Methylethylketon (MEK), dimethylketon (aceton).
Objectifs. N'utilisez pas ces solvants de nettoyage : méthyléthylcétone (MEK), diméthylcétone (acétone).
Objektive. Verwenden Sie diese Reinigungsmittel nicht: Methylethylketon (MEK), Dimethylketon (Aceton).
Obiettivi. Non utilizzare i seguenti solventi per la pulizia: metiletilchetone (MEK) e dimetilchetone (acetone).
Objetivos. No utilice los siguientes disolventes de limpieza: metiletilcetona (MEK) o dimetilcetona (acetona).

Intended Product Use

Beoogd productgebruik

Utilisation prévue du produit

Vorgesehene Produktverwendung

Uso previsto del prodotto

Uso previsto del producto

WARNING **Software Quality Control.** The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct quality control checks could result in erroneous test data.

Softwarekwaliteitscontrole. Bij het wijzigen van de softwareparameters en het vaststellen van afleesmethoden moet de operator de bijsluiter van de test van de fabrikant volgen. Het wordt beschouwd als een goede laboratoriumpraktijk om laboratoriummonsters te onderzoeken volgens de instructies en specifieke aanbevelingen die zijn opgenomen in de bijsluiter van de verpakking van de uit te voeren test. Het niet uitvoeren van kwaliteitscontroles kan leiden tot foutieve testgegevens.

Contrôle de qualité du logiciel. L'opérateur doit respecter la notice présente dans l'emballage du test lorsqu'il modifie les paramètres du logiciel et établit les méthodes de lecture. L'exécution d'échantillons de laboratoire conformément aux instructions et aux recommandations spécifiques présentées dans la notice de l'emballage du test à réaliser est considérée comme une bonne pratique de laboratoire. Ne pas exécuter les vérifications de contrôle de qualité peut produire des données de test erronées.

Qualitätskontrolle der Software. Beim Ändern von Softwareparametern und Festlegen der Leseverfahren muss der Bediener die Vorschriften des Herstellers auf der Packungsbeilage des Assays beachten. Es gilt als bewährte Laborpraxis, Messungen an Laborproben gemäß den Anweisungen und speziellen Empfehlungen der Packungsbeilage des Assay-Pakets für den beabsichtigten Test durchzuführen. Das Versäumnis, Qualitätskontrollprüfungen vorzunehmen, kann zu falschen Messergebnissen führen.

Controllo qualità del software. L'operatore deve attenersi alle istruzioni del fabbricante contenute nel foglietto della confezione del test quando modifica i parametri software e stabilisce i metodi di lettura. È considerata una buona pratica di laboratorio eseguire campioni di laboratorio in base alle istruzioni e alle raccomandazioni specifiche incluse nel foglietto della confezione del test relativo al test da condurre. La mancata esecuzione delle verifiche di controllo qualità

potrebbe dare luogo a dati di test errati.

Control de calidad del software. El operador tiene que seguir las instrucciones del folleto del paquete del ensayo cuando modifique parámetros del software y establezca métodos de lectura. Se considera una buena práctica de laboratorio efectuar las muestras de laboratorio siguiendo las instrucciones y las recomendaciones específicas incluidas en el folleto del paquete del ensayo para cada prueba que se va a realizar. Si no se realizan las comprobaciones de control de calidad, la prueba puede arrojar datos erróneos.

WARNING

Data Reduction. No limits are applied to the raw measurement data. Data exported via computer control must be analyzed by the operator. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. Users must evaluate this instrument and PC-based software in conjunction with their specific assay(s). This evaluation must include the confirmation that performance characteristics for the specific assay(s) are met.

Gegevensreductie. Er worden geen grenzen toegepast op de onbewerkte meetgegevens. Gegevens die via computerbesturing worden geëxporteerd, moeten door de operator worden geanalyseerd. De prestatiekenmerken van de gegevensreductiesoftware zijn voor geen enkele diagnostische laboratoriumtest vastgesteld. Gebruikers moeten dit instrument en de pc-gebaseerde software evalueren in samenhang met hun specifieke test(s). Deze evaluatie moet de bevestiging omvatten dat aan de prestatiekenmerken voor de specifieke test(s) is voldaan.

Réduction des données. Aucune limite n'est appliquée aux données de mesure brutes. Les données exportées par commande informatique doivent être analysées par l'opérateur. Les caractéristiques de performance du logiciel de réduction des données n'ont pas été établies par un test de diagnostic en laboratoire. Les utilisateurs doivent évaluer l'instrument et le logiciel pour PC conjointement à leur(s) test(s) spécifique(s). Cette évaluation doit comprendre la confirmation que les caractéristiques de performance pour le ou les tests spécifiques sont remplies.

Datenauswertung. Auf die Rohdaten der Messung sind keine Grenzwerte anzuwenden. Computergesteuert exportierte Daten müssen vom Bediener analysiert werden. Die Leistungsmerkmale der Datenauswertungs-Software wurden bei keinem Labordiagnostik-Assay bestimmt. Die Evaluierung dieses Geräts und der PC-basierten Software durch den Anwender muss in Verbindung mit dessen speziellem/speziellen Assay(s) erfolgen. Diese Evaluierung muss die Bestätigung einschließen, dass die Leistungsmerkmale für den/die speziellen Assay(s) erfüllt sind.

Riduzione dei dati. Non sono previsti limiti ai dati di misurazione grezzi. I dati esportati tramite il computer devono essere analizzati dall'operatore. Le caratteristiche di prestazione del software di riduzione dei dati non sono state stabilite con alcun test di diagnostica

di laboratorio. Gli utenti devono valutare questo strumento e il software basato su PC congiuntamente ai loro test specifici. Tale valutazione deve comprendere la conferma che siano rispettate le caratteristiche di prestazione per i test specifici.

Reducción de datos. No se aplican límites a los datos de medición no procesados. El operador debe analizar los datos exportados a través del control informático. Las características de rendimiento del software de reducción de datos no se han establecido con ningún ensayo de diagnóstico de laboratorio. Los usuarios deberán evaluar este instrumento y el software basado en PC junto con sus ensayos específicos. Esta evaluación deberá incluir la confirmación de que se cumplen las características de rendimiento de los ensayos específicos.

WARNING

Unspecified Use. Failure to operate equipment according to the guidelines and safeguards specified in the product user documentation could result in a hazardous condition.

Ongespecificeerd gebruik. Als de apparatuur niet wordt gebruikt volgens de richtlijnen en voorzorgsmaatregelen die in de gebruikersdocumentatie van het product staan vermeld, kan dat leiden tot een gevaarlijke situatie.

Utilisation non spécifiée. Ne pas utiliser l'équipement conformément aux recommandations spécifiées dans la documentation utilisateur relative au produit peut entraîner des situations dangereuses.

Von den Vorschriften abweichende Verwendung. Die Verwendung des Geräts und der zugehörigen Komponenten in Abweichung von den Vorschriften und Sicherheitshinweisen in diesem Dokument für Produktanwender kann gefährliche Situationen verursachen.

Uso non specificato. Il mancato utilizzo delle apparecchiature in base alle linee guida e le misure di protezione specificate nella documentazione per l'utente del prodotto potrebbe causare pericoli.

Uso no especificado. Si no se utiliza el equipo de conformidad con las directrices y salvaguardias especificadas en la documentación del producto para el usuario, se puede producir una situación de peligro.

CAUTION

Use of labware other than described in this document can result in positioning errors during program execution.

Gebruik van labware anders dan beschreven in dit document kan leiden tot positioneringsfouten tijdens de uitvoering van het programma.

L'utilisation de matériel de laboratoire autre que celui décrit dans ce document peut entraîner des erreurs de positionnement lors de l'exécution du programme.

Die Verwendung anderer als in diesem Dokument beschriebener Laborgeräte kann zu Positionierungsfehlern bei der Programmausführung führen.

L'uso di vetreria diversa da quella descritta in questo documento può causare errori di posizionamento durante l'esecuzione del programma.

El uso de material de laboratorio diferente al descrito en este documento puede dar lugar a errores de posicionamiento durante la ejecución del programa.

Index

E

F

G

H

I

Q

R

S

T

U

W

In This Book

This document contains installation, operation, maintenance, and qualification information for all models of the Cytation 5.

Document Revision History			
Part Number	Revision	Date	Modifications
1321000N	A	December 2021	First issue.

Original Language – EN

Manufactured by Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

BioTek®