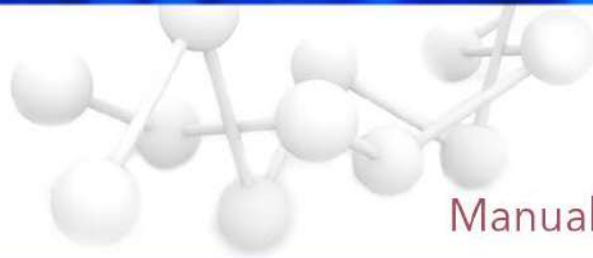


# Cytation™ 3

## Operator's Manual



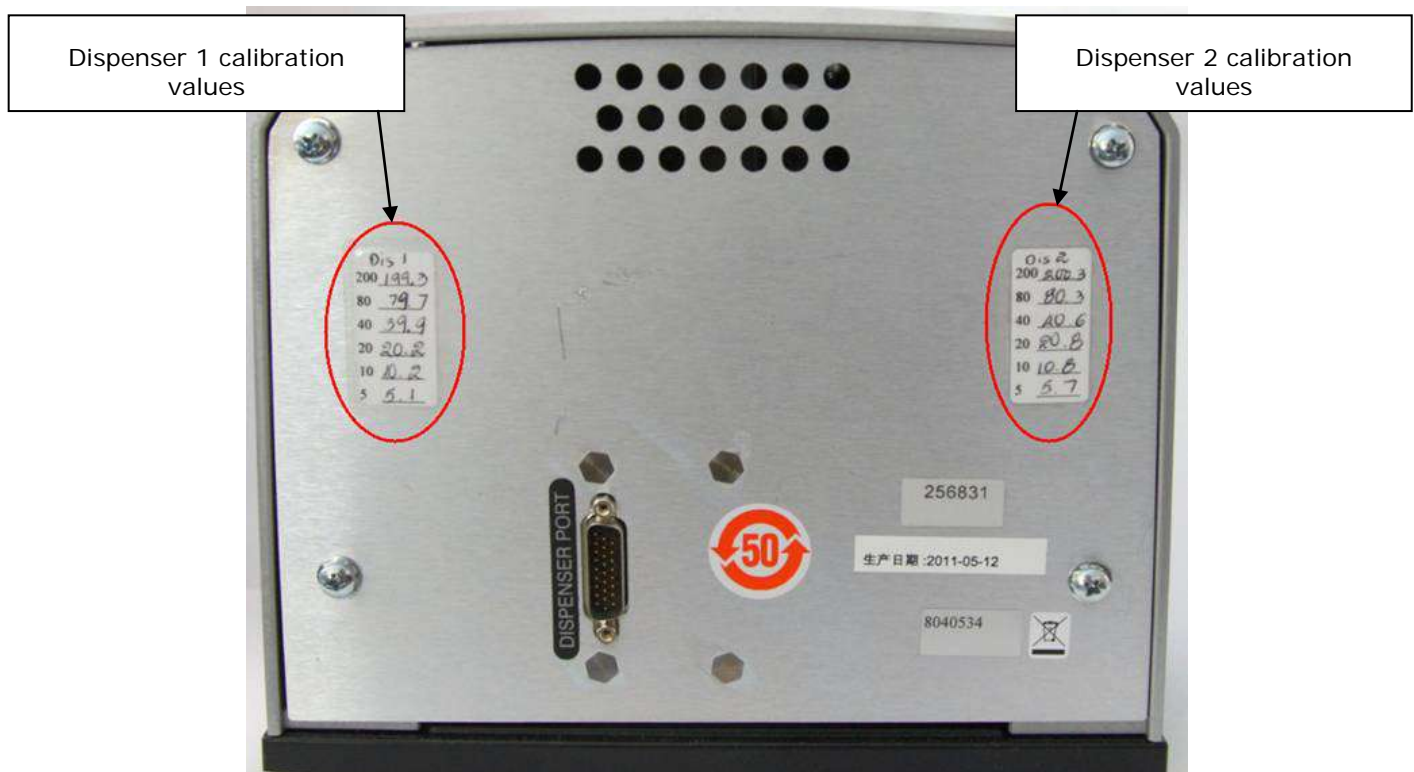




## Setting the Dispenser Calibration Values

Before you use the dispenser with a BioTek reader, you **must** set the calibration values in Gen5.

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



Please contact BioTek's Technical Assistance Center with any questions, [tac@biotek.com](mailto:tac@biotek.com).



# Cytation™ 3

Cell Imaging Multi-Mode Reader  
Operator's Manual



BioTek® Instruments, Inc.  
June 2013  
© 2013  
PN 1221000  
Revision A

## Notices

BioTek<sup>®</sup> Instruments, Inc.  
Highland Park, P.O. Box 998  
Winooski, Vermont 05404-0998 USA

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## Contact Information

### Customer Service and Sales

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 Phone: 888-451-5171 (toll-free in the U.S.)  
 802-655-4740 (outside the U.S.)  
 Fax: 802-655-7941  
 Email: [customercare@biotek.com](mailto:customercare@biotek.com)

### Global Service and Support

BioTek instrument service and repair is available worldwide at one of BioTek's International Service Centers and in the field at your location. For technical assistance, contact the Technical Assistance Center (TAC) at BioTek World Headquarters US. To arrange for service or repair of your instrument, contact the office nearest you.

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## Revision History

Rev	Date	Changes
A	6/2013	Initial release to production

## Document Conventions

	This icon calls attention to important safety notes.
Warning!	A Warning indicates the potential for bodily harm and tells you how to avoid the problem.
Caution	A Caution indicates potential damage to the instrument and tells you how to avoid the problem.
Note	Bold text is primarily used for emphasis.
<i>italic</i>	Topics that apply only to specific Cytation3 models are preceded by a notice in <i>italic</i> , for example, <i>Applies only to Cytation3 models with injectors.</i>
	This icon calls attention to important information.

## Intended Use Statement

The Cytation3 is a hybrid multi-mode microplate reader. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. The user 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.

- BioTek Gen5 software package provides the user with instrument control and data reduction capabilities.
- The Cytation3 can operate with standard robotic systems, such as the BioStack 3.
- If the instrument has an "IVD" label, it may be used for clinical and non-clinical purposes, including research and development. If there is no such label, the instrument may be used only for research and development or other non-clinical purposes.

## 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

Please take a moment to review the warranty information that shipped with your product. Please also register your product with BioTek to ensure that you receive important information updates about the product(s) you have purchased.

You can register online through the Customer Resource Center (CRC) at [www.biotek.com](http://www.biotek.com) or by calling 888-451-5171 or 802-655-4740.

## Repackaging and Shipping



If you need to ship the instrument to BioTek for service or repair, contact BioTek for a **Return Materials Authorization (RMA)** number, and be sure to use the original packing materials. Other forms of commercially available packaging are not recommended and can void the warranty. If the original packing materials have been damaged or lost, contact BioTek for replacement packing.



## Warnings



Operate the instrument on a level, stable surface away from excessive humidity.

Bright sunlight or strong incandescent light can reduce the linear performance range of the instrument.

Measurement values may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these may vary depending on the instrument model. See Hazards and Precautions.

## Hazards

The following hazards are provided to help avoid injury:



**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.

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

**Warning! Lubricants.** Do not apply lubricants to the microplate carrier or carrier track. Lubricant on the carrier mechanism or components in the carrier compartment will attract dust and other particles, which may obstruct the carrier path and cause the instrument to produce an error.

**Warning!** The instrument with all available modules weighs up to **80 lbs. (36.3 kg)**. Use two people when lifting and carrying the instrument.

**Warning! Liquids.** Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard. If a spill occurs while a program is running, abort 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. Contact BioTek TAC for assistance.

**Warning! Unspecified Use.** Failure to operate the equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.

**Warning! Software Quality Control.** The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. Failure to conduct quality control checks could result in erroneous test data.

**Warning! Reader Data Reduction Protocol.** No limits are applied to the raw measurement data. All information exported via computer control must be thoroughly analyzed by the operator.



**Warning! Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument or removing its top case.



**Warning! Potential Biohazards.** Some assays or specimens may pose a biohazard. This hazard is noted by the symbol shown here. 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! LED Lights.** Serious eye injury may occur if you stare directly at the LED during operation of the light. This hazard is noted by the symbol shown here.



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

## Precautions

The following precautions are provided to help avoid damage to the instrument:



**Caution: Service.** The instrument should be serviced by BioTek-authorized service personnel. Only qualified technical personnel should perform service procedures on internal components.

**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: Environmental Conditions.** Do not expose the system to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in **Appendix A, Specifications**. Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

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

**Caution: Power Supply.** Use only the power supply shipped with the instrument. Operate this power supply within the range of line voltages listed on it.

**Caution: Disposal.** This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)" or local ordinances.

**Caution: Warranty.** Failure to follow preventive maintenance protocols may void the warranty. See **Chapter 5, Preventive Maintenance**.

**Caution: Shipping Hardware.** The shipping brackets must be removed before operating the instrument. They must be reinstalled before shipping the instrument. See **Chapter 2, Installation**.

**Caution: Electromagnetic Environment.** Per IEC 61326-2-6 it is the user's responsibility to ensure that a compatible electromagnetic environment for this instrument is provided and maintained in order that the device will perform as intended.

**Caution: Electromagnetic Compatibility.** Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), because these may interfere with the proper operation.

## CE Mark



Refer to the Declaration of Conformity for specific details.

### **Directive 2004/108/EC: Electromagnetic Compatibility**

#### **Emissions—Class A**

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1: Class A for Radiated Emissions and Line Conducted Emissions.

Verification of compliance was conducted to the limits and methods of EN 55011 – (CISPR 11) Class A. In a domestic environment it may cause radio interference, in which case you may need to mitigate the interference.

#### **Immunity**

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 and EN 61326-2-6 for Immunity. Verification of compliance was conducted to the limits and methods of the following:

- EN 61000-4-2, Electrostatic Discharge
- EN 61000-4-3, Radiated EM Fields
- EN 61000-4-4, Electrical Fast Transient/Burst
- EN 61000-4-5, Surge Immunity
- EN 61000-4-6, Conducted Disturbances from RFI
- EN 61000-4-8, Power Frequency Magnetic Field Immunity Test
- EN 61000-4-11, Voltage Dips, Short Interruptions and Variations

### **Directive 2006/95/EC Low Voltage (Safety)**

The system has been type-tested by an independent testing laboratory and was found to meet the requirements of this Directive. Verification of compliance was conducted to the limits and methods of the following:

- EN 61010-1. "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."
- EN 61010-2-081. "Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."
- EN 61010-2-010. "Particular requirements for laboratory equipment for the heating of materials."
- EN 608250-1, "Safety of laser products. Part 1: Equipment classification and requirements."

### **Directive 2002/96/EC: Waste Electrical and Electronic Equipment**

**Disposal Notice:** This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, “on waste electrical and electronic equipment (WEEE)” or local ordinances.

### **Directive 98/79/EC: In Vitro Diagnostics (if labeled for this use)**

- Product registration with competent authorities
- EN 61010-2-101. “Particular requirements for in vitro diagnostic (IVD) medical equipment.”
- Traceability to the U.S. National Institute of Standards and Technology (NIST).

## **Electromagnetic Interference and Susceptibility**

### **USA FCC CLASS A**

#### RADIO AND TELEVISION INTERFERENCE

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and television reception.

### **Canadian Department of Communications Class A**

This digital apparatus does not exceed Class A limits for radio emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

*Le present appareil numerique n'emet pas du bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.*











## User Safety

This device has been type-tested by an independent laboratory and found to meet the requirements of the following:

- Underwriters Laboratories UL 61010-1, “Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: General requirements.”
- Canadian Standards Association CAN/CSA C22.2 No. 61010-1, “Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: General requirements.”
- EN 61010 Standards, see **CE Mark** starting on page xvi

## Safety Symbols

Some of the following symbols may appear on the instrument or accessories:

	<p>Alternating current Courant alternatif Wechselstrom Corriente alterna Corrente alternata</p>		<p>Warning, risk of crushing or pinching</p>
			<p>Attention, risque d'écrasement et pincement</p>
			<p>Warnen, Gefahr des Zerquetschens und Klemmen</p>
			<p>Precaución, riesgo del machacamiento y sejeción</p>
			<p>Attenzione, rischio di schiacciare ed intrappolarsi</p>
	<p>Direct current Courant continu Gleichstrom Corriente continua Corrente continua</p>		<p>Warning, hot surface</p>
			<p>Attention, surface chaude</p>
			<p>Vorsicht, heiße Oberfläche</p>
			<p>Precaución, superficie caliente</p>
			<p>Attenzione, superfice calda</p>
	<p>Both direct and alternating current Courant continu et courant alternatif Gleich- und Wechselstrom Corriente continua y corriente alterna Corrente continua e corrente alternata</p>		<p>Laser radiation: Do not stare into beam</p>
			<p>Rayonnement laser: Ne pas regarder dans le faisceau</p>
			<p>Laserstrahlung: nicht in den strahl blicken</p>
			<p>Radiación de laser: No mire fijamente al rayo</p>
			<p>Radiazione di laser: Non stare nel fascio</p>
	<p>Earth ground terminal Borne de terre Erde (Betriebserde) Borne de tierra Terra (di funzionamento)</p>		<p>Warning, potential biohazards</p>
			<p>Attention, risques biologiques potentiels</p>
			<p>Warnung! Moegliche biologische Giftsoffe</p>
			<p>Atención, riesgos biológicos</p>
			<p>Attenzioni, rischio biologico</p>



Protective conductor terminal  
 Borne de terre de protection  
 Schutzleiteranschluss  
 Borne de tierra de protección  
 Terra di protezione



Caution (refer to accompanying documents)  
 Attention (voir documents d'accompagnement)  
 Achtung siehe Begleitpapiere  
 Atención (vease los documentos incluidos)  
 Attenzione, consultare la doc annessa



On (Supply)  
 Marche (alimentation)  
 Ein (Verbindung mit dem Netz)  
 Conectado  
 Chiuso



Consult instructions for use  
 Consulter la notice d'emploi  
 Gebrauchsanweisung beachten  
 Consultar las instrucciones de uso  
 Consultare le istruzioni per uso



Off (Supply)  
 Arrêt (alimentation)  
 Aus (Trennung vom Netz)  
 Desconectado  
 Aperto (sconnessione dalla rete di alimentazione)



In vitro diagnostic medical device  
 Dispositif médical de diagnostic in vitro  
 Medizinisches In-Vitro Diagnostikum  
 Dispositivo médico de diagnóstico in vitro  
 Dispositivo medico diagnostico in vitro



Warning, risk of electric shock  
 Attention, risque de choc électrique  
 Gefährliche elektrische schlag  
 Precaución, riesgo de sacudida eléctrica  
 Attenzione, rischio di scossa elettrica



Separate collection for electrical and electronic equipment  
 Les équipements électriques et électroniques font l'objet d'une collecte sélective  
 Getrennte Sammlung von Elektro- und Elektronikgeräten  
 Recogida selectiva de aparatos eléctricos y electrónicos  
 Raccolta separata delle apparecchiature elettriche ed elettroniche



## Chapter 1

# Introduction

This chapter introduces the Cytation3 Cell Imaging Multi-Mode Reader, describes its hardware and software features, and provides contact information for technical assistance.

---

Product Description .....	2
Package Contents .....	3
Optional Accessories .....	4
Product Support and Service .....	6

## Product Description

The Cytation3 is a cell imaging multi-mode reader. Depending on the model, Cytation3 detection modes include fluorescence intensity (FI), fluorescence polarization (FP), time-resolved fluorescence (TRF), luminescence, UV-visible absorbance, and imaging. The instrument is modular, and upgrade options are available; contact BioTek Customer Care for more information.

The reader is computer-controlled using Gen5 software for all operations, including data reduction and analysis. The Cytation3 is robot accessible and compatible with the BioTek BioStack 3 Microplate Stacker. Gen5 supports OLE automation to facilitate the Cytation3's integration into an automated system.

The Cytation3 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 Cytation3 also has a direct-to-PMT channel (no filtering, white light only).

The imaging system supports LED filter-based fluorescence reads and a bright field read. The reader can use up to four LED cubes and four filter cubes and two objectives.

The Cytation3 has 4-Zone temperature control from 4°C over ambient to 45°C. Internal plate shaking, with both linear and orbital modes, is supported to ensure that reagents are properly mixed prior to reading.

The Cytation3 supports the reading of 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry, the Take3 and Take3 Trio Multi-Volume Plates, and microscope slides and flasks (using the adapters available from BioTek).

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

Models with injectors support dual-reagent dispensing 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 that support the gas controller can control the CO<sub>2</sub> or O<sub>2</sub> concentrations in the reading chamber for CO<sub>2</sub>- or O<sub>2</sub>-sensitive assays.

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

\* This dual light path capability is protected by U.S. patent number 8,218,141.

## Package Contents

Package contents and part numbers are subject to change. Please contact BioTek Customer Care with any questions.

Item	Part #
<i>Cytation3 Operator's Manual</i>	1221000
Power cord set (specific to installation environment):	
Europe (Schuko)	75010
USA/International	75011
United Kingdom	75012
Australia/New Zealand	75013
RS-232 serial cable	75034
USB cable with Virtual COM Driver Software	75108/7090204
#2 Phillips screwdriver	01188
9/64" Hex wrench	01623
Shipping bracket storage bracket	1222208
<i>Models with the imaging module:</i>	
FireWire desktop interface	01604
OR	
FireWire laptop card and power supply	1220535 Power supply only: 01062
FireWire cable	1220538
Microplate slide holder	1220526
Flask adapter	1222210

Item	Part #
Isolation table	1220521
<i>Models with an external dispense module (packed separately), with the following accessories:</i>	
Injector	8040541
Inlet tubes (2) from supply bottles to syringe drives	7082121
250- $\mu$ L syringes (2)	7083000
Syringe thumbscrews	19511
Priming plate	8042202
Injector tip priming trough	8042068
Dispense module communication cable	75107
Dispense module front cover	8042197
Dispense module box	8040534
Supply bottles (2, 30 mL)	7122609
Supply bottle holders (2)	8042193
Injector tip cleaning stylus and plastic storage bag	2872304
Strap reagent racks (6)	7212035
<i>Models with the gas controller ("G" models)(packed separately):</i>	
Gas controller unit, CO <sub>2</sub> /O <sub>2</sub> control	1210500
Shipping accessories, CO <sub>2</sub> /O <sub>2</sub> control	1210010
Gas Controller Unit, CO <sub>2</sub> only	1210504
Shipping accessories, CO <sub>2</sub> only	1210009

## Optional Accessories

Accessory availability and part numbers are subject to change. Please contact BioTek Customer Care if you have any questions or visit [www.biotek.com](http://www.biotek.com) and use the Accessories search tool.

<b>Item</b>	<b>Part #</b>
7-filter Absorbance Test Plate for absorbance measurement testing	7260522
Cytation3 Product Qualification (IQ-OQ-PQ) package	1220514
Carrier Level and Contrast jig	1222520
Take3 Micro-Volume Plate	TAKE3
Take3 Trio Micro-Volume Plate	TAKE3TRIO
PCR Tube Adapter Plates	6002072 6002076
BioCell Quartz Vessel	7272051
BioCell Adapter Plate	7270512
Harta Luminometer Reference Microplate	8030015
Harta Plate Adapter for the Cytation3	1222205
Gas-ready upgrade kit	Call BioTek Sales
Laptop computer	COMPLAPCYT3
Filters, filter cubes, LED cubes, and imaging filter cubes are available at <a href="http://www.biotek.com/products/accessories/">www.biotek.com/products/accessories/</a> ; contact BioTek for part numbers and availability.	
The Cytation3 is compatible with the BioStack 3 Microplate Stacker. The BioStack 3 rapidly and systematically transfers a "stack" of microplates to and from the instrument's microplate carrier. Contact BioTek or visit our website to learn more.	

<b>Solutions for Liquid Tests</b>	<b>Part #</b>
<b>Absorbance Liquid Test Solutions:</b>	
BioTek Wetting Agent Solution (PN 7773002)	7773002
BioTek QC Check Solution No. 1 (25 mL) <b>or</b> BioTek QC Check Solution No. 1 (125 mL)	7120779 7120782
$\beta$ -NADH Powder ( $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form)	Sigma #N6785-10VL (or BioTek PN 98233)
Phosphate-Buffered Saline (PBS) Tablets (pH 7.2–7.6)	Sigma #P4417
Dispense Module Liquid Test Solution: BioTek Green Test Dye	7773003
<b>Fluorescence Liquid Test Solutions, if using Sodium Fluorescein:</b>	
Fluorescence Liquid Test Kit	7160010 (contains 7160011, 7160012, and 7160013)
Sodium Fluorescein Powder	98155
Sodium Fluorescein Kit	7160013
Fluorescence Polarization Kit	7160014
TRF Europium Kit	7160011
<b>Fluorescence Liquid Test Solutions, if using Methylumbelliferone:</b>	
10-mg vial of Methylumbelliferone (MUB)	98156
Carbonate-Bicarbonate Buffer ("CBB") capsules	Sigma #3041
Refill for MUB Kit	7160012

## Product Support and Service

### Technical Assistance Center (TAC)

If your instrument or software fail to function properly, if you have questions about how to use or maintain our products, or if you need to send an instrument to BioTek for service or repair, please contact our Technical Assistance Center ("TAC").

TAC is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays.

- **Phone:** (800) 242-4685 or (802) 655-4740
- **Fax:** (802) 654-0638
- **E-Mail:** tac@biotek.com
- **Web:** www.biotek.com

Please be prepared to provide the following information:

- Your name and company information, along with a daytime phone or fax number, and/or an e-mail address
- The product name, model, and serial number (the serial number is located inside the filter cube access door)
- The onboard software part number and basecode version (available via Gen5 for the Cytation3 by selecting **System > Instrument Configuration > Get Basecode Information**)
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed in Gen5 (see also **Appendix B, Error Codes**)
- 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**)

If you need to return an instrument to BioTek for service or repair, please contact the TAC for a Return Materials Authorization (RMA) number and the shipping address. Repackage the instrument according to the instructions at the end of **Chapter 2, Installation**.

## Applications Support

BioTek's fully equipped Application Laboratory provides our on-staff scientists with the means to assist you with the integration of our instrumentation and software with your unique scientific applications. If you are having difficulty with optimizing fluorescence sensitivity or integrating a unique data reduction transformation, or you are just looking for a recommendation on an appropriate fluorophore, contact us.

**Phone:** (888) 451-5171

**E-Mail:** applications@biotek.com





## Chapter 2

# Installation

This chapter includes instructions for unpacking and setting up the Cytation3 and, if applicable, the external dispenser. Instructions are also included for preparing the reader and dispenser for shipment.

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## Product Registration

Please register your product(s) with BioTek to ensure that you receive important information and updates about the product(s) you have purchased.

Register online through BioTek's Customer Resource Center (CRC) at [www.biotek.com](http://www.biotek.com) or by contacting BioTek Customer Care at (888) 451-5171 or (802) 655-4740.

## Important Pre-Installation Information

	<p>This chapter contains installation and setup tasks for a Cytation3 reader that has all of the available modules (the "CYT3MFV" model). Your Cytation3 may be different. Perform the installation and setup tasks in the order presented, skipping those that do not apply to your reader's configuration.</p> <p><b>Materials:</b> You will need a Phillips screwdriver to perform some of the steps in this section. You will also need a small wrench; this item is supplied with the instrument.</p>
	<p>Remove the shipping hardware before turning on the instrument.</p> <p>Reinstall the shipping hardware before repackaging the instrument for shipping.</p>
	<p>The instrument with all available modules weighs up to <b>80 lbs. (36.3 kg)</b>. Use two people when lifting and carrying the instrument.</p>

## 1: Unpack and Inspect the Reader



The Cytation3 should be removed from the box by two people. The instrument with all available modules weighs up to **80 pounds (36.6 kg)**.

Save all packaging materials. If you need to ship the reader to BioTek for repair or replacement, you must use the original materials. 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, reader, and accessories for shipping damage. If the reader is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. BioTek will arrange for repair or replacement immediately.

1. Open the shipping box, remove the instrument from the box, and place it on a level, stable surface.
2. Place the packaging materials back into the shipping box for reuse if the instrument needs to be shipped again.
3. *For the instruments with the imaging module:* Open the accessories box, and remove the isolation table.

## 2: Unpack and Inspect the Dispenser



Save all packaging materials. If you need to ship the dispenser to BioTek for repair or replacement, you must use the original materials. 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, the dispenser, and accessories for shipping damage. If the dispenser is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. BioTek will arrange for repair or replacement of your dispense module immediately.

If applicable, perform these steps to unpack the dispenser. Refer to **Repackaging and Shipping Instructions** on page 34.

1. Open the shipping box. Remove the accessories box and foam insert that contains 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. The accessories should include the dispenser-related items listed under **Package Contents and Accessories** in Chapter 1.
4. Place all packaging materials into the shipping box for reuse if the dispenser needs to be shipped.

### 3: Unpack and Inspect the Gas Controller



Save all packaging materials. If you need to ship the gas controller to BioTek for repair or replacement, you must use the original materials. 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, the gas controller, and accessories for shipping damage. If the gas controller is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. BioTek will arrange for repair or replacement of your gas controller immediately.

If applicable, perform these steps to unpack the 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.

### 4: Select an Appropriate Location

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

Performance measurements, including detection limits, were verified up to 25°C.

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 reader 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.

### Installing Instruments with the Imaging Module

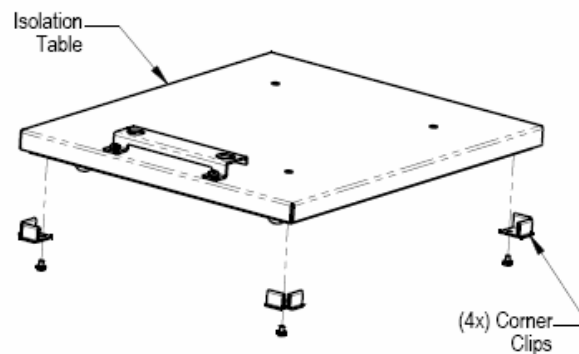


Instruments using the BioStack3 cannot be operated with the isolation table. Store the isolation table in a clean, dry location.

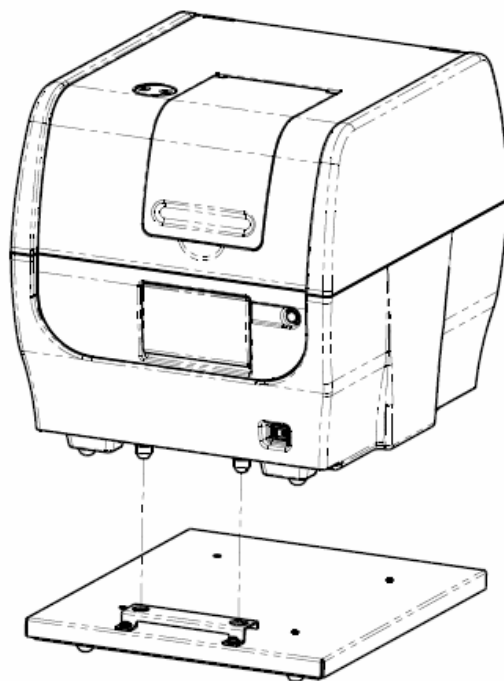
If you are installing the BioStack3 for operation with the Cytation3, you may wish to seat the instruments in their alignment plates now. Refer to the *BioStack3 Operator's Manual* for more information.

Cytation3 models with the imaging module can be used with an isolation table, which helps to eliminate vibration during image reads.

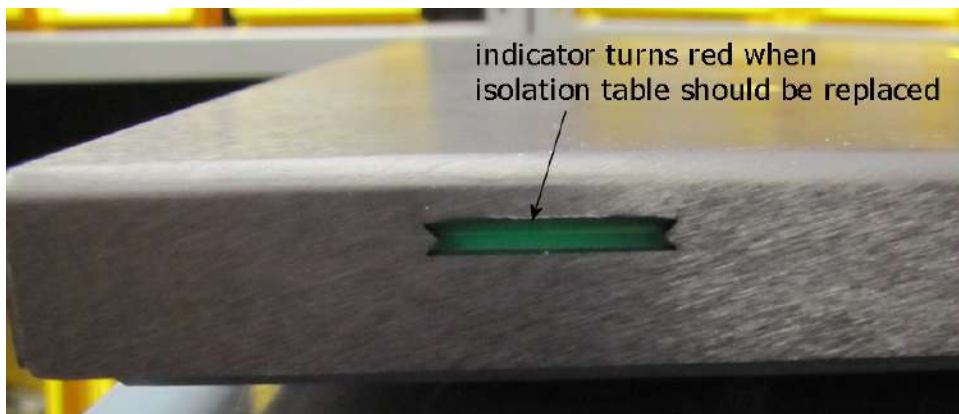
1. Remove the four corner clips from the isolation table.



2. Place the isolation table in the selected installation location.
3. Place the instrument on the table as shown below.



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.



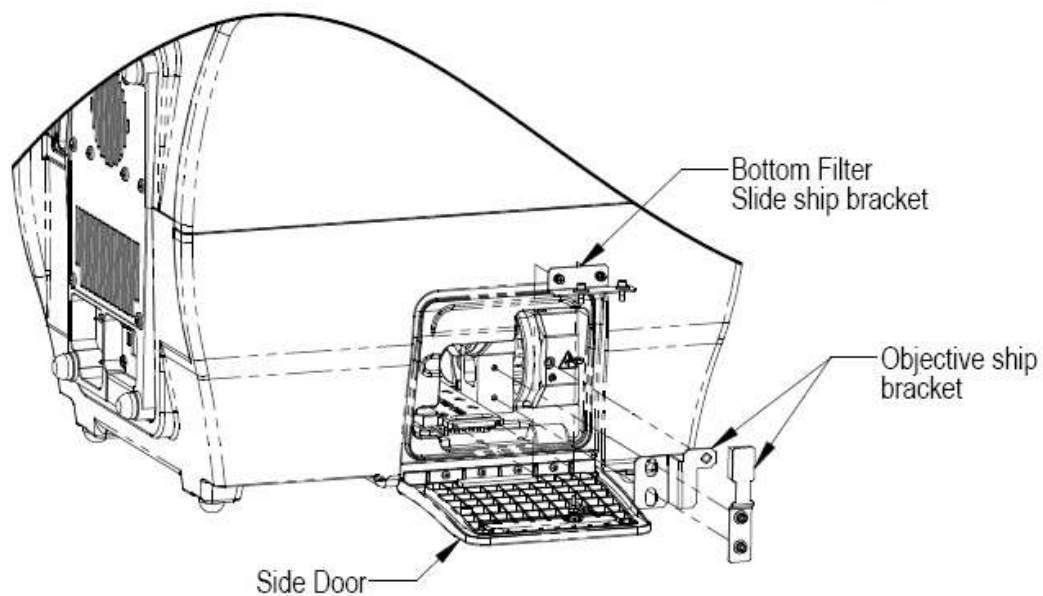
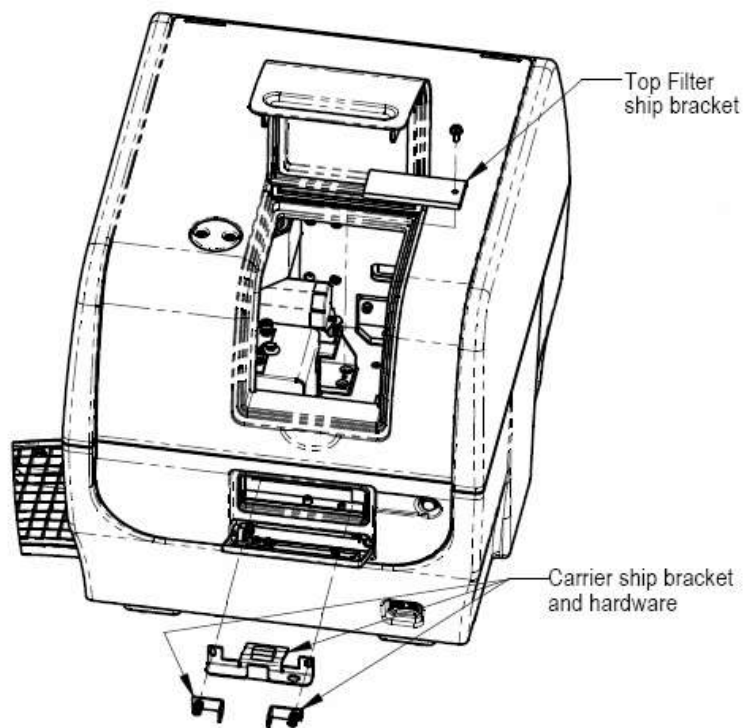
## 5: Remove the Shipping Hardware



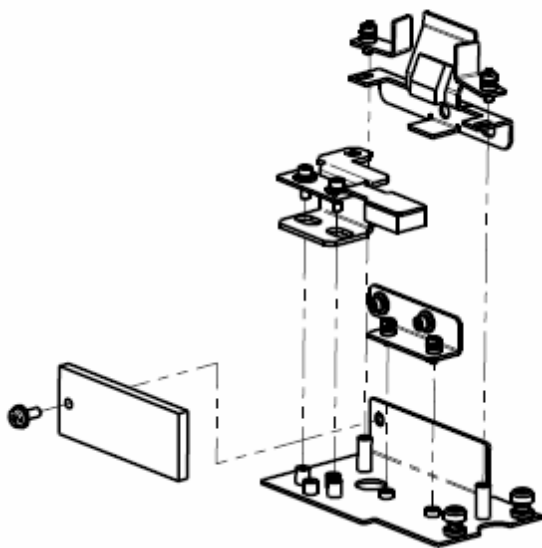
Remove all shipping hardware before you turn on the reader.

1. Locate the shipping hardware, shown in the next figure.

The figures below depict a Cytation3 with all modules installed.

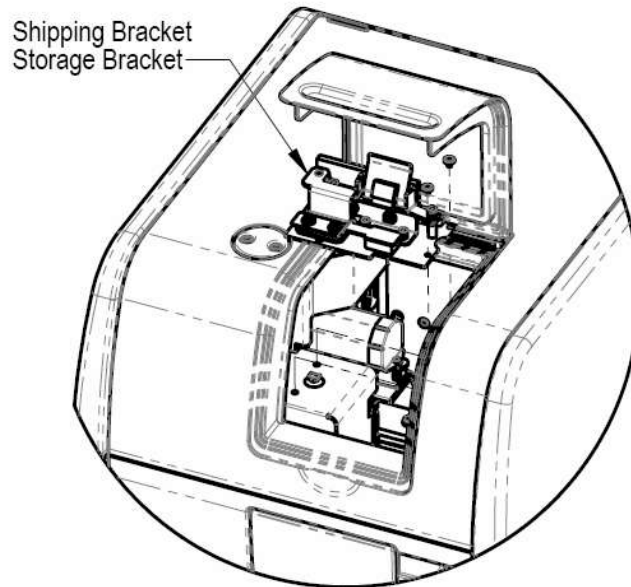


2. Using a #2 screwdriver, remove the carrier shipping bracket.
3. If equipped with the filter module, use the #2 Phillips screwdriver to remove the filter shipping bracket.
4. If equipped with the imaging module, use a 9/64" hex wrench to remove the bottom filter slide shipping bracket and objective turret shipping bracket.
5. Assemble all shipping hardware as shown in the next figure:



6. Install the assembled shipping brackets inside the instrument for safe keeping.





## 6: Install the Power Supply



**Power Rating.** The instrument 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.

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

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.

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

## 7: Install the Gas Controller (if applicable)

The gas controller is an external module that enables the user to control CO<sub>2</sub> and O<sub>2</sub> concentrations inside the attached instrument's reading chamber. If you purchased the module for operation with the Cytation3, refer to the *Gas Controller User Guide* for installation instructions.

## 8: Install the Dispenser

If you are installing the dispense module in addition to the gas controller module, place the dispenser on top of the gas controller unit. If you are not installing the gas controller module, place the dispenser on top of the reader.

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.

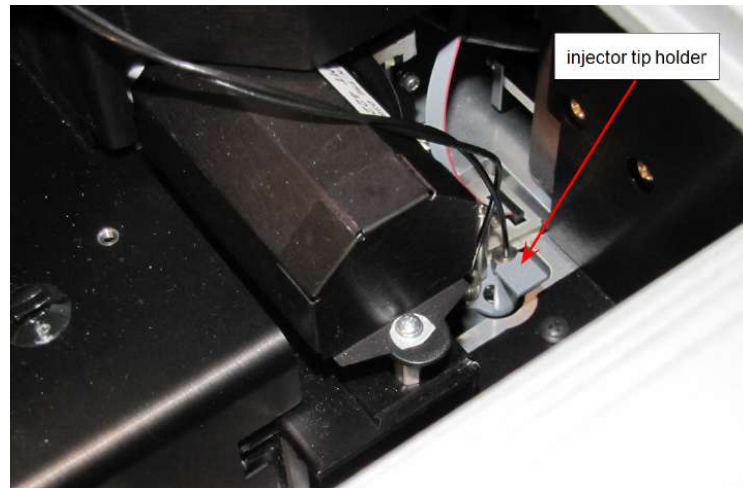
When installing the inlet and outlet tubes, do not use any tools. Finger-tighten only!

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

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

8. Remove the 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.
9. Thread the injector tip holder, with outlet tubing connected to both ports, through the hole in the top of the reader.

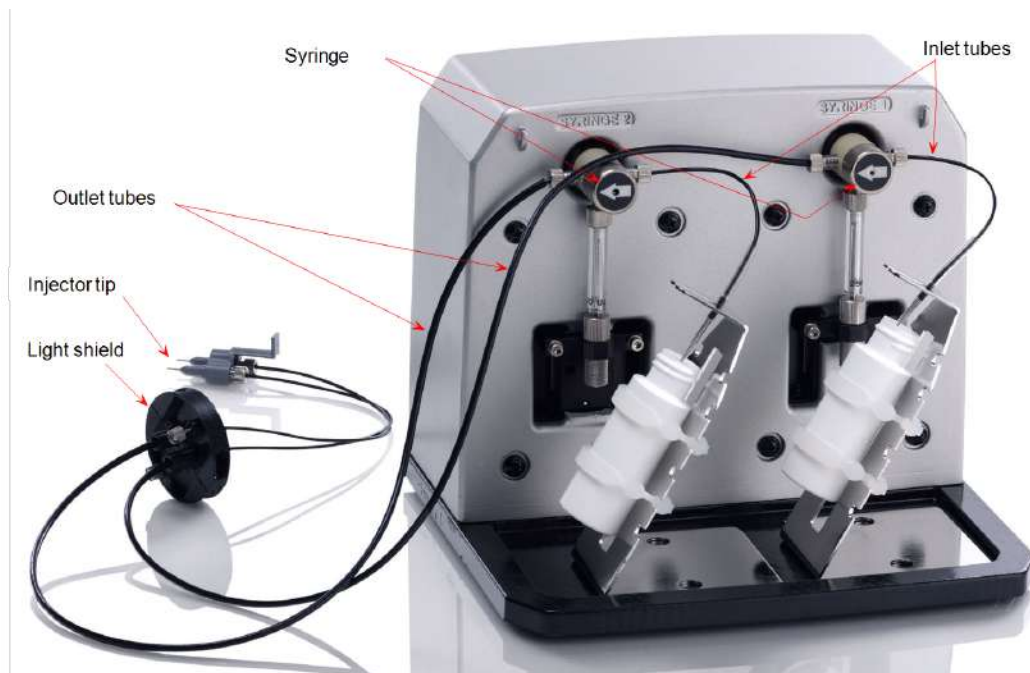
10. Open the door on the front of the reader, and, holding the injector tip holder by the tab, insert the injector tips into the appropriate holes inside the reader.



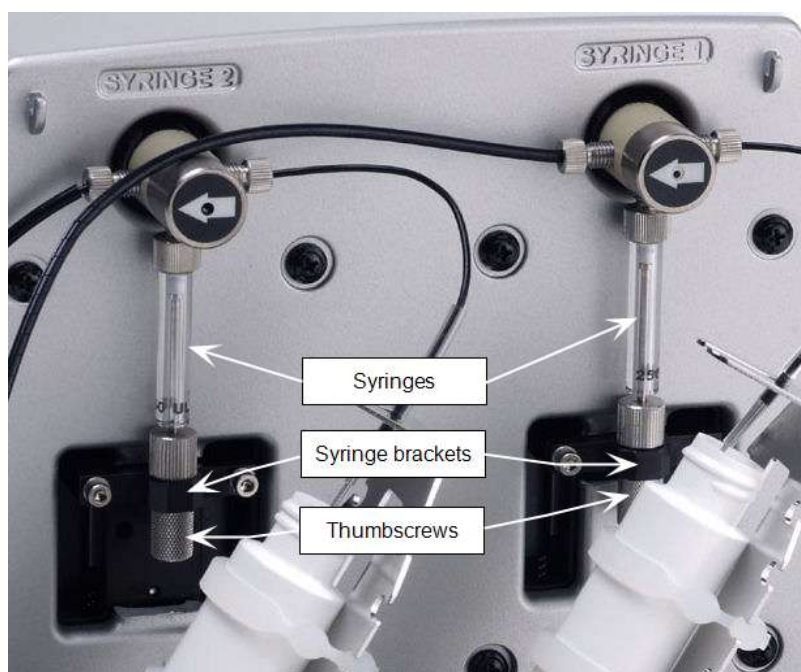
The injector tip holder in its socket

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

11. Place the light shield over the hole in the top of the reader and finger-tighten the thumbscrews to secure it.



12. Remove the two syringes from their protective boxes. They are identical and interchangeable. Each syringe should already be assembled in one piece, but if for some reason there are two separate pieces, assemble them now: Insert the white tip of the syringe plunger into the barrel of the syringe and gently push it all the way into the barrel.
13. 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.



14. 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.
15. Locate the injector tip-cleaning stylus, packaged in a small cylinder. Attach the cylinder to the back of the dispenser for storage.

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

## 9: Connect the Host Computer

The Cytation3 is equipped with up to three communication ports: Serial (RS-232) USB, and, for models with imaging capability, FireWire. All three ports are located on the back of the reader.

- The communication cables are included in the accessories box. Determine which cable is supported by the host computer.
- For RS-232 and USB connections: Connect one end to the appropriate port on the reader and the other end to the appropriate port on the host computer.
- For FireWire connection: See **Install the Imager Module** on page 21.

## 10: Install Gen5



The Cytation3 is controlled by Gen5 software running on a host computer. There is a certain sequence of events that **must** be followed to ensure that the software is properly installed and configured. Please follow the instructions provided in *Gen5 Getting Started Guide* to install the software.

## 11: Install the Imager Module

### 1. PC/Camera Setup

#### Install the PCI Express Card/IEEE-1394b in a Desktop Computer

To avoid electrostatic discharge and damage to internal components, ground yourself by using wrist grounding straps or by touching a metal surface on the computer's chassis.

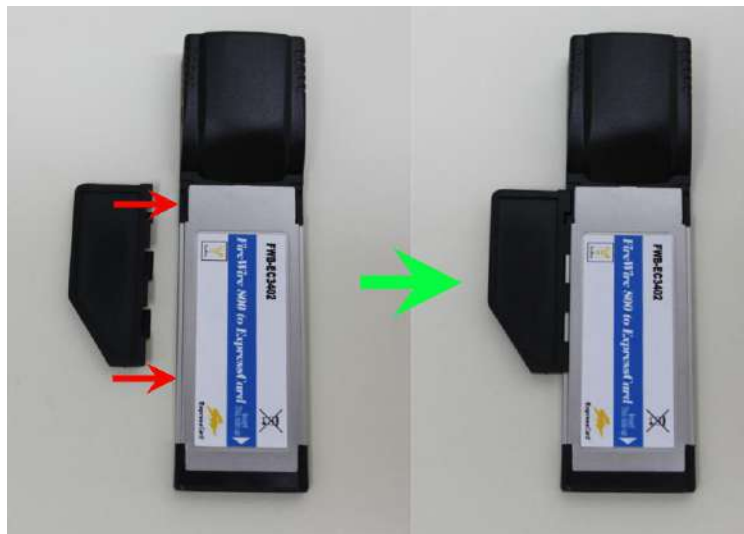
The following directions provide general steps for installing a PCI Express card. Talk to your company's IT representative for assistance with these steps. For more detailed help, contact BioTek TAC.

1. Turn off your computer, then remove the outside case.
2. Locate the PCI Express slot. Open the card retainer, and remove any existing graphics card (if necessary) or blank port located in the PCI Express slot.
3. Insert the PCI Express card, ensuring that it is aligned with the slot.
4. Press the card firmly into place, and rotate the card retainer back into place.
5. Replace your computer's outside case, then power on the computer.

### **Install the PCI Express Card/IEEE-1394b in a Laptop Computer**

1. Remove the PCI Express port cover or any existing PCI Express card from your laptop.
2. Touch a metal object to discharge any static electricity.
3. Remove the PCI Express card from the packaging.
4. Attach the spacer to the FireWire PCI Express card.

The spacer does not fit tightly. Ensure that it does not fall off.



5. Insert the FireWire Express card, with the label facing up, into the appropriate slot on your laptop.
6. If necessary, plug the power cord into the card, then plug the cord into a power outlet.

## Install the FireWire Driver

You must install the PCI Express card before performing this step.

The FireWire driver is included with the Gen5 installation files.

1. Navigate to the Gen5 program files on your computer, for example, C:\Program Files\BioTek\Gen5 2.04.
2. Open the Firewire Drivers folder, and then open the folder appropriate for your computer: Windows\_32 (for Windows 32-bit) or Windows\_64 (for Windows 64-bit).
3. Double-click InstallPGRDriver.bat to run the driver installer. When the installer is finished, a message, "SUCCESS: Installed package <path to package>". If you do not see this message, contact BioTek TAC.
4. Insert one end of the FireWire cable in the back of the reader, and insert the other end in the outlet on your computer (located in the card you installed).

## Establish Communication with the Camera

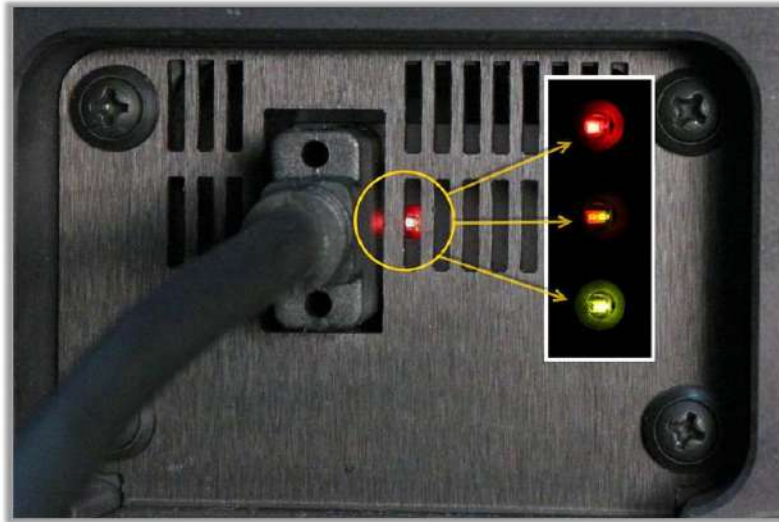
1. From the Gen5 main screen, select **System > Instrument Configuration**, select **Cytation3**, and then click **View/Modify**.
2. Click **Camera Info**. If communication is successful, Gen5 displays information about the camera.

For instructions on how to install the LED cubes and filter cubes and the objectives, see **Install the LED Cubes and Filter Cubes** on page 25 and **Install the Imaging Objectives** on page 27.

## Troubleshooting

- Have you established communication to the instrument first?
- Did you install the FireWire driver?
- Is the FireWire cable installed?
- Is there an LED light lit on the camera?
  - Red: Power to the camera from the FireWire card
  - Half Red/Half Green: Ready state

- Green: Communicating/activity



Successful communication between Gen5 and the FireWire cable

## 2. Hardware Setup

### Setting the Objective, LED Cube, and Imaging Filter Cube Configuration

When you install an LED cube, an imaging filter cube, or an objective, you must set the cubes' and objectives' configuration before physically changing or installing them.

If you physically install an LED cube, an imaging filter cube, or an objective before setting the new configuration, the instrument will fail its self-test.

1. From the main Gen5 screen, click **System > Instrument Configuration**.
2. Select the **Cytation3**, click **View/Modify > Setup**, and select the **Imaging Configuration** tab.
3. In the Objective Configuration area, select the objective for the position or positions you want to define, or select **None** if you are removing an objective from the instrument. Click **Move to Access Position**.
4. In the LED Filter Cube Configuration area, select the filter cube for the position you want to define, or select **None** if you are removing the filter cube from the instrument. The corresponding LED cube part number is filled in automatically.



5. Click **Send Values**.
6. Power down the instrument.

### **Install the LED Cubes and Imaging Filter Cubes**

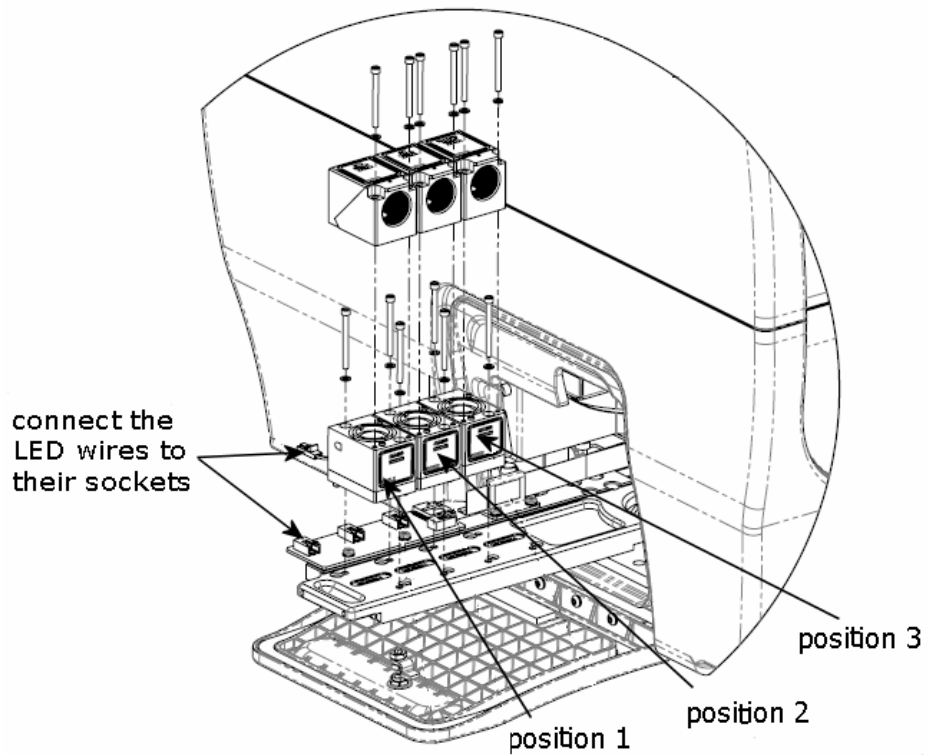
Do not open the side access door during operation. Doing so may affect measurements.

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.

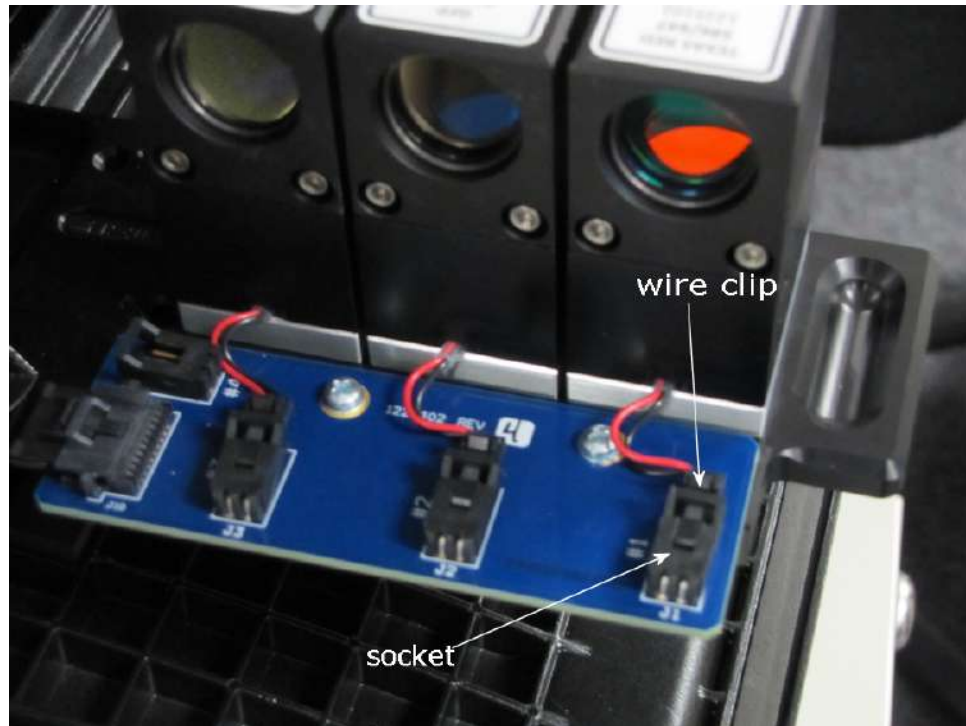
If you are changing the LED cubes and imaging filter cubes, see **Removing the LED Cubes and Imaging Filter Cubes**, below, for instructions, then continue with this procedure.

3. Place the new LED cube in the appropriate position (Position 1, 2, 3, or 4) on the filter slide.
4. Insert the screws into the LED cube, and screw them into the filter slide.
5. Place the imaging filter cube on top of the LED cube you installed in the previous step.
6. Using a hex wrench, attach the filter cube to the LED cube.

7. Insert the LED cube's wire clip into the socket on the carrier.



LED cubes and imaging filter cubes installed in positions 1, 2, and 3



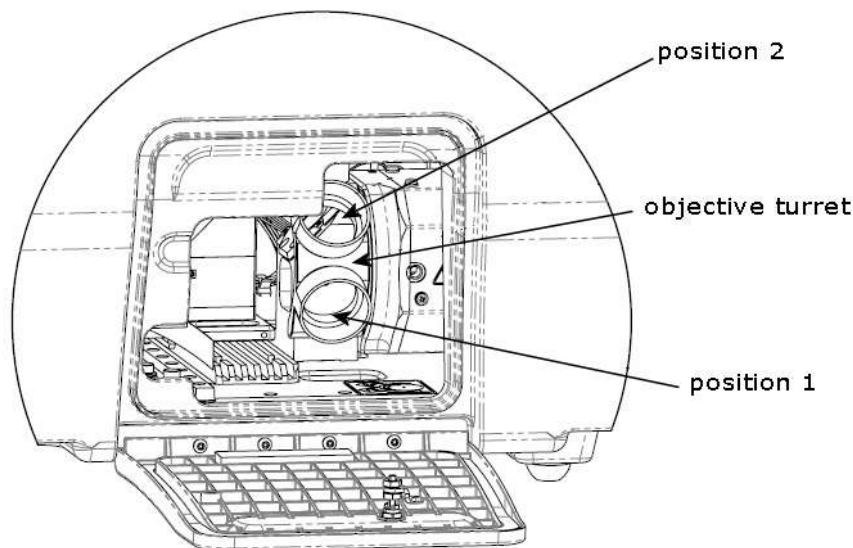
Rear view of LED cubes and filter cubes

8. Slide the filter slide back into the instrument.

### **Install the Imaging Objectives**

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

1. Turn the knob on the side access door to release the latch, and open the door.



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.

Some objectives use an adapter to fit the Cytation3 objective turret. Make sure to remove both the objective and its adapter.

3. Position the objective you want to install in the objective holder, and screw it in to secure it.
4. Close the side door

### Run Auto Calibration

After you have physically installed the LED cubes, imaging filter cubes, and objectives, you must run Auto Calibration before you can use the imaging module.

1. Turn on your instrument.
2. After the self-test is complete, in Gen5, go to **System > Instrument Configuration**, select Cytation3, then click **View/Modify > Setup**.
3. On the Instrument Configuration tab, click **Auto Calibration**.

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

## 12: Turn on the Reader

1. If Gen5 is open, close it now.
2. The reader's power switch is located on the lower-right corner of the front panel. Turn on the reader. The reader performs a system test. When the test is completed, the reader extends the microplate carrier.

The carrier eject button, located above to the reader's power switch, can be used to extend/retract the microplate carrier.

## 13: Establish Communications

If using the USB cable, refer to the instructions that shipped with the USB Driver Software CD to install the necessary drivers. The driver must be installed on the computer before you connect the instrument.

1. Start Gen5 and log in if prompted. The default System Administrator password is **admin**.
2. From the Gen5 main screen, select **System > Instrument Configuration** and click **Add**.
3. Set the Reader Type to **Cytation3**.
4. Perform one of the following steps:
  - Set the Com Port to the computer's COM port to which the reader is connected.

If using the USB cable, the information can be found via the Windows Control Panel, under Ports in the Hardware/Device Manager area of System Properties (e.g., Serial Port (COM5)).

- Select **Plug & Play**.

A Cytation3 must be connected via USB to the computer and turned on to appear in the Available Plug & Play Readers list.

5. To test that Gen5 can communicate with the instrument, click **Test Comm** and **Camera Info**. If the communication attempt is successful, Gen5

displays a success message or information about the camera. Return to Gen5's main screen.

## Communication Errors

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

- Is the reader connected to the power supply and turned on?
- Is the communication cable firmly attached to both the reader and the computer?
- Did you select the correct Reader Type in Gen5?

### USB Cable:

- Try a different COM Port.
- Did you install the USB driver software?

If you remain unable to get Gen5 and the reader to communicate with each other, contact BioTek's Technical Assistance Center.

## 14: Run a System Test

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

1. Turn on the incubator:
  - From the Gen5 main screen, select **System > Instrument Control > Cytation3**.
  - Click the **Pre-Heating** tab.
  - Enter a Requested temperature of at least 37°C and click **On**.
  - Return to Gen5's main view.

Wait until the incubator temperature reaches the set point before continuing.

2. Select **System > Diagnostics > Run System Test**. If prompted to select a reader, select Cytation3 and click **OK**.
3. When the test is completed, a dialog requesting additional information appears. Enter the information and click **OK**.

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

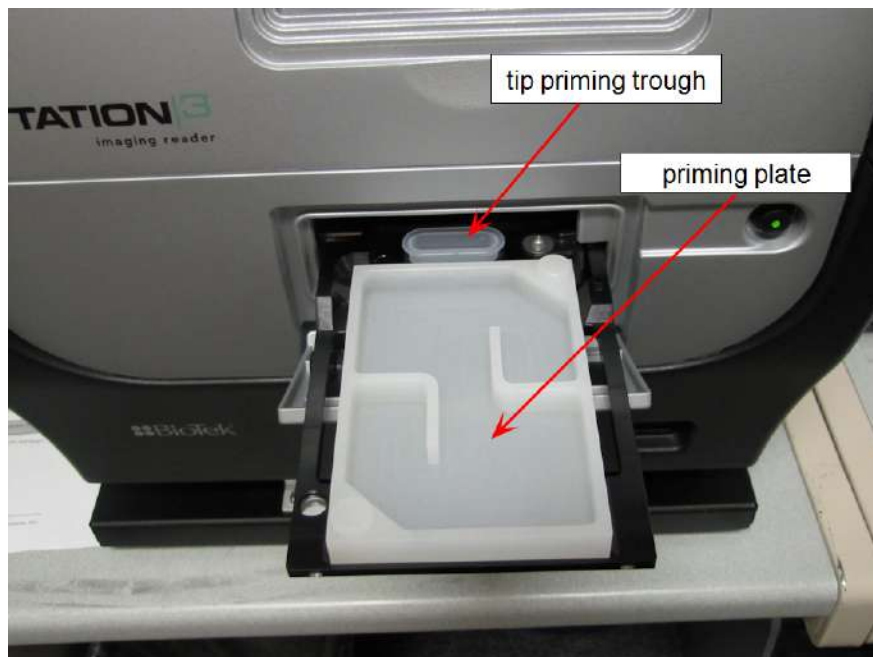
4. The results report appears. Scroll down toward the bottom; the text should read "SYSTEM TEST PASS."
  - You may wish to print the report and store it with your records.
  - The Gen5 software stores system test information in its database; you can retrieve it at any time.

If an error code is returned, refer to **Appendix B** and look up the code. If the problem is something you can fix, do so now and run another system test. If the problem is something you cannot fix, or if the test continues to fail, contact BioTek's Technical Assistance Center at (800) 242-4685 or (802) 655-4740.

5. Turn off the incubator:
  - Select **System > Instrument Control > Cytation3**.
  - Click the **Pre-Heating** tab and click **Off**.
  - Return to Gen5's main view.

## 15: Test the Injector System

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. From the Gen5 main screen, select **System > Instrument Control > Cytation3**.
6. Click the **Dispenser** tab.
7. With Dispenser set to **1**, set the Volume to **5000  $\mu\text{L}$**  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 BioTek's Technical Assistance Center.
8. When the prime finishes, set Volume to **2000  $\mu\text{L}$**  and click **Purge** to clear the fluid lines.
9. Set Dispenser to **2** and repeat steps 7 and 8.
10. When finished, remove and empty the priming plate.
11. Close the software.

The installation and setup process is complete.



## Operational/Performance Qualification




Your Cytation3 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, turn to **Chapter 7, Instrument Qualification** now to learn about BioTek's recommended OQ/PQ procedures for Cytation3.

A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Cytation3 is available for purchase (PN 1220514). Contact your local BioTek dealer for more information.

## Repackaging and Shipping Instructions

**Important! Please read all of the information provided below before preparing the Cytation3 for shipment.**

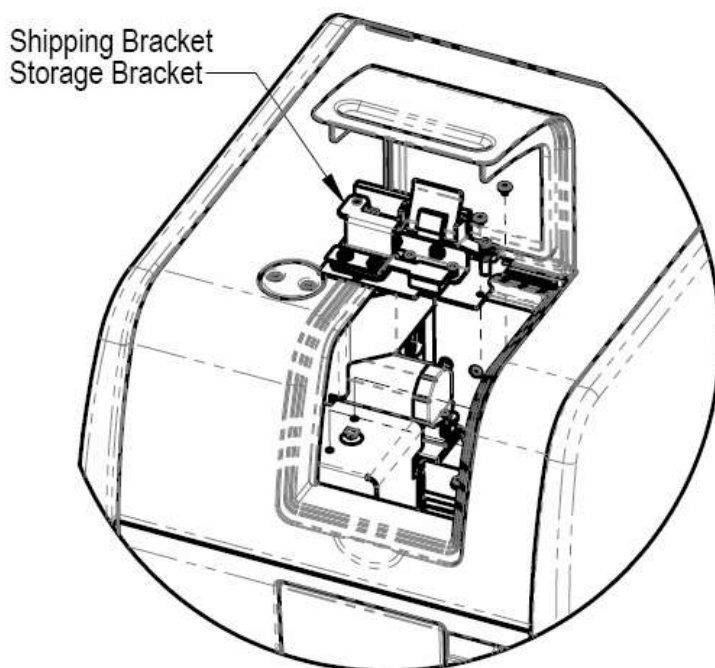
	<p>If the reader and/or dispenser has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the reader during shipping, handling, and servicing. Decontamination prior to shipping is required by the U.S Department of Transportation regulations. See the <b>As-Needed Maintenance</b> chapter for decontamination instructions.</p> <p>Remove the microplate and tip prime trough (if equipped) from the carrier before shipment. Spilled fluids can contaminate the optics and damage the instrument.</p>
	<p>The instrument with all available modules weighs up to <b>80 lbs. (36.3 kg)</b>. Use two people when lifting and carrying the instrument.</p>
	<p>The instrument's packaging design is subject to change. If the instructions in this section do not appear to apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance.</p> <p>Replace the shipping hardware before repackaging the reader. Please contact BioTek if you have misplaced any of these items.</p> <ul style="list-style-type: none"> <li>• Carrier shipping bracket (PN 1220510)</li> <li>• Top filter shipping bracket (PN 8042187)</li> <li>• Bottom Filter Slide shipping bracket (PN 1220516)</li> <li>• Objective shipping bracket (PN 1220515)</li> <li>• Shipping bracket storage bracket (PN 1222208)</li> </ul> <p>If you need to ship the Cytation3 and/or the dispense module to BioTek for service or repair, be sure to use the original packaging materials. Other forms of commercially available packaging are not recommended and can void the warranty.</p> <p>The shipping materials are designed to be used no more than five times. If the original materials have been damaged, lost, or used more than five times, contact BioTek to order replacements.</p>

1. Contact BioTek's Technical Assistance Center for an RMA (Return Materials Authorization) number and the shipping address 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 you will also be shipping the dispense module, see **Preparing the Dispenser for Shipment** on page 38.

If you are not shipping the dispenser, disconnect it from the reader now.

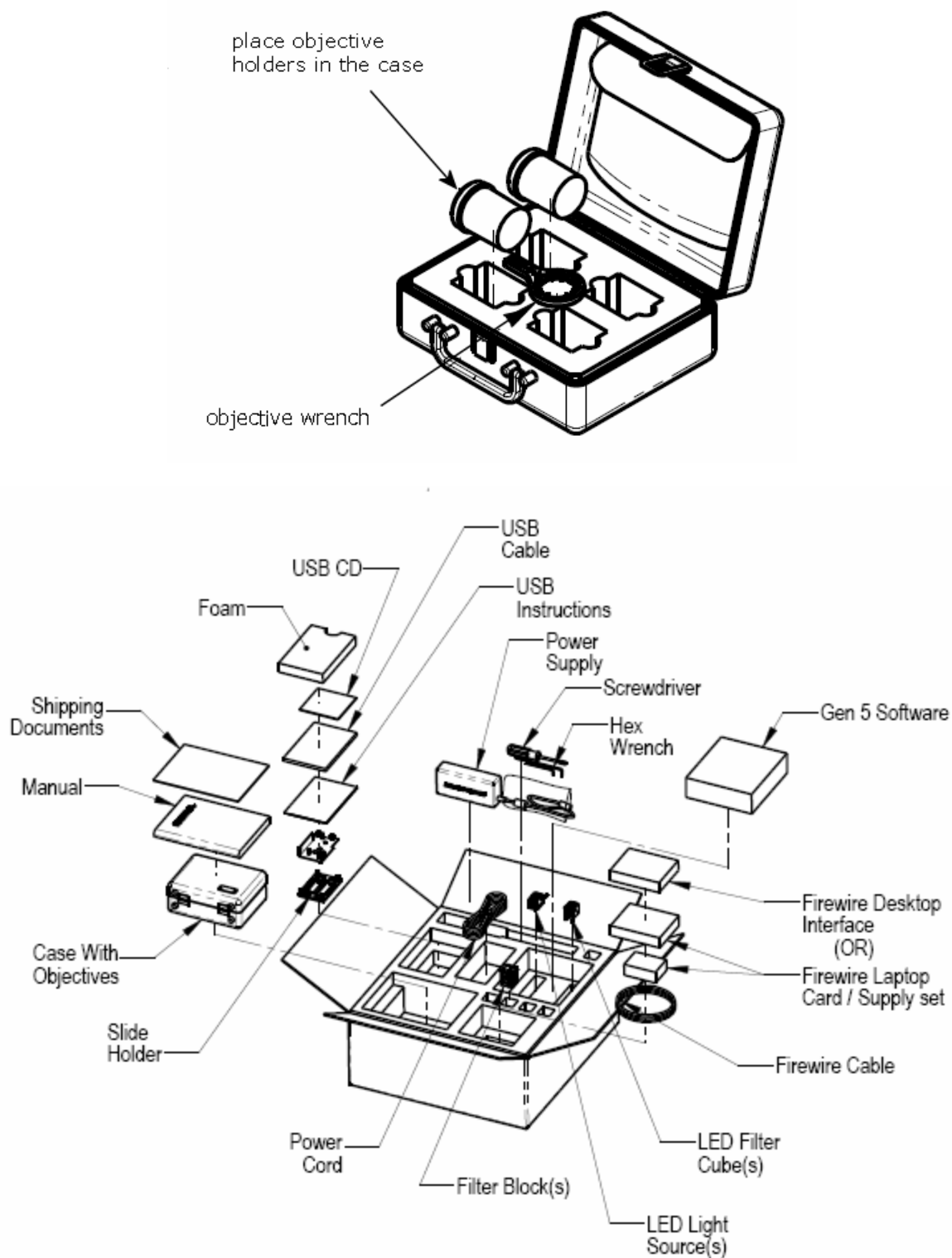
4. If applicable, disconnect the gas controller, and lift it off the top of the instrument.
5. If you have not already done so, retract the microplate carrier and then turn off and unplug the reader.
6. Remove the shipping bracket storage bracket from the instrument, and remove each shipping bracket from the storage bracket.

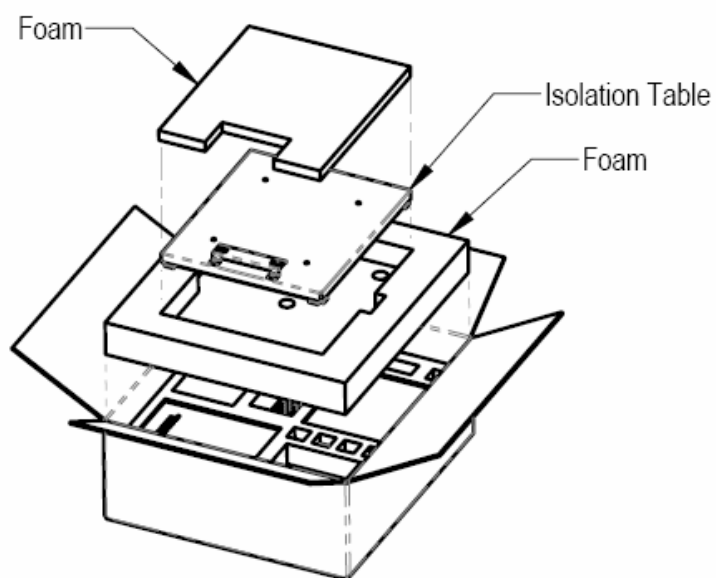


Removing the shipping bracket storage bracket

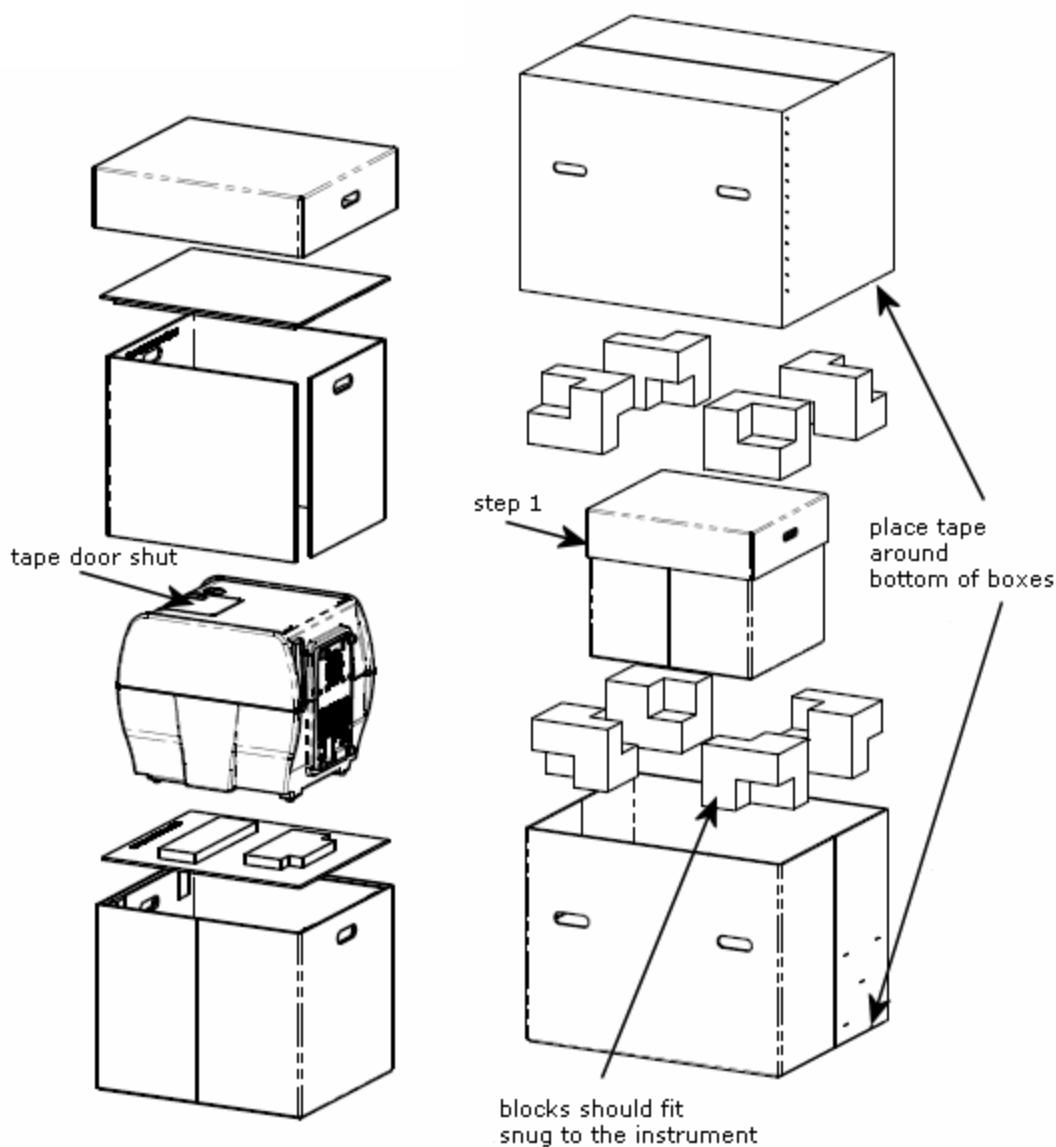
7. Install the carrier shipping bracket and, if applicable, the filter reader shipping bracket, the bottom filter slide shipping bracket, and the objective shipping bracket. See "Remove the Shipping Hardware" on page 14 for descriptions of this hardware.

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. Refer to the next figure.

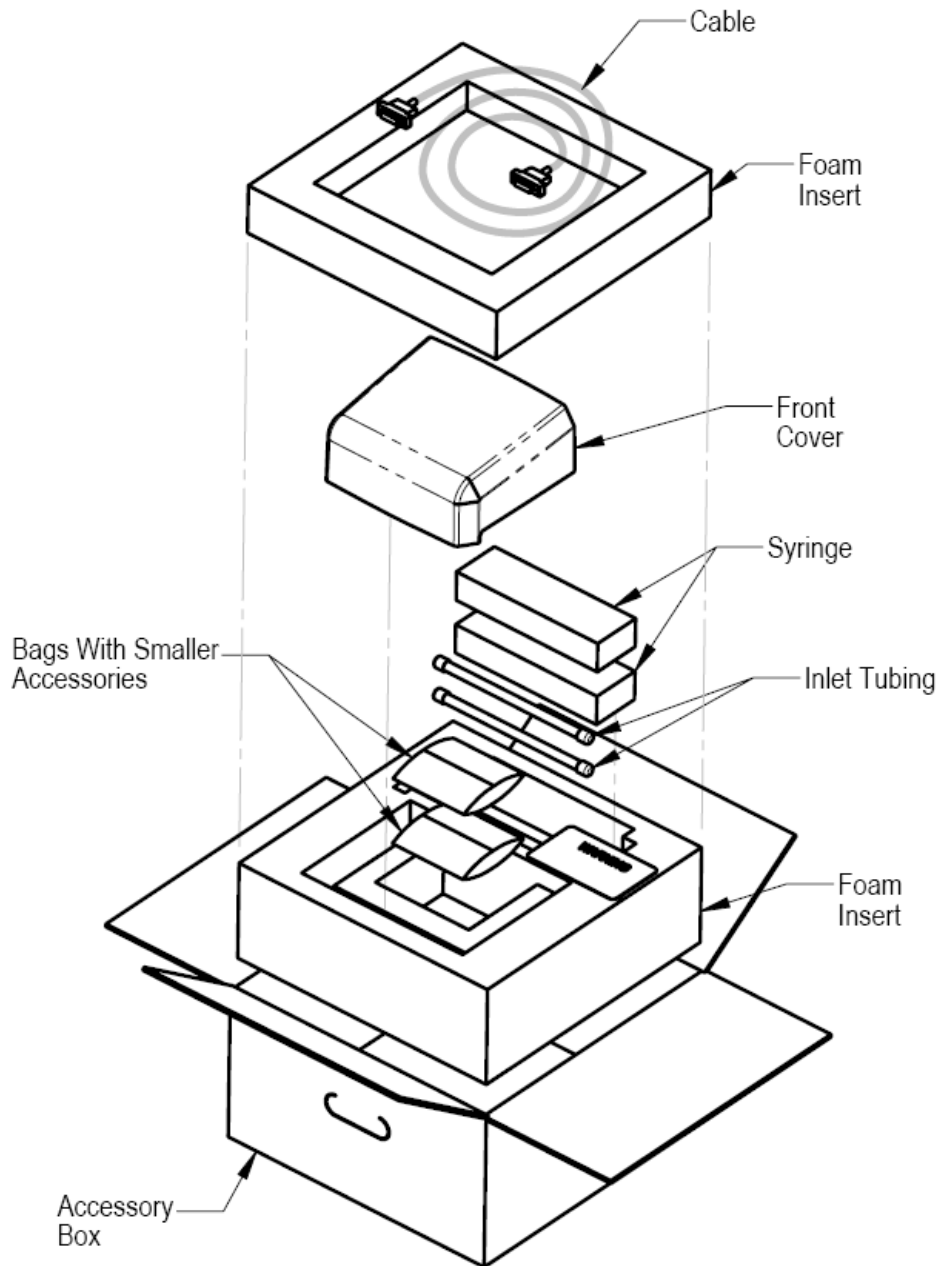


## Preparing the Dispenser for Shipment

1. If you have not already done so, contact BioTek's Technical Assistance Center for an RMA (Return Materials Authorization) number and the shipping address before returning equipment for service.
2. Decontaminate the dispenser according to the instructions in the **As-Needed Maintenance** chapter. Be sure to purge the dispenser of all fluid when finished.
3. With the reader on, start Gen5 and select **System > Instrument Control > Cytation3**.

4. Perform this step twice, for both dispensers: Click the **Dispenser** tab and set the dispenser number (1 or 2). Click **Maintenance**. The syringe bracket lowers. Remove the thumbscrew from underneath the bracket. Carefully unscrew the top of the syringe from the syringe valve. Lift out the syringe and store it in its original box.
5. Fully detach the dispenser from the reader. (The screws are stored in the plastic bag attached to the back of the dispenser.) Set the dispenser aside for the moment.
6. Remove the tip priming trough and store it in the dispenser accessories bag.
7. Remove the two inlet tubes from the syringe valves and store them in their plastic canisters.
8. Remove the two outlet tubes from the syringe valves. Attach the clear plastic shrouds to the fittings of the outlet tubes. Place the tubes in a plastic bag.
9. Remove the front cover from the dispenser.

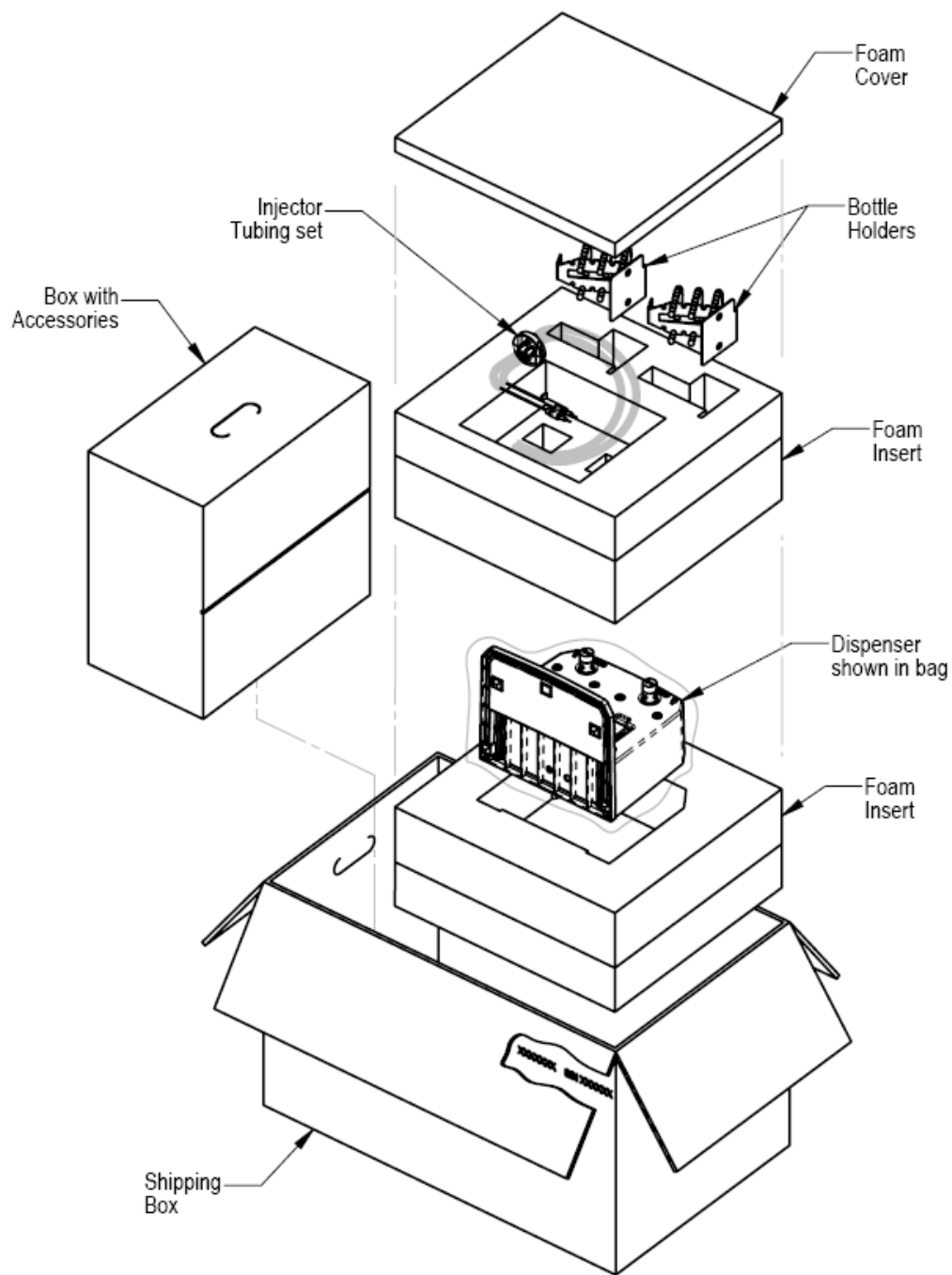
10. Insert the bottom foam end cap in the dispenser accessories shipping box and place the accessories in the insert.



11. Insert the bottom foam end cap in the shipping box, and place the dispenser inside the end cap.
12. Insert the foam insert that holds the reagent bottle holders and injector tubing into the shipping box and place the bottle holders and tubing in it.
13. Slide the dispenser accessories box into the shipping box.



14. Insert the top foam end cap. Close and seal the outer box with tape.
15. Write the RMA number on the outside of the box. Ship the box to BioTek.





## Chapter 3

# Getting Started

This chapter describes some of the Cytation3's external and internal components, and provides an introduction to using BioTek Gen5 software to control the instrument.

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## Modular Design

The Cytation3 is a multi-mode 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 Customer Care 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 Cytation3 model; for example, a reader with all capabilities is an **CYT3MFV**. This is indicated on a label on the reader.

Identifier	Module Description
CYT3V	Base model: imaging, incubation, shaking, and gas ready
CYT3MV	Base model with top and bottom monochromator-based optics
CYT3FV	Base model with top filter-based optics
CYT3MFV	Base model with both top and bottom monochromator-based optics and top filter-based optics
CYT3M	Non-imager base model with top and bottom monochromator-based optics
CYT3F	Non-imager base model with top filter-based optics
CYT3MF	Non-imager base model with both top and bottom monochromator-based optics and top filter-based optics

## External Components



1	The entry for the dispense outlet tubes and injectors (if equipped)
2	Filter cube access door
3	The microplate carrier eject button
4	The light-blocking microplate carrier access door
5	The power switch
6	LED cubes, imaging filter cubes, and objectives access door
7	Gas controller hookup (on back of instrument)

## Internal Components

Component	Description	Page
Filter Cube	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.  <b>Note:</b> These cubes are not to be confused with the imaging LED cubes and filter cubes.	page 46
Injector System	<b>Applies to models with the dispense module.</b> The syringes may require replacement over time. The tubing and injectors require cleaning at regular intervals.	page 47
Imaging System	<b>Applies to models with the imaging module.</b> The imaging system, comprising a CCD camera, objectives, LED cubes, and filter cubes, allows you to run experiments with imaging reads as well as view images in live mode.	page 50

### Filter Cube

The Cytation3 is 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 through a hinged door on the front of the instrument.

Synergy H1 filter cubes are interchangeable with the filter cubes for the Cytation3.

Do not open the doors to access the filter cube during instrument operation. Doing so may result in invalid data.

### Define the Filters

Gen5 keeps track of each cube's contents and communicates this information to the instrument during operation. If you change the filter cube, you must update Gen5's Filter Cube Table (**System > Instrument Configuration > Setup**).

- Select **Band Pass**, **Long Pass**, or **Short Pass**, as appropriate for each filter type.

- **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.
- Select **PLUG** to indicate the presence of a plug.
- Select **HOLE** to indicate an empty location.

Learn how to change the filter cube in **Chapter 4, Filters and Mirrors**.

See page 50 for information on updating the Gen5 Filter Cube Table.

### 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.

1. Click **System > Optics Library > Filter Cubes**.
2. Select the checkbox in the On Site column for the filter cubes you have available.

### Injector System

The tubing and injectors should be cleaned at least every three months. See **Chapter 5, Preventive Maintenance**, for instructions.

Inspect the injector system daily for leaks, preferably immediately after priming and whenever tubing changes have been made.

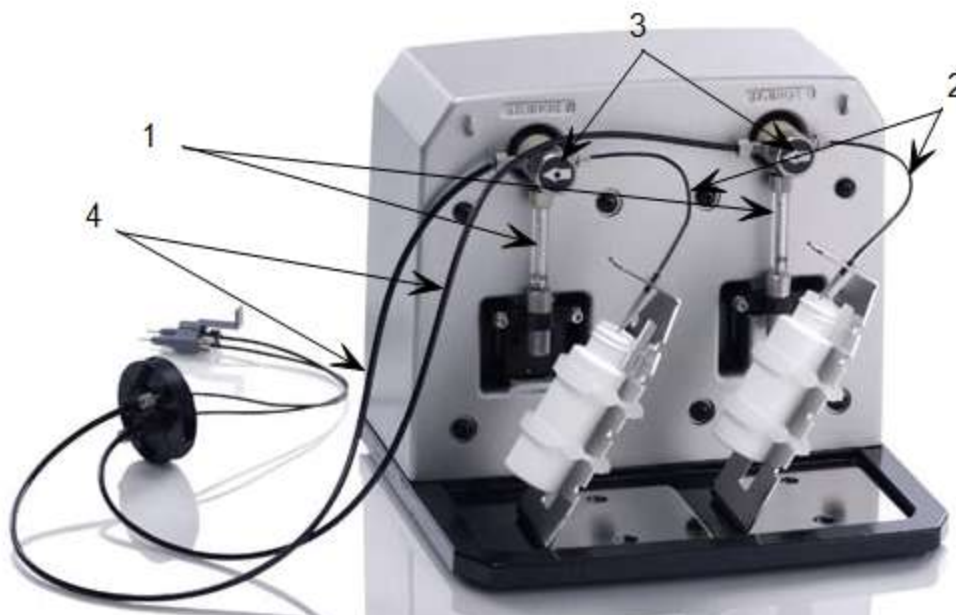
If a syringe is leaking, it may need to be replaced. See **Chapter 6, As Needed Maintenance**, for instructions.

### Dispense Module



Each dispense module is calibrated to perform with a specific Cytation3 reader. Make sure the dispense module and the reader have the same serial number.

The dispense module sits on top of the reader 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  $\mu$ L 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 Cytation3's top cover. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

### Dispense Module Components and Materials Composition

Continuous contact with harsh chemicals is not recommended. Always rinse the fluid path with deionized water after contact with any strong acid, base, or solvent.

#### Components

#### Material Composition

Tubing, syringe fittings

PTFE (polytetrafluoroethylene)



Components	Material Composition
Injector tips	315 stainless steel
Injector body	PVC (polyvinyl chloride)
Priming plate and trough	Polypropylene
Valve diaphragms	Ethylene propylene (EPDM)
Valve body	PEEK (polyether ether ketone)
Syringe barrel	Borosilicate glass

See the **Preventive Maintenance** chapter for cleaning instructions.

### 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.

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, and is used for performing 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.

**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 tray should be empty before priming and contain fluid after priming.

## Imaging Module

Instruments with the imaging module can perform image reads, view and capture images in manual mode, and save the images for later analysis. The imaging module comprises a CCD digital camera, up to four LED cubes and four imaging filter cubes, a bright field channel, and two objectives. The imaging module supports two modes, manual mode and experiment mode.

### Camera

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

### LED Cubes and Filter Cubes

The LED cubes and imaging filter cubes are located behind a door on the left side of the instrument and are user-changeable. See **LED Cubes and Imaging Filter Cubes** on page 63 for more information.

### Objectives

The objectives are also located next to the LED cubes and imaging filter cubes and are held in an objective turret. Gen5 supports two user-changeable objectives being installed simultaneously.

### 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 include an image read step as an endpoint read or in a kinetic block in your procedures and experiments. A procedure containing an image read step can also include other steps, such as a different detection mode read, dispense steps, shake steps, and incubation, if supported by the instrument.

## Gen5 Software

BioTek Gen5 software supports all Cytation3 reader models. Use Gen5 to control the reader, the imaging module (if equipped), the dispense module (if equipped), and the stacker (if equipped); perform data reduction and analysis on the measurement values; print or export results; and more. This section provides brief instructions for working with Gen5 to create protocols and experiments and read plates. Refer to the Gen5 Help system for more information.

## Define the Filter Cube

The reader's onboard software is configured with the filter and mirror values and their locations in the filter cube. When Gen5 communicates with the reader, it "asks" for this information and then stores it in a Filter Cube table. If you make any changes to the filter cube, you must define the changes in Gen5 and send the information to the reader.

- Select **System > Instrument Configuration**. Highlight the Cytation3, and click **View/Modify**.
- Click **Setup** and then click the **Filter Cube** tab. Refer to the Gen5 Help system for additional instructions.

## Protocols and Experiments

In Gen5, a protocol contains instructions for controlling the reader and (optionally) instructions for analyzing the data retrieved from the reader. At a minimum, a protocol must specify the procedure for the assay you wish to run. After creating a protocol, create an experiment that references the protocol. You'll run the experiment to read plates and analyze the data.

These instructions briefly describe how to create a protocol in Gen5. See the Gen5 Help system for complete instructions.

1. Open a new protocol.
2. Open the Procedure dialog. If prompted to select a reader, select the **Cytation3** and click **OK**.
3. Select a Plate Type.

The assay plate must match the plate type selected in Gen5. Otherwise, the results of the read may be invalid.

For imaging reads, you must first define the bottom elevation in the Plate Type Database. See the Gen5 Help for instructions.

4. Add steps to the procedure for shaking or heating the plate, dispensing fluid, reading the plate, and more. Click **Validate** to verify that the reader supports the defined steps, and then click **OK**.

Optionally, perform the next steps to analyze and report the results:

5. Open the Plate Layout dialog and assign blanks, samples, controls, and/or standards to the plate.

6. Open the Data Reduction dialog to add data reduction steps. Categories include Transformation, Well Analysis, Curve Analysis, Cutoff, and Validation.
7. Create a report or export template via the Report/Export Builders.
8. Select **File > Save** and give the file an identifying name.

These instructions briefly describe how to create an experiment and then read a plate in Gen5. See the Gen5 Help system for complete instructions.


1. Open a new experiment.
2. Select the desired protocol and click **OK**.
3. Select a plate in the menu tree and read it.
4. When the read is complete, measurement values appear in Gen5. Select the desired data set from the Data list.
5. Select **File > Save** and give the file an identifying name.

## Imaging Module: Manual Mode

*Applies only to models with the imaging module.*

The following sections **briefly** describe how to use the Gen5 imaging module in manual mode. See the Gen5 Help for more complete instructions and descriptions of these features.



### Focusing the Camera

1. From the Task Manager, select **Read Now > Run Imager Manual Mode**. Alternatively, from the main Gen5 screen, click  .
2. Click **Auto Expose**, or manually adjust the Exposure settings until you see an image.
3. Click **Auto Focus**, or click the arrow buttons until the image is in focus.
4. Repeat these two steps until the image is exposed and focused to your liking.

You can also select **Find Image** to have Gen5 automatically expose and focus the image.

## Capture and Save an Image

After you have exposed and focused an image, you can capture and save it for later analysis.

1. When an image in the Capture dialog is exposed and focused to your liking, click  to capture it.
2. To save the image or images you capture, click **Review**, click  to perform any additional brightness and contrast adjustments, then click **Save**.

Any adjustments made using the B&C (brightness and contrast) dialog are for display purposes only; the changes do not affect the data measurements from the images.

## Analyze Captured and Saved Images

1. With your captured or saved images displayed in the left panel of the Capture dialog, click **Review**.
2. In the Review dialog, you can further adjust the brightness and contrast of the image, if necessary.
3. Click **Analyze**.
4. In the Analyze Tool, select which type of analysis you want to perform and define the parameters for the analysis, then click **Start**. The results are displayed in the right pane.

## Imaging Module: Experiment Mode

*Applies only to models with the imaging module.*

The following sections **briefly** describe how to use the Gen5 imaging module in experiment mode. See the Gen5 Help for more complete instructions and descriptions of these features.

### Perform an Image Read

The following steps briefly describe how to set up a protocol with an imaging read step. See the Gen5 help for more detailed instructions.

1. Create a new protocol.
2. Select **Image** as the Read Method.

3. (Optional) Enter a step label or unique name for this step. Data sets of the reading results will use the label in online views, reports, and export files.
4. Select the Objective.
5. Define whether to read a full plate or specific wells.
6. Define up to four channels to read, defining the color filter and exposure settings and selecting which well are to be read for each color channel.
7. Select whether to create a single image for each well. If this box is cleared, you are prompted to define a montage read.
8. Define the horizontal and vertical offset from the center of the well.
9. Define advanced options, if needed.

After you have defined the protocol, you can create an experiment and read the plate:

1. Open a new experiment.
2. Select the protocol you created with the imaging read step, and click **OK**.
3. Select a plate in the menu tree and read it.
4. When the read is complete, images and measurement values appear in Gen5. Select the desired data set from the Data list.
5. Select **File > Save** and give the file an identifying name.

## Using the Slide Holder

### Manual Mode


1. When prompted upon entering manual mode, select **Slide Holder** as your plate type.
2. The slide holder plate map contains two well positions that correspond with the two slide locations on the slide holder. Select the well position that contains the slide you want to image.
3. By default, the carrier moves so that the middle of the selected slide position is below the objective. However, the sample on your slide may be in a different location on the slide. To find the sample:
  - a. Select your lowest-power objective.
  - b. Select either **Small step** or **Large step**, then use the arrow buttons to move the slide around until you find your sample. The cross in the slide display to the right of the arrow buttons shows the general position of

the image in relation to the slide. The back arrow to the left of the slide display shows the direction that the slide enters the read chamber.



4. After you find your sample, you can change to a higher-power objective, if desired, or define a Read step in a protocol using the x- and y-coordinates of your sample, which are displayed below the Well button.

### Experiment Mode

1. In an experiment, select **Slide Holder** 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. Follow steps 2 and 3 in the manual mode procedure (above) to find your samples, then click **Save settings**. Gen5 imports your exposure settings and the values for Horizontal and Vertical offset from the center of the well to your read step.
4. Select **Auto** to turn Auto Exposure back on. Gen5 will auto-expose your image, retaining the well offset imported from manual mode.

You can also enter the values for Horizontal and Vertical offset from center of well if you had determined them during previous testing.

### Dispense Module Control

*This section applies only to models with injectors.*

Gen5 is used to perform several dispense functions, such as initialize, dispense, prime, and purge. The Prime and Purge functions are introduced here. See the Gen5 Help system for more information.

Priming and purging routines are used to clean the fluid paths; see also **Flushing/Purging the Fluid Path** in the **Preventive Maintenance** chapter.

### 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, select **System > Instrument Control > Cytation3** and click the **Dispenser** 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  $\mu\text{L}$ .
6. Select a prime Rate, in  $\mu\text{L}/\text{second}$ .
7. Click **Prime** to start the process.
8. When finished, carefully remove the priming plate from the carrier and empty it.

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, select **System > Instrument Control > Cytation3** and click the **Dispenser** tab.
2. Select the Dispenser number (1 or 2) associated with the supply bottle.
3. Enter the desired purge Volume in  $\mu\text{L}$  (e.g., 2000).
4. Select a prime Rate in  $\mu\text{L}/\text{second}$ .
5. Click **Purge** to start the process.

## Recommendations for Optimum Performance

### General

- Microplates should be clean and free from 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.
- Although the Cytation3 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.

- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results in most cases, use at least 100  $\mu$ L per well in a 96-well plate and 25  $\mu$ L 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.

## Read Direction

The Cytation3 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 Cytation3'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 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 **Preventive Maintenance** chapter for more information.
- When dispensing volumes less than or equal to 20  $\mu\text{L}$ /well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20  $\mu\text{L}$ /well, we recommend a tip prime volume of 20  $\mu\text{L}$ .
- 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.

## Chapter 4

# Filters, Filter Cubes, Mirrors, LED Cubes, and Objectives

The **Getting Started** chapter provided an overview of the filters, mirrors, and objectives installed in some Cytation3 models. This chapter provides more detailed information on working with these components.

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## Filter Cubes

For information about the LED cubes and imaging filter cubes, see page 63.

### Overview

Most Cytation3 models are equipped with excitation and emission filters and mirrors for use when taking fluorescence and luminescence measurements. Each filter cube contains two filter sets, each of which contains one excitation filter, one emission filter, and one mirror. The filter cube is accessed through a hinged door on the front of the instrument. You can mark the label on the front of the filter cube with the contents of the cube.



You can easily exchange the Cytation3's filter cube to meet your assay requirements. If you regularly need to change the filters or mirrors on the reader, consider purchasing additional filter cubes from BioTek to make the process easier and faster.

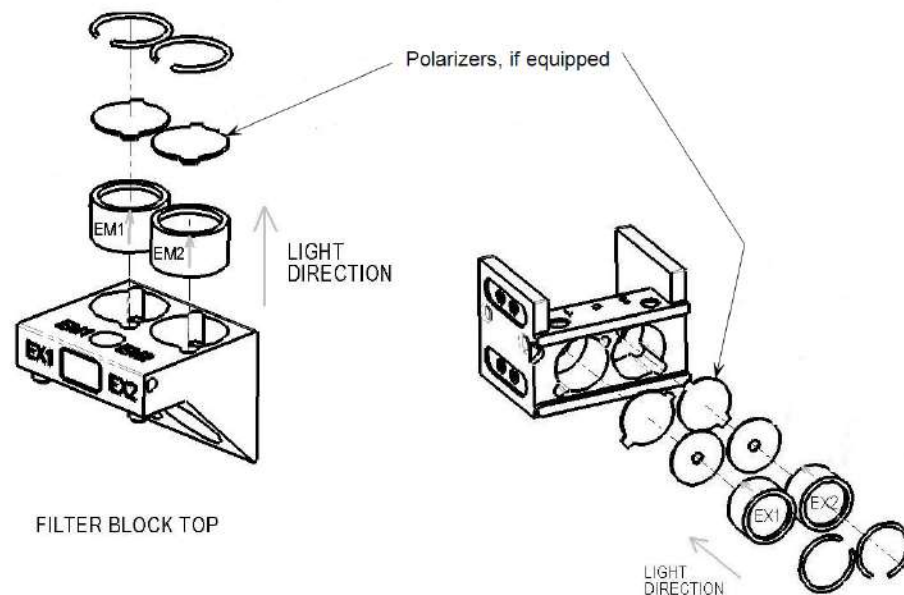
Gen5 has a Filter Cube Library in which you must manage the contents of your filter cubes. See **Adding a Filter Cube to the Optics Library** on page 63.

The Synergy H1 filter cubes are interchangeable with the filter cubes for the Cytation3.

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

	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.

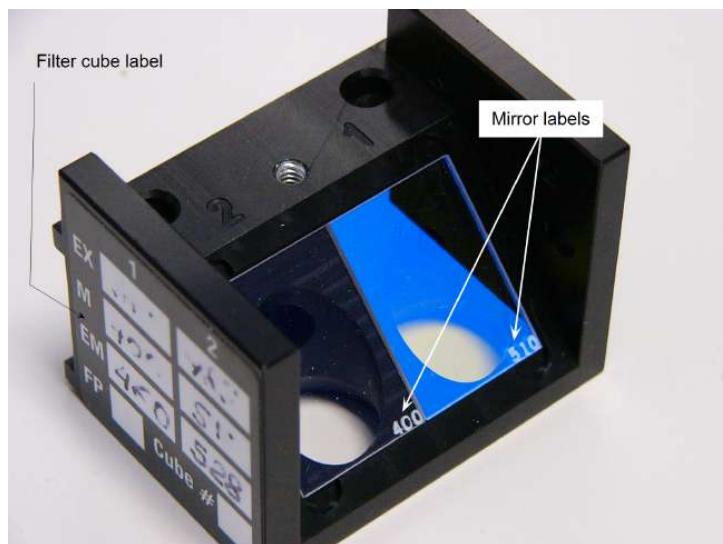
For Cytation3 models with FP capability, the cube is equipped with up to four polarizers of the following types:

- Excitation polarizer (visible-range or 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.

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.



## Filter Cubes Library

The Cytation3 Filter Cubes Library lists the filter cubes available for use with the Cytation3. The cubes are arranged numerically, with short descriptions of their purposes and filter configurations.

To open the Filter Cubes Library, from the Gen5 main screen, click **System > Optics Library > Filter Cubes**. In this dialog, you can

- Indicate which cubes are on site by selecting the checkbox to the left of the filter cube number
- Import filter cube configurations for new cubes
- Export a filter cube configuration
- Delete unnecessary filter cubes

## Cleaning the Filters and Mirrors

Instructions are provided in the **Preventive Maintenance** chapter.

## Installing or Removing a Filter Cube

Do not open the access door on the front of the instrument during operation. Doing so may affect measurements.

Lift up the hinged door on the front of the reader.

- To install a filter cube, slide it gently into its chamber.
- To remove a filter cube, slide it out of its chamber.

## Adding a Filter Cube to the Optics Library

1. Use Gen5 to update the reader's internal software with the current filter cube configuration; see "Define the Filters" in the **Getting Started** chapter.
2. Click **System > Optics Library > Filter Cubes**.
3. Click **Add**, and enter a name for the filter cube. This is then available for selection on the protocol/experiment procedure.
4. Enter a name for Filter Set 1.

If you select Fluorescence Polarization Cube, only Filter Set 1 is available for definition; the filters and mirrors of Filter Set 2 must be identical to those of Filter Set 1 for fluorescence polarization cubes.

5. 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.
6. Select the mirror type and enter the excitation and emission ranges.
7. Define Filter Set 2, if necessary.
8. Click **OK**.

## Filter Cubes Available from BioTek

Preconfigured filter cubes, LED cubes, and imaging filter cubes are available for purchase from BioTek. See the list at [www.biotek.com/products/accessories](http://www.biotek.com/products/accessories). Please note that part numbers are subject to change, and new cubes may become available. Custom filters are also available. Contact BioTek Customer Care with any questions.

## LED Cubes and Imaging Filter Cubes

Cytation3 models with imaging capability are equipped with up to four LED cubes and four imaging filter cubes. The LED cubes hold an LED light source; the filter

cubes contain excitation and emission filters and a dichroic mirror. The preconfigured LED cubes and filter cubes are available from BioTek at [www.biotek.com/products/accessories](http://www.biotek.com/products/accessories).

The LED cubes and filter cubes are accessed through a door on the left side of the instrument and are seated on a filter slide that you can pull out of the instrument. See **Install the LED Cubes and Filter Cubes** on page 25 for more information.

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



## LED Cube and Imaging Filter Cube Library

### System > Optics Library > LED Filter Cubes

The LED Cube and Imaging Filter Cube Library lists the LED cubes and filter cubes available for use with the Cytation3. The cubes are listed by color, filter cube part number, excitation and emission wavelengths, and LED cube part number.

### Import and Export LED Cube and Imaging Filter Cube Definitions

The Gen5 installation package includes an XML file that contains the definitions of the LED cubes and imaging filter cubes available with your reader. If you purchase additional LED cubes and filter cubes and do not have their corresponding XML file, you must import their definitions into Gen5.

1. Go to **System > Optics Library > LED Filter Cubes**.
2. Click **Import**, and navigate to the folder in which your Gen5 application files are stored (for example, C:\Program Files\BioTek\Gen5 x.x).



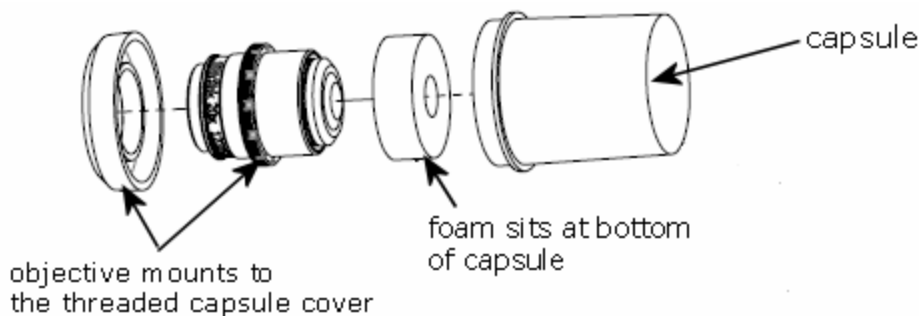
3. Select the **InitialLEDFilterCubes.xml** file, and click **Open**. Any new LED cube and filter cube definitions are imported into Gen5.

## About Imaging Objectives

Readers with the imaging module support fluorescence and bright field reads using objectives. Up to two objectives can be installed in the reader simultaneously.

The objectives are defined in the Imaging Configuration tab in the Reader Setup dialog. Gen5 communicates with the reader to get and set the currently configured objectives. If you select an objective that is not defined as being installed in the reader, when validating an imaging read step, Gen5 displays an error message. See **Install the Imaging Objectives** on page 27 for more information.

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.



## Import and Export Objective Definitions

The Gen5 installation package includes an XML file that contains the definitions of the objectives available with your reader. If you purchase additional objectives and do not have their corresponding XML files, you must import their definitions into Gen5.

1. Go to **System > Optics Library > Objectives**.
2. Click **Import**, and navigate to the folder in which your Gen5 application files are stored (for example, C:\Program Files\BioTek\Gen5 x.x).
3. Select the **InitialObjectives.xml** file, and click **Open**. Any new objective definitions are imported into Gen5.



## Chapter 5

# Preventive Maintenance

This chapter provides instructions for maintaining the Cytation3 and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

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## Preventive Maintenance Overview

A general preventive maintenance regimen for all Cytation3 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 described in the **As Needed Maintenance** chapter.

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

## Recommended Maintenance Schedule

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










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

Task	Daily	Quarterly	As Needed
All models:			
Decontamination	<i>before shipment or storage</i>		
Clean exposed surfaces			✓
Models with filter-based fluorescence capability:			
Inspect/clean excitation and emission filters		✓	
Inspect/clean mirrors			<i>annually</i>
Models with injectors:			
Flush/purge the fluid path	✓		
(Optional) Run Dispense protocol			✓
Empty/clean tip prime trough	✓		
Clean priming plate			✓
Clean dispense tubes and injectors		✓	✓
Models with imaging:			
Clean objectives		✓	✓

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

## Warnings and Precautions

Read the following before performing any maintenance procedures:

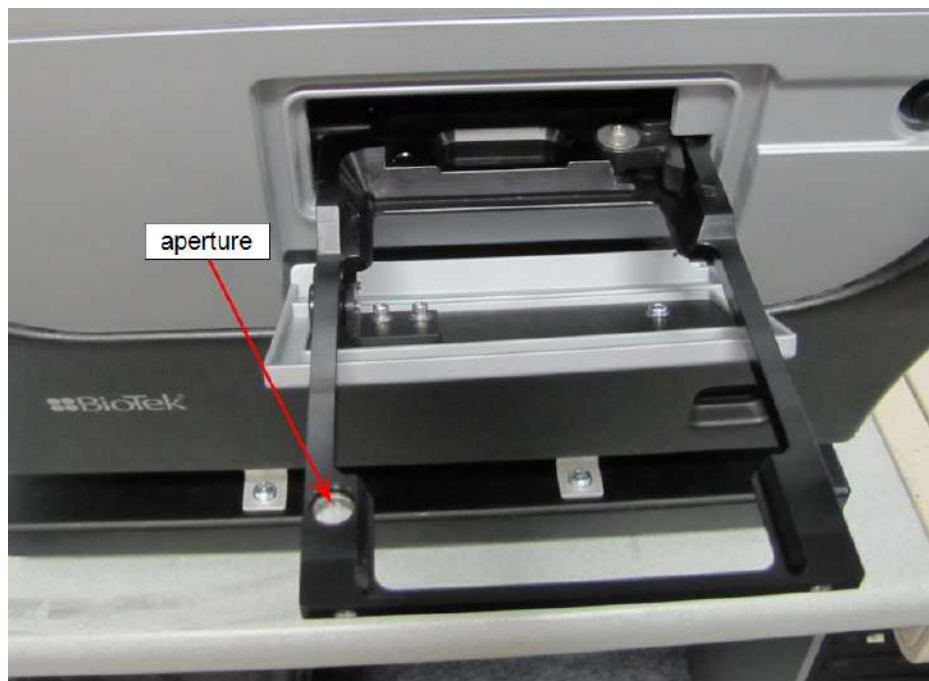
	<b>Warning! Internal Voltage.</b> Turn off and unplug the instrument for all maintenance and repair operations.
	<b>Important!</b> Do not immerse the instrument, spray it with liquid, or use a “wet” cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact BioTek’s Technical Assistance Center.
	<b>Important!</b> Do not apply lubricants to the microplate carrier or carrier track. Lubricant attracts dust and other particles, which may obstruct the carrier path and cause errors.
	<b>Warning!</b> 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.
	<b>Warning!</b> 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.
	<b>Caution!</b> The buildup of deposits left by the evaporation of spilled fluids within the read chamber can impact measurements. Be sure to keep System Test records before and after maintenance so that changes can be noted.
	<b>Warning!</b> The instrument with all available modules weighs up to <b>80 lbs. (36.3 kg)</b> . Use two people when lifting and carrying the instrument.

## 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. For instruments with imaging capability: Used canned air to blow any debris from the aperture on the carrier. Do not wipe with liquid because liquid can seep inside the glass plates surrounding the aperture and negatively 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.

**Models with injectors:** If the Tip Priming Trough overflows or other spills occur 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 BioTek's Technical Assistance Center with any questions about your particular model.

## Inspect/Clean Excitation and Emission Filters

*Applies only to Cytation3 models with filter-based fluorescence capabilities.*

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

Do not touch the filters with your bare fingers!

1. Turn off and unplug the instrument.
2. Open the access door on the front of the instrument. Slide the filter cube out of its compartment.
3. 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.

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

4. 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.
5. Use a magnifying glass to inspect the surface; remove any loose threads left from the cotton ball.
6. Replace the filter cube and close the door.

## Inspect/Clean Mirrors

*Applies only to Cytation3 models with filter-based fluorescence capabilities.*

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

These optical elements are delicate and should be handled as carefully as possible. 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
- Magnifying glass
- 100% pure cotton balls (for the polarizing filters)

## Procedure

1. Turn off and unplug the reader.
2. Open the access door on the front of the instrument and slide the filter cube straight out of its compartment.
3. Set the filter cube on the work surface. Using a 7/64" hex key, remove the screw and washer located between the emission filter positions.

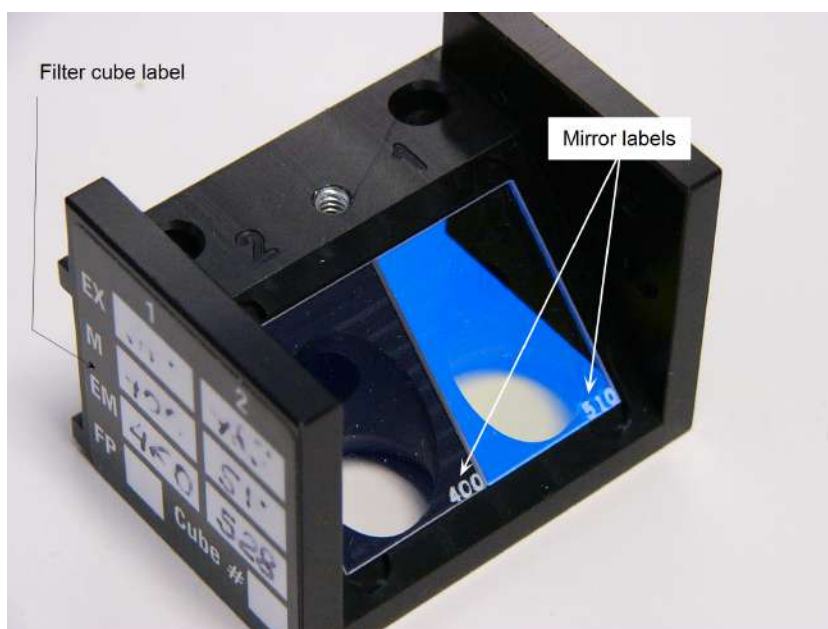


Remove the screw from the filter cube

- Carefully lift the filter cube top from the cube.
- Wearing linen or cloth gloves, grasp the mirror by its edges and lift it out of the cube.

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

- 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.
- 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.
- Use the magnifying glass to inspect the surface; if debris is still visible, repeat with a new Kimwipe.
- 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.



Mirrors in filter cube

- Place the filter cube top back onto the cube and replace the screw and washer.

- When finished, reinstall the filter cube in the reader.

## Clean the Objectives

*Applies only to models with the imaging module.*

The objectives used in the Cytation3 should be cleaned 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	Non-recommended Cleaning Solvents
HyperClean (hexamethyldisiloxane and ethanol), available from Olympus	Methyl ethyl ketone (MEK)
Isopropyl alcohol, 70%/30% with deionized water	Dimethyl ketone (acetone)
Methyl alcohol, 70%/30% with deionized water	
Ethyl alcohol, 70%/30% with deionized water	

- From the Gen5 main screen, go to **System > Instrument Configuration**, select **Cytation3**, click **View/Modify > Setup**.
- In the Objective Configuration area in the Imaging Configuration tab, click **Move to Access Position**. The objective turret rotates to its access position.
- Open the side door of the instrument. Grasp one of the objectives, unscrew it from the objective holder, 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.

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

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 holder, and screw it in to secure it.
9. Repeat these steps to clean the second objective, if necessary.
10. In the Imaging Configuration tab on Reader Setup dialog (which you opened in step 1), click **Auto Calibration** to calibrate the objectives.

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

### Tips

- 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.

## Flush/Purge the Fluid Path

*Applies only to Cytation3 models with injectors.*

At the end of each day that the dispense module is in use, 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.

This flushing and purging routine is also recommended before disconnecting the outlet tubes from the reader, 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. From the Gen5 main screen, select **System > Instrument Control > Cytation3**.
4. Click the **Dispenser** tab and select **Dispenser 1**.
5. Set the Volume to **5000 µL**. Keep the default prime rate.
6. Click **Prime** to start the process. When the process is complete, carefully remove the priming plate from the carrier and empty it.
7. Repeat the process for Dispenser 2.

Leave the water in the system overnight or until the instrument will be used again. Purge the fluid from the system (see below) 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 **System > Instrument Control > Cytation3**.
3. Click the **Dispenser** 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.

After purging the system, you may wish to run a quick Dispense protocol to visually verify the dispense accuracy (see below) or the more thorough Dispense Accuracy and Precision Tests (see **Chapter 7, Instrument Qualification**).

## Run a Dispense Protocol (Optional)

*Applies only to Cytation3 models with injectors.*

After flushing/purging the system and before running an assay that requires dispense, 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  $\mu$ L**.
    - Set the Dispense Volume to **100  $\mu$ L** (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; select 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.

## Empty/Clean the Tip Priming Trough

*Applies only to Cytation3 models with injectors.*

The tip priming trough is a removable cup located in the 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 by the user. Gen5 will instruct you to do this at the start of an experiment that requires dispensing.

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.

## Clean the Priming Plate

*Applies only to Cytation3 models with injectors.*

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.

## Clean the Dispense Tubes and Injectors

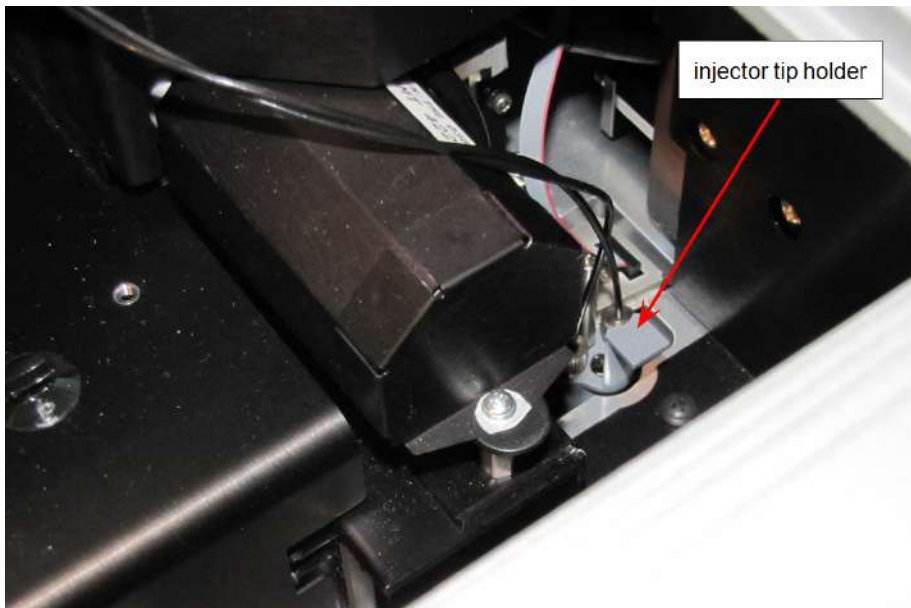
*Applies only to Cytation3 models with injectors.*

The Cytation3'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 front 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.

Be careful not to bend the injector tips. A bent tip might not dispense accurately.



## Chapter 6

# As-Needed Maintenance

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

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## Decontamination

Any laboratory instrument that has been used for research or clinical analysis is considered a biohazard and requires decontamination prior to handling.

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. 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.

	<p>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.</p>
	<p>Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, and nose. Eating and drinking while decontaminating instruments is not advised.</p>
	<p>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 performing the decontamination procedure.</p>

### Required Materials

For all Cytation3 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 Models without a Dispenser

	<p>The sodium hypochlorite (bleach) solution is caustic; wear gloves and eye protection when handling the solution.</p> <p>Do not immerse the instrument, spray it with liquid, or use a “wet” cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department.</p>
	<p>Turn off and unplug the instrument for all decontamination and cleaning operations.</p>

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.

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 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 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

Perform the Routine Procedure when the Cytation3 is functioning normally. If you are unable to perform a prime due to a system failure, perform the Alternate Procedure described on page 88.

### Routine Procedure



If disinfecting with sodium hypochlorite (bleach), be sure to flush repeatedly with deionized water to remove the bleach. After disinfecting with sodium hypochlorite, perform the rinse procedure provided on page 87.

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.

### Clean Exposed Surfaces

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.

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 the exposed surfaces of the dispenser.
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 (see **Flush/Purge the Fluid Path** on page 76) 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.
9. Perform the decontamination procedures described below through page 88.

### 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.
2. Place the SYRINGE 1 inlet tube in the beaker.
3. 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.
4. Launch Gen5 and from the main screen select **System > Instrument Control**, and click the **Dispenser** tab.
5. Select Dispenser **1**, enter a Volume of **5000 µL**, and keep the default dispense Rate.
6. Place the priming plate on the carrier.
7. Run two prime cycles, for a total of 10,000 µL.
8. Wait at least 20 minutes to allow the solution to disinfect the tubing.
9. Remove the inlet tube from the beaker of disinfectant solution.
10. From the Instrument Control dialog, change the Volume to 1000 µL.
11. Run one prime cycle, to flush the disinfectant out of the fluid lines.
12. Empty the beaker containing the outlet tubes. Put the tubes back in the empty beaker.
13. If sodium hypochlorite (bleach) was used, perform Rinse the Fluid Lines.

Otherwise (or after performing the Rinse procedure), repeat steps 1–13 for SYRINGE 2/Dispenser 2.

### Rinse the Fluid Lines

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 1 or 2 inlet tube in the beaker.
3. If you have not already done so, place the outlet tubes in an empty beaker.
4. From the Instrument Control dialog, select Dispenser **1** or **2**, 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  $\mu\text{L}$ . 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 the procedures under **Clean the Dispense Tubes and Injectors** in **Chapter 5, Preventive Maintenance**.

### **Decontaminate the Tip Priming Trough and Priming Plate**

1. Remove the tip priming trough from the instrument's microplate carrier.
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 the trough and plate with DI 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.

### **Alternate Procedure**

If you are unable to prime the Cytation3 due to a system failure, decontaminate the instrument and the dispenser as follows:

1. Perform the procedures under **Clean the Dispense Tubes and Injectors** in **Chapter 5, Preventive Maintenance**.
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.

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 **Preventive Maintenance** chapter for cleaning procedures you must perform regularly and also 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 BioTek TAC to order replacement syringes.

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

### Syringe Maintenance Position

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

Gen5 provides access to syringe setup functions for maintenance and calibration purposes. When a syringe needs to be installed or replaced, it must first be moved to its “maintenance position.”

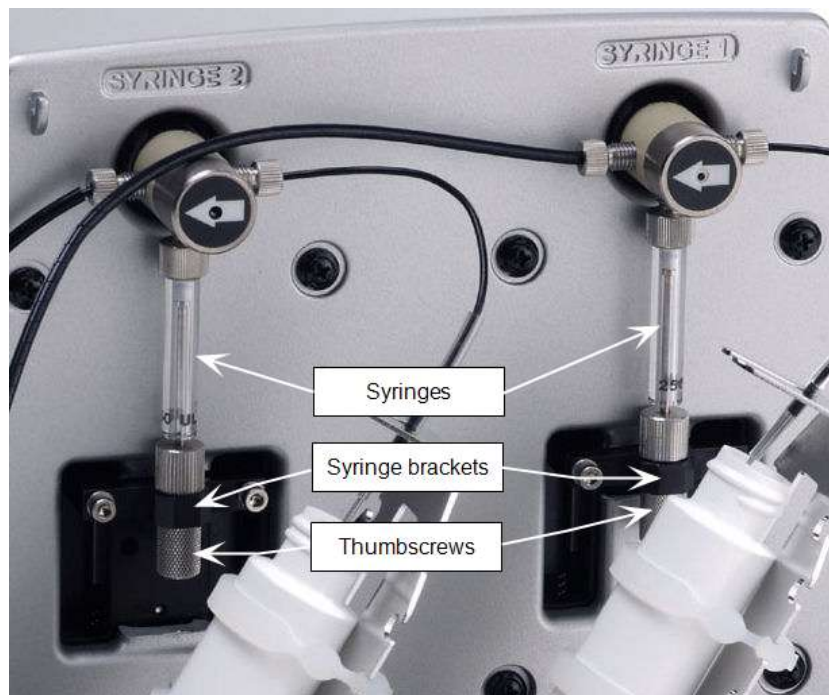
1. From the Gen5 main screen, select **System > Instrument Control > Cytation3** and click the **Dispenser** tab.

2. Select the appropriate 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; if it is not, see “Install the Dispenser” in the **Installation** chapter.



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 > Cytation3**. Click the **Dispenser** tab and click **Initialize**.



## Chapter 7

# Instrument Qualification

This chapter contains procedures for qualifying the initial and ongoing performance of the Cytation3 and the external dispense module (if equipped).

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## Instrument Qualification Overview

This chapter contains BioTek Instruments' recommended Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) procedures for all models of the Cytation3 Microplate Reader.

Every Cytation3 reader and external dispense module 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.

A Product Qualification Package (PN 1220514) for the Cytation3 is available for purchase. The package contains complete procedures, Gen5 protocols, checklists, and logbooks for performing Installation Qualification, Operational Qualification, Performance Qualification, and Preventive Maintenance. Contact your local BioTek dealer for more information.

## IQ/OQ/PQ Description

**Installation Qualification** confirms that the reader 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 liquid 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 reader 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 Liquid 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 Cytation3 is properly maintained as outlined in the **Preventive Maintenance** section.



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
<b>All models:</b>				
Unpacking, installation, setup, and verification	✓			
Software Documentation/Verification	✓	✓	✓	
Software Wavelengths Table Verification	✓	✓	✓	
System Test	✓	✓	✓	
Plate Shaker Test		✓		
"Run Assay" Test			✓	
<b>Absorbance capability*:</b>				
Absorbance Plate Test		✓	✓	
Absorbance Liquid Test 1		✓		✓
Absorbance Liquid Test 3 (optional)		✓		✓
<b>Fluorescence capability*:</b>				
Corners, Sensitivity, Linearity Tests		✓	✓	
Fluorescence Polarization Test		✓		✓
Time-Resolved Fluorescence Test		✓		✓
<b>Luminescence capability*:</b>				
Luminescence Test		✓	✓	
<b>Dispenser system*:</b>				
Injection System Test	✓			
Dispense Accuracy and Precision Test		✓		✓
<b>Imaging capability*:</b>				
Carrier Level Test		✓		✓
Contrast Test		✓		✓
Auto Focus Test		✓		✓

\* If applicable to your reader model



## System Test

Each time the Cytation3 is turned on, it automatically performs a series of tests on the reader's motors, lamp, the PMT, and various subsystems. The duration of this system test depends on the reader model, and can take a few minutes to complete. If all tests pass, the microplate carrier is ejected 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 reader beeps repeatedly and the red LED on the carrier switch flashes. If this occurs, press the carrier eject button to stop the beeping. If necessary, initiate another system test using Gen5 to try to retrieve an error code from the reader. Refer to **Appendix B, Error Codes** for information on error codes and for troubleshooting tips.

If the power-up system test fails, when you initiate a system test using Gen5, Gen5 displays a message stating that the reader 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.

1. Turn on the reader and launch Gen5.
2. If your assays use incubation, we recommend enabling Temperature Control and allowing the incubator to reach its set point before running the system test. To access this feature, from the Gen5 main screen, select **System > Instrument Control** and click the **Pre-Heating** tab.
3. Select **System > Diagnostics > Run System Test**.

If the test fails during execution, a message box appears in the software. Close the box; 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. Scroll down toward the bottom of the report; it shows either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL \*\*\* ERROR (error code) DETECTED."
6. Print the report if desired.
  - Gen5 stores the results in a database, so the results can be retrieved at any time. We recommend that you print and save the reports to document that the test was performed.

7. If the test failed, look up the error code in **Appendix B, Error Codes** to determine its cause. If the cause is something you can fix, turn off the reader, fix the problem, and then turn the reader back on and retry the test.

If the test continues to fail, or if the cause is not something you can fix, contact BioTek's Technical Assistance Center.

## 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 Cytation3. The Absorbance Test Plate compares the reader's optical density and wavelength measurements to NIST-traceable values.

An alternate method for confirming accuracy, linearity, and repeatability is Liquid Test 2, described on page 114.

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

- 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). For the Cytation3, use the values in the 2.4 nm Spectral Bandpass table.

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

### Define Absorbance Test Plate Parameters

1. Obtain the certificates 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.

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), based on the number of expected peak wavelength values provided on the certificate.
8. Enter the Expected Peak value(s) from the certificate and set the Test Range - and + values.

The glass in each test plate is unique. Therefore, the expected peaks may differ slightly from plate to plate.

- If the C6 filter is Holmium or Erbium glass, the certificate contains two Spectral Band Pass tables. The Cytation3 has a band pass wider than 5 nm for wavelengths greater than 285 nm and less than 4 nm for 230–285 nm. As a result, we recommend you use the expected peak values in the 5.0 nm table for your tests.
  - For the Erbium glass, any peak can be used.
  - For the Holmium glass, use the expected peak values closest to 242, 279, 362, 417, and 538 nm. For example, if your certificate looks like the one below, you might choose to run the test at four of the five highlighted Expected Peak/Test Range combinations:

2.4 nm Spectral Band Pass		5.0 nm Spectral Band Pass	
Expected Peak	Test Range	Expected Peak	Test Range
241	-5+5	242	-5+5
278	-6+4	279	-6+4
287	-4+6	288	-4+6
334	-5+5	334	-5+5
360	-5+5	362	-5+5
417	-5+5	417	-5+5
484	-5+5	485	-5+5
537	-5+5	538	-5+5
642	-5+5	643	-5+5

- If your C6 filter is Didymium glass, a single peak wavelength value is provided. Enter this value and set the Test Range - and + values so the range displayed in parentheses is 580 to 590, as shown here:

Peak wavelength tests: 1

Expected Peak: 586    -    Test Range: 6    +    (580 to 590)

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.

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". See **Results and Troubleshooting Tips** for information on results and troubleshooting tips in the event of failures.

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 range(s) 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 Peak Wavelength Certificate supplied with the Test Plate. The accuracy of the wavelength should be  $\pm 3$  nm ( $\pm 2$  nm instrument,  $\pm 1$  nm filter allowance). If the reader fails this test:
  - Make sure the information entered into Gen5 matches the Test Plate's Peak Wavelength Certificate.
  - Verify that the Test Plate has a filter in location C6. (Test Plates with the part number 9000547 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 0.990 is expected.

## Imaging Test Procedures

Gen5 provides qualification procedures for testing the level position of the carrier and the reliability of the Auto Focus feature.

### Importing the Plate Maps for Imaging Test Procedures

The qualification protocols require the use of the Tilt/Contrast jig, available from BioTek (PN 1222520). To use the jig, you must import the following plate maps that are installed with Gen5: **1222520.ptf** and **1222520\_AF.ptf**.

1. In Gen5, click **System > Plate Types**, then click **Import**.
2. Navigate to the location where your Gen5 installation files are store, such as C:\Program Files\BioTek\Gen5 v2.x.

3. Select the file **1222520.ptf**, then click **Open**. The plate map is added to your Plate Type database.
4. Repeat steps 1 through 3 to import the **1222520\_AF.ptf** plate map.

## Required Materials

- Tilt/Contrast jig, PN 1222520
- Plate map files, **1222520.ptf** and **1222520\_AF.ptf**
- Gen5 protocols described on page 105
  - **<x>X\_CarrierLevel.prt**
  - **AF\_Reliability\_<x>X.prt**
- DAPI 377/447 cube (PN 1225100)

## Carrier Level Test

This test determines how level the carrier is in relation to the imaging system. You will run this test using the lowest-power objective you have available (10X or lower is preferred).

### Procedure

1. Place the Tilt/Contrast jig on the carrier with well A1 facing up in the right-rear corner of the carrier.
2. If you have not already done so, create the protocol described on page 105
3. Create a new experiment in Gen5 using the **<x>X\_CarrierLevel.prt** protocol, with <x> representing the lowest-power objective you have available.
4. Click **Plate > Read Plate**, then click **OK**.
5. When prompted by Gen5, rotate the jig 180 degrees in the carrier.
6. When the read is finished, save the experiment, and 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.  $-1 * (A1 \text{ Normal Mean} - H12 \text{ Turnaround Mean})$
  - b.  $-1 * (H12 \text{ Normal Mean} - A1 \text{ Turnaround Mean})$
  - c.  $-1 * (A12 \text{ Normal Mean} - H1 \text{ Turnaround Mean})$
  - d.  $-1 * (H1 \text{ Normal Mean} - A12 \text{ Turnaround Mean})$
4. For A1/H12:
  - Calculate the Mean Delta ( $\mu\text{m}$ ): (step **a** results + step **b** result) / 2
  - Calculate the Carrier Tilt: Mean Delta / 25400  $\mu\text{m}$
  - The Carrier Tilt must be less than 0.004" to Pass.
5. For A12/H1:
  - Calculate the Mean Delta ( $\mu\text{m}$ ): (step **c** results + step **d** result) / 2
  - Calculate the Carrier Tilt: Mean Delta / 25400  $\mu\text{m}$
  - The Carrier Tilt must be less than 0.004" to Pass.

### **AutoFocus Test**

The AutoFocus test confirms the imaging system's ability to repeatedly focus on a known target.

You must run this test for each objective you have onsite.

1. Place the Tilt/Contrast jig on the carrier with well A1 facing up in the right-rear corner of the carrier.
2. If you have not already done so, create the protocol described on page 1.
3. Create a new experiment in Gen5 using the **AF\_Reliability\_<x>.prt** protocol, with <x> representing the objective you are using. for each test.
4. Click **Plate > Read Plate**, then click **OK**.
5. When the read is finished, save the experiment, and analyze the results as described below.



### Analyze the Results

1. For the set of 100 data points in well F7, calculate the Mean, Slope, and Intercept. (If using Microsoft Excel, use the SLOPE and INTERCEPT functions.)
2. For each data point, calculate the Residual:

$$[\text{value}] - ((\text{Slope} * [\text{data point sequence \#}]) + \text{Intercept})$$

Example:  $325 - ((0 * 1) + 325.9) = -0.9$  Residual for data point #1

3. For each data point, compare the absolute value of the Residual with the objective's Limit ( $\mu\text{m}$ ) value in the chart below (e.g., 95.4 for 2.5X). If the absolute value of the Residual is greater than the Limit, note "1" for that data point; otherwise, note "0".

Depth of Field Objective	NA	e ( $\mu\text{m}$ )	$\lambda$ (nm)	DOF ( $\mu\text{m}$ )	Limit ( $\mu\text{m}$ )
20	0.45	6.45	377	2.6	5.2
10	0.3	6.45	377	6.3	12.7
4	0.13	6.45	377	34.7	69.4
2.5	0.12	6.45	377	47.7	95.4
2	0.05	6.45	377	215.3	430.6

4. After conducting this comparison for all 100 data points, add up the notations and divide the sum by 100; this is the Measured Autofocus Failure Rate. The Measured Autofocus Failure Rate must be  $\leq 1.0\%$  to Pass.
5. In the Gen5 plate matrix, select the **FM Ratio [0:DAPI 377,447]** data set. Refer to the chart below for passing criteria for the value displayed in well F7.

Objective	FM Ratio must be
2X	> 2
2.5X	> 1.4
4X	> 25
10X	> 50
20X	> 75

## Troubleshooting

If any of the imaging tests fail:

- Ensure the Tilt/Contrast jig is clean. You can use canned air to blow any debris from the surface of the jig.
- Ensure your objectives are clean. See **Clean the Objectives** on page 75.
- If your imaging tests still fail after cleaning the jig and the objectives, contact BioTek TAC.

## Imaging Protocols

The information in the following tables represents the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

The Plate Type setting in each Gen5 protocol should match the plate you are actually using.

### Carrier Level Tests

**2X\_CarrierLevel.prt, 2.5X\_CarrierLevel.prt, 4X\_CarrierLevel.prt, 10X\_CarrierLevel.prt**

Parameter	Default Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520 (Tilt/Contrast jig)
<b>Read Steps 1–4</b>	
Kinetic Step:	Run Time: 1:12 Interval: 8 seconds Reads: 10
Objective:	2X/2.5X/4X/10X
Read Wells:	Read Step 1: A1 Read Step 2: A12 Read Step 3: H1 Read Step 4: H12
Color Channel:	Bright Field

<b>Parameter</b>	<b>Default Setting</b>
Exposure:	LED: 5 Integration time: 50 Gain: 0
Auto Focus Options:	Focus method: Auto focus without optional scan Minimum focus metric ratio: 3 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 100 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:	0 msec
<b>After Read Step 4</b>	
Plate Out, In	"Turn jig around"
<b>Read Steps 5–8</b>	
Kinetic Step:	Run Time: 1:12 Interval: 8 seconds Reads: 10
Objective:	2X/2.5X/4X/10X
Read Wells:	Read Step 5: A1 Read Step 6: A12 Read Step 7: H1 Read Step 8: H12
Color Channel:	Bright Field
Exposure:	LED: 5 Integration time: 50 Gain: 0

Parameter	Default Setting
Auto Focus Options:	Focus method: Auto focus without optional scan Minimum focus metric ratio: 3 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 100 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:	0 msec

### AutoFocus Reliability Tests

#### AF\_Reliability\_2X.prt, AF\_Reliability\_2.5X.prt

Parameter	Default Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520_AF
Kinetic Step:	Run Time: 19:59, Interval: 12 seconds, Reads: 100
Objective:	2X/2.5X
Read Wells:	F7
Color Channel:	DAPI 377, 447
Exposure:	Auto

Parameter	Default Setting
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: -50
Auto-Exposure Options:	Target exposure %: 75 Skip %: 0.1 Integration threshold: 100
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 5
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well	Enabled
Delay after plate movement:	0 msec

**AF\_Reliability\_4X.prt**

Parameter	Default Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520_AF
Kinetic Step:	Run Time: 19:59, Interval: 12 seconds, Reads: 100
Objective:	4X
Read Wells:	F7
Color Channel:	DAPI 377, 447
Exposure:	Auto

Parameter	Default Setting
Auto-Focus Options:	Focus method: Auto focus with optional scan Minimum focus metric ratio: 25 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 75 Offset from bottom of well: -50
Auto-Exposure Options:	Target exposure %: 75 Skip %: 0.1 Integration threshold: 100
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 5
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well	Enabled
Delay after plate movement:	0 msec

**AF\_Reliability\_10X.prt**

Parameter	Default Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520_AF
Kinetic Step:	Run Time: 19:59, Interval: 12 seconds, Reads: 100
Objective:	10X
Read Wells:	F7
Color Channel:	DAPI 377, 447
Exposure:	Auto

Parameter	Default Setting
Auto-Focus Options:	Focus method: Auto focus with optional scan Minimum focus metric ratio: 50 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 75 Offset from bottom of well: -50
Auto-Exposure Options:	Target exposure %: 75 Skip %: 0.1 Integration threshold: 100
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 5
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well	Enabled
Delay after plate movement:	0 msec

**AF\_Reliability\_20X.prt**

Parameter	Default Setting
Detection Method:	Imaging
Read Type:	Endpoint
Plate Type:	1222520_AF
Kinetic Step:	Run Time: 19:59, Interval: 12 seconds, Reads: 100
Objective:	20X
Read Wells:	F7
Color Channel:	DAPI 377, 447
Exposure:	Auto

Parameter	Default Setting
Auto-Focus Options:	Focus method: Auto focus with optional scan Minimum focus metric ratio: 75 Scan distance: 600 Scan increment: 30 Minimum focus delta: 8 % of capture exposure for focus: 75 Offset from bottom of well: -50
Auto-Exposure Options:	Target exposure %: 75 Skip %: 0.1 Integration threshold: 100
Vibration Detection Options:	CV Threshold: 0.01 Images to average: 5
Horizontal offset from center of well:	0
Vertical offset from center of well:	0
Single image per well	Enabled
Delay after plate movement:	0 msec

## 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

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 (A) or from the ingredients listed below (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 a 5-mL aliquot of 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  $\mu$ L 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 out 0.092 g of FD&C Yellow No. 5 dye powder into 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  $\mu$ L in a flat-bottom microwell.

### Prepare the Plate

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  $\mu\text{L}$  of the concentrated solution (A or B) into the first column of wells in the microplate.
3. Pipette 200  $\mu\text{L}$  of the diluted solution into the second column of wells.

After pipetting the diluted test solution into the microplate and before reading the plate, we strongly recommend shaking the plate for four minutes. This will 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. The spreadsheet reports pass or fail for the tests performed. See the calculation descriptions below and **Section 6, System Description** for troubleshooting tips.

### 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:  $\pm 1\% \pm 0.005$  OD from 0.000 to 2.000 OD ( $\text{Mean} * 0.010 + 0.005$ ). 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:  $\pm 1.0\% \pm 0.010$  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  $\pm 2.0\% \pm 0.010$  OD, then the expected range for the mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD.  $1.902 \times 0.010 + 0.010 = 0.029$ ;  $1.902 - 0.029 = 1.873$ ;  $1.902 + 0.029 = 1.931$ .

**Repeatability Specification:**

- $\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD
- $\pm 3.0\% \pm 0.005$  OD from 2.000 OD to 2.500 OD

**Accuracy Specification:**

- $\pm 1.0\% \pm 0.010$  OD from 0.000 to 2.000 OD
- $\pm 3.0\% \pm 0.010$  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 Test Plate (see page 63). If the Test Plate is not available, however, Liquid Test 2 can be used for these tests.

### 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 $\mu$ L	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

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  $\mu$ L 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  $\mu$ L 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.

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 0.99 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  $\pm 1.0\% \pm 0.005$  OD. If above 2.000 OD, apply the  $\pm 3.0\% \pm 0.005$  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% ( $1.951 \times 0.010$ ) = 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:**

$\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD

$\pm 3.0\% \pm 0.005$  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  $\pm 1.0\% \pm 0.010$  OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. ( $1.902 \times 1.0\% = 0.019 + 0.010 = 0.029$ , 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:**

$\pm 1.0\% \pm 0.010$  OD from 0.000 to 2.000 OD

$\pm 3.0\% \pm 0.010$  OD from 2.000 to 2.500 OD

### Absorbance Liquid Test 3

This test verifies operation of the reader at 340 nm and is provided for sites requiring proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test is optional because the reader has good “front end” linearity throughout its wavelength range.

#### Materials

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)
  - $\beta$ -NADH Powder ( $\beta$ -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  $\beta$ -NADH powder and mix thoroughly. This is the **100% Test Solution**.
  3. (Optional) Read a 150- $\mu$ L 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  $\mu$ L of the 100% Test Solution into all wells of columns 1 and 2
  - 150  $\mu$ L of the 75% Test Solution into all wells of columns 3 and 4
  - 150  $\mu$ L 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. The spreadsheet reports pass or fail for the tests performed. See the calculation descriptions below and **Section 6, System Description** for troubleshooting tips.

### Analyze the Results

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:  $\pm 1\% \pm 0.005$  OD from 0.000 to 2.000 OD (Mean  $\times$  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  $\times$  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 Square value of at least 0.99 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 Liquid Tests

**Required Materials**

Microplates should be perfectly clean and free from dust or bottom scratches. Use new microplates from sealed packages.

Manufacturer part numbers are subject to change.

**All Tests:**

- 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 in the next table and described starting on page 122



For the Filter-Based Fluorescence System	
CYT3_FI_T_SF.prt	Corners, Sensitivity, and Linearity tests, using the top optics
CYT3_FI_T_MUB.prt	Alternative top optics test, using methylumbelliferone
CYT3_FP.prt	Fluorescence Polarization test
CYT3_FIFP_T.prt	Combination of Corners, Sensitivity, and Linearity tests and the Fluorescence Polarization test, using the top optics
CYT3_TRF.prt	Time-Resolved Fluorescence test

For the Monochromator-Based Fluorescence System	
CYT3_M_FI_T_SF.prt	Corners, Sensitivity, and Linearity tests, using the Top optics
CYT3_M_FI_B_SF.prt	Corners, Sensitivity, and Linearity tests, using the Bottom optics
CYT3_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).

The image displays two side-by-side screenshots of the Gen5 software's Filter Set Setup interface. Each screenshot shows the configuration for a specific filter set.

**Left Screenshot (Filter Set 1 - Green):**

- Filter Set Name:** Green
- Excitation:** Band Pass, Wavelength: 485, Bandwidth: 20
- Mirror:** Dichroic, Top 510 nm
- Ex (Min/Max):** 440, 505
- Em (Min/Max):** 515, 640
- Emission:** Band Pass, Wavelength: 528, Bandwidth: 20

**Right Screenshot (Filter Set 1 - Blue):**

- Filter Set Name:** Blue
- Excitation:** Band Pass, Wavelength: 360, Bandwidth: 40
- Mirror:** Dichroic, Top 400 nm
- Ex (Min/Max):** 320, 390
- Em (Min/Max):** 410, 800
- Emission:** Band Pass, Wavelength: 460, Bandwidth: 40

### Corners/Sensitivity/Linearity Tests

BioTek offers a liquid test kit (PN 7160010) containing the microplates and solutions used in all (SF/MUB/Eu) fluorescence liquid test procedures. Kits for each individual procedure are also available; see the **Optional Accessories** section in the **Introduction** chapter. Sodium Fluorescein Test Kit PN 7160013 contains the buffer and the SF already pre-diluted.

Manufacturer part numbers are subject to change.

Methylumbelliferone can be used as an alternative or supplemental method for performing these tests. See page 139.

- 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

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

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

The kit also includes two red polarization solutions; these 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

BioTek offers a pre-configured qualification TRF filter cube for purchase. Contact BioTek Customer Care and ask about part number 8040555.

- 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  $\mu\text{m}$  europium luminescent, 2  $\mu\text{L}$ ) is available from Invitrogen Corporation (PN F20881) or from BioTek (PN 7160011)

## Fluorescence Test Solutions

### Corners/Sensitivity/Linearity Tests



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.
  - Ensure that 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 $\mu$ M	
110 $\mu$ L of 50.2 $\mu$ M SF	13.89 mL	400 nM	
110 $\mu$ L of 50.2 $\mu$ M SF	10.5 mL	100 nM	
0.46 mL of 100 nM SF	13.54 mL	3.3 nM	<i>Corners Test</i>
4.24 mL of 3.3 nM SF	9.76 mL	1 nM	<i>Sensitivity/Linearity Tests</i>

### Fluorescence Polarization (FP) Test

As described in **Fluorescence Polarization (FP) Tests**, 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) Tests**, 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  $\mu$ L 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  $\mu$ L 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.

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.

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 134.
2. If you have not already done so, prepare the solutions for the tests you plan to perform. See **Fluorescence Test Solutions** on page 124.

Refer to the pipette maps starting on page 127 for the remaining steps.

3. Perform the Corners/Sensitivity/Linearity tests using the Top optics of the filter-based fluorescence system:
  - Pipette 200  $\mu$ L/well for each of the solutions for the Corners Test into a clean 96-well microplate.
  - Pipette 150  $\mu$ L/well of the solutions for the Sensitivity and Linearity Tests.
  - Create an experiment based on the **CYT3\_FI\_T\_SF.prt** protocol. Read the plate, and then save the experiment.
4. 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 the **CYT3\_FP.prt** protocol. Read the plate, and then save the experiment.

You can use the single "combination" **CYT3\_FIFP\_T.prt** protocol instead of separately running **CYT3\_FI\_T\_SF.prt** and **CYT3\_FP.prt**.

5. Perform the Corners/Sensitivity/Linearity tests for the monochromator-based fluorescence system:
  - Using the same plate as was used in step 4, pipette 50  $\mu$ L/well of buffer into wells C1-F5 and C10-F12.
  - Create experiments based on the **CYT3\_M\_FI\_T\_SF.prt** (for the top optics) and **CYT3\_M\_FI\_B\_SF.prt** (for the bottom optics) protocols. 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 the **CYT3\_TRF.prt** protocol. Read the plate and then save the experiment.
7. Calculate and evaluate results as described under **Results Analysis**, starting on page 130.

## Pipette Maps

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.

The Corners, Sensitivity, Linearity, and “FP” pipette maps are designed so that multiple tests can be run using the same microplate. Some examples:

- Corners, Sensitivity, and Linearity tests for the top and bottom optics for the monochromator-based fluorescence systems can be performed using the same quartz or glass-bottom plate.
- Corners/Sensitivity/Linearity (top optics) and the FP test can be performed using the same solid black plate.
- Corners/Sensitivity/Linearity (top optics) for the filter- and monochromator-based fluorescence systems can be performed using the same solid black plate.

## Corners Test

You can omit the buffer when using a solid black plate or the Greiner SensoPlate.

- Pipette 200  $\mu\text{L}$  of the 3.3 nM SF solution into the “corner” wells.
- Pipette 200  $\mu\text{L}$  of the buffer in the wells surrounding the SF.

## Sensitivity and Linearity Tests

Use an eight-channel pipette with just four tips installed. Perform these instructions carefully, and refer to the plate map.

- Pipette 150  $\mu\text{L}$  of the buffer into columns 2–5 and 10–12. Discard the tips.
- Pipette 150  $\mu\text{L}$  of the 1 nM SF solution into column 1.

- Pipette 150  $\mu\text{L}$  of the 1 nM SF solution into column 2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 2 and dispense into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 3 and dispense into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 4 and dispense into column 5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 5. Discard the solution and the tips.

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
Corners	<b>A</b>	3.3 nM	3.3 nM	3.3 nM						3.3 nM	3.3 nM	3.3 nM
	<b>B</b>											
Sensitivity/ Linearity	<b>C</b>	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF	BUF	BUF
	<b>D</b>	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF	BUF	BUF
	<b>E</b>	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF	BUF	BUF
	<b>F</b>	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM				BUF	BUF	BUF
Corners	<b>G</b>											
	<b>H</b>	3.3 nM	3.3 nM	3.3 nM						3.3 nM	3.3 nM	3.3 nM

If your model is equipped with the monochromator-based fluorescence system, after testing the top optics of the filter-based system, you will pipette 50  $\mu\text{L}$  of buffer on top of the existing SF solutions and buffer. This will dilute the wells as shown in the following map:

Sensitivity/ Linearity	<b>C</b>	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM				BUF	BUF	BUF
	<b>D</b>	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM				BUF	BUF	BUF
	<b>E</b>	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM				BUF	BUF	BUF
	<b>F</b>	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM				BUF	BUF	BUF



**Fluorescence Polarization (FP) Test**

- Pipette 200  $\mu$ L of the (green) polarization buffer (BUF) into wells A6–H6.
- Pipette 200  $\mu$ L of the green high polarization reference (HPR) into wells A7–B7.
- Pipette 200  $\mu$ L 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  $\mu$ L of deionized water into wells A6–H6.
- 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  $\mu$ L of the 20 pM europium suspension (Eu) into wells A8–B8.

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

## Results Analysis

### Corners Test

1. Calculate the Mean of the 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

#### Filter-Based Fluorescence System

1. Calculate the Mean and Standard Deviation for the buffer wells (C10-F12).
2. Calculate the Mean for the 1000 pM (1 nM) SF solution wells (C1-F1).
3. Calculate the Detection Limit, in pM:

$$1000 / ((\text{Mean SF} - \text{Mean Buffer}) / (3 * \text{Standard Deviation Buffer}))$$

Optic Probe	To pass, the Detection Limit must be less than or equal to:
Top, with 510 nm dichroic mirror	10 pM (3 pM typical))

#### Monochromator-Based Fluorescence System

1. Calculate the Mean and Standard Deviation for the buffer wells (C10-F12).
2. Calculate the Mean for the 750 pM (0.75 nM) SF solution wells (C1-F1).
3. Calculate the Detection Limit, in pM:

$$750 / ((\text{Mean SF} - \text{Mean Buffer}) / (3 * \text{Standard Deviation Buffer}))$$

Optic Probe	To pass, the Detection Limit must be less than or equal to:
EX 485 nm, EM 528 nm	Top/Bottom: 20 pM (5 pM typical)

### 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-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

Monochromator-Based Fluorescence System	
x	y
750	Mean of the 750 pM wells
375	Mean of the 375 pM wells
187.5	Mean of the 187.5 pM wells
93.75	Mean of the 93.75 pM wells
46.875	Mean of the 46.875 pM wells

3. Calculate the R-Square value; it must be  $\geq 0.950$  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 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.
3. Calculate the G-Factor for each LPR well:

$$(\text{Parallel LPR Signal} * (1-0.02)) / (\text{Perpendicular LPR Signal} * (1+0.02))$$

- Calculate the Mean G-Factor.
- Calculate the Polarization value in mP for each HPR well (“PHPR”):

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

- 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

- Calculate the Polarization value in mP for each LPR well (“PLPR”):

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

- Calculate the Standard Deviation of the “PLPR,” in mP.

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

### Time-Resolved Fluorescence (TRF) Test

- Calculate the Mean and Standard Deviation of the wells containing the deionized water (wells A6–H6).
- Calculate the Mean and Standard Deviation of the wells containing the europium solution (wells A8–B8).
- Calculate the Detection Limit, in fM:

$$20000 / ((\text{Mean Eu} - \text{Mean DI water}) / (3 * \text{Standard Deviation DI water}))$$

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 BioTek’s Technical Assistance Center.

- Are the solutions fresh? Discard the plate 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. Contact BioTek TAC.
- 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. Contact BioTek's Technical Assistance Center for instructions.
- Review the pipetting instructions to verify the plate was correctly prepared.
- 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. If you suspect this is a problem, contact BioTek TAC.
- When testing Fluorescence Polarization capability using a solid black plastic microplate, if the standard deviation for the buffer wells is too high, try either 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 50–200, this may indicate a problem.

If the value is less than 50:

- 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. Contact BioTek's Technical Assistance Center to confirm that this may be the case.

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. If you suspect this is a problem, contact BioTek TAC.
- The PMTs or optical path(s) may be deteriorating, or the optics or other hardware may be misaligned. Contact BioTek's Technical Assistance Center.

## Gen5 Protocol Reading Parameters

The information in the following tables represents 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 132).

The Plate Type setting in each Gen5 protocol should match the plate you are actually using.

### CYT3\_FI\_T\_SF.prt

This procedure contains two Read steps using filters to test the top optics: one for the Corners Test and one for the Sensitivity/Linearity Test.

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96 black opaque (#3915)
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds after shake
Read Wells:	Corners Read step: Full plate Sensitivity/Linearity Read Step: Wells C1-F12
Step Label:	Corners Read: "Corners Read" Sensitivity/Linearity: "Sensitivity Read"
Filter Set:	1 (filter cube)
Filters:	EX 485/20 nm, EM 528/20 nm
Optics Position:	Top 510 nm

Parameter	Default Setting
Gain:	Corners Read: Auto, Scale to High Wells, A1, 50000 Sensitivity/Linearity Read: Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal Delay after plate movement: 0 msec Measurements per data point: 50
Delay After Plate Movement:	350 msec
Measurements per Data Point:	40
Lamp Energy:	Low (faster)
Read Height:	7.00 mm

**CYT3\_FP.prt**

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

Parameter	Default 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
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

Parameter	Default Setting
Measurements Per Data Point:	60
Lamp energy:	Low (faster)
Read Height:	7.0 mm

**CYT3\_FIFP\_T.prt**

Parameter	Default Setting
Plate Type:	Costar 96-well black opaque
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds
<b>Read Step 1:</b>	
Detection Method:	Fluorescence intensity
Read Type:	Endpoint
Step Label:	Corners Read
Read Wells:	Full Plate
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, A1, 50000
Read Speed:	Normal
Delay After Plate Movement:	350 msec
Measurements per Data Point:	50
Lamp energy:	Low (faster)
Read Height:	7.00 mm
<b>Read Step 2:</b>	
Detection Method:	Fluorescence intensity
Read Type:	Endpoint
Step Label:	Sensitivity Read



<b>Parameter</b>	<b>Default Setting</b>
Read Wells:	C1..F12
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, C1, 50000
Read Speed:	Normal
Delay After Plate Move- ment:	350 msec
Measurements per Data Point:	40
Lamp energy:	Low (faster)
Read Height:	7.00 mm
<b>Read Step 3</b>	
Stop/Resume Step:	Prepare to install the FP filter cubes and then resume the experiment
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds
Synchronized Mode:	Plate Mode with Timing Control
Detection Method:	Fluorescence polarization
Read Type:	Endpoint
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 Move- ment	350 msec

Parameter	Default Setting
Measurements Per Data Point:	60
Lamp energy:	Low (faster)
Read Height:	7.00 mm

**CYT3\_TRF.prt**

Parameter	Default Setting
Plate Type:	Costar 96-well white opaque
Detection Method:	Time-resolved fluorescence
Read Type:	Endpoint
Delay Step:	3 minutes
Shake Step:	Linear for 30 seconds
Read Wells:	A5-H9
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:	350 msec
Measurements per data point:	20
Lamp energy:	Low (faster)
Read Height:	7.00 mm

**CYT3\_M\_FI\_T\_SF.prt and CYT3\_M\_FI\_B\_SF.prt**

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	96-well Greiner SensoPlate

Parameter	Default Setting
Shake Step:	Linear for 15 seconds at 567 cpm
Delay Step:	5 seconds after shake
Read Wells:	Corners 1: A1..A3 Corners 2: A10..A12 Corners 3: H1..H3 Corners 4: H10..H12 Gain: C1..F12
Wavelength:	1, EX 485 nm, EM 528 nm
Optics Position:	Top/Bottom
Gain	Corners 1: Auto, Scale to High Wells, A1, 50000 Corners 2-4: Auto, Use first filter set gain from FIRST Read Step Gain: Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal
Delay after plate movement:	350 msec
Measurements per data point:	100
Lamp Energy:	Low (faster)
Read Height (for top optics):	7.00 mm

### Fluorescence Test Procedure (Methylumbelliferone)

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

#### Required Materials

Microplates should be perfectly clean and free from dust or bottom scratches. Use new microplates from sealed packages.

Manufacturer part numbers are subject to change over time.

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

- 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. The same plate is used to test both filter- and monochromator-based systems.
- 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
- (Optional) Black polyethylene bag(s) to temporarily store plate(s)
- Gen5 protocols described on page 145:
  - **CYT\_FL\_T\_MUB.prt** tests the top filter-based fluorescence system
  - **CYT\_M\_FL\_T\_MUB.prt** tests the top optics of the monochromator-based fluorescence system

### Test Solutions



Filter solutions to remove particulates that could cause erroneous readings. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate.

Wrap the vial containing the MUB stock solution in foil to prevent exposure to light.

Discard any open, unused solutions after seven days.

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 as described on page 145.
2. If you have not already done so, prepare the test solutions. See page 140.
3. Perform the Sensitivity/Linearity tests using the Top optics of the filter-based fluorescence system:
  - Refer to the pipette map in the next section and pipette the solutions into a clean, 96-well plate.
  - Create an experiment based on the **CYT3\_FI\_T\_MUB.prt** protocol. Read the plate and then save the experiment.
4. Perform the Sensitivity/Linearity tests for the monochromator-based fluorescence system:
  - Using the same plate as was used in step 3, pipette 50 µL/well of buffer into wells C1-F5 and C10-F12.

- Create an experiment based on the **CYT3\_M\_FI\_T\_MUB.prt** protocol. Read the plate and then save the experiment.
5. Calculate and evaluate the results as described under Results Analysis, starting on page 143.

### Pipette Map

Using a multi-channel pipette with just four tips installed to process rows C–F:

- Pipette 150  $\mu\text{L}$  of buffer into columns 10–12.
- Pipette 150  $\mu\text{L}$  of buffer into columns 2–5 (**not column 1**). Discard the tips.
- Pipette 150  $\mu\text{L}$  of the 17.6 ng/mL (100 nM) solution into column 1. Discard the tips.
- Pipette 150  $\mu\text{L}$  of the 17.6 ng/mL (100 nM) solution into column 2. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 2 and dispense it into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 3 and dispense it into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 4 and dispense it into column 5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu\text{L}$  from column 5. 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	BUF	BUF
D	100 nM	50 nM	25 nM	12.5 nM	6.25 nM					BUF	BUF	BUF
E	100 nM	50 nM	25 nM	12.5 nM	6.25 nM					BUF	BUF	BUF
F	100 nM	50 nM	25 nM	12.5 nM	6.25 nM					BUF	BUF	BUF
G												
H												

For the monochromator-based fluorescence system, after testing the top optics of the filter-based system, you will pipette 50  $\mu\text{L}$  of buffer on top of the existing MUB solutions and buffer. This will dilute the wells as shown next.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	75 nM	37.5 nM	18.75 nM	9.375 nM	4.687 nM					BUF	BUF	BUF
D	75 nM	37.5 nM	18.75 nM	9.375 nM	4.687 nM					BUF	BUF	BUF
E	75 nM	37.5 nM	18.75 nM	9.375 nM	4.687 nM					BUF	BUF	BUF
F	75 nM	37.5 nM	18.75 nM	9.375 nM	4.687 nM					BUF	BUF	BUF
G												
H												

### Results Analysis

If you are using the Gen5 protocols that are supplied on the Product Qualification CD, the calculations and pass/fail determinations are performed automatically.

### Sensitivity Test

#### Filter-Based Fluorescence System:

1. Calculate the Mean and Standard Deviation for the buffer wells (C10-F12).
2. Calculate the Mean for the 17.6 ng/mL MUB solution wells (C1-F1).
3. Calculate the Detection Limit, in ng/mL:

$$17.6 / ((\text{Mean MUB} - \text{Mean Buffer}) / (3 * \text{Standard Deviation Buffer}))$$

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:

1. Calculate the Mean and Standard Deviation for the buffer wells (C10-F12).
2. Calculate the Mean for the 13.2 ng/mL (75 nM) MUB solution wells (C1-

F1).

3. Calculate the Detection Limit, in ng/mL:

$$13.2 / ((\text{Mean MUB} - \text{Mean Buffer}) / (3 * \text{Standard Deviation Buffer}))$$

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-based Fluorescence System	
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

Monochromator-based Fluorescence System	
x	y
75	Mean of the 75 nM wells
37.5	Mean of the 37.5 nM wells
18.75	Mean of the 18.75 nM wells
9.375	Mean of the 9.375 nM wells
4.6875	Mean of the 4.6875 nM wells

3. Calculate the R-Square value; it must be  $\geq 0.950$  to pass.



### Gen5 Protocol Reading Parameters

The information in the following table represents the recommended reading parameters. It is possible that your test will require modifications to some of these parameters, such as the Plate Type or Sensitivity value (see Troubleshooting on page 132).

The Plate Type setting in the Gen5 protocol should match the plate you are actually using.

#### CYT3\_FI\_T\_MUB.prt

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96-well black opaque
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds after shake
Read Wells:	Wells C1–F12
Filter Sets:	1
Filters:	EX 360/40 nm, EM 460/40 nm
Optics Position:	Top 400 nm
Gain:	Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal
Delay After Plate Movement:	350 msec
Measurements Per Data Point:	40
Lamp Energy:	Low (faster)
Read Height:	7.00 mm

#### CYT3\_M\_FI\_T\_MUB.prt

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96-well black opaque

Parameter	Default Setting
Shake Step:	Linear for 15 seconds
Delay Step:	5 seconds
Read Wells:	Wells C1–F12
Wavelengths:	1, EX 360 nm, EM 460 nm
Optics:	Top
Gain:	Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal
Delay after plate movement:	350 msec
Measurements per data point:	100
Lamp Energy:	Low (faster)
Read Height:	7.00 mm

## Luminescence Test

BioTek provides two methods for verifying the performance of luminescence reads. One method measures a Harta Luminometer Reference Microplate, which is an LED-based test plate. Contact BioTek to purchase a plate, or go to [www.hartainstruments.com](http://www.hartainstruments.com) for more information. The other method measures a LUX Biotechnology, Ltd., Glowell unit, which is a small, sealed cylinder with a gaseous tritium light source.

Before using the **F-LumTest\_Harta.prt** or **F-LumTest\_Glowell.prt** protocols described in this section, create the filter set shown below.

The screenshot shows a configuration window titled "Filter Set 1" with the following settings:

- Filter Set Name:** Open
- Excitation:** Plug
- Mirror:** <none>
- Ex (Min/Max):** Two empty input boxes.
- Em (Min/Max):** Two empty input boxes.
- Emission:** Hole

## Harta Plate Test

### Materials

- Harta Luminometer Reference Microplate, PN 8030015
- Harta Plate Adapter, PN 1222205
- Gen5 protocol (see page 150)

### 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 simultaneously on the two test buttons on the back of the plate and ensuring that the test light turns on.
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 **F-LumTest\_Harta.prt** or **M-LumTest\_Harta.prt** protocol and read the plate.
5. Calculate and evaluate results as described under **Results Analysis** below.

### Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A		A2 meas					battery check	battery check				
B												
C												
D												
E	BUF	BUF	BUF	BUF								
F	BUF	BUF	BUF	BUF								
G	BUF	BUF	BUF	BUF								
H	BUF	BUF	BUF	BUF								

## Harta Plate Results Analysis

A manual ATP correlation process determined that 11,000 RLU from the Harta plate is equivalent to approximately 1800 attomoles of ATP.

1. On the Harta plate's Calibration Certificate, locate the NIST measurement for the A2 position and convert it to attomoles:  
 $(A2 \text{ NIST measurement} / 11,000) * 1800$
2. Determine if the plate's battery is still functioning properly:
  - If  $A8 > A7$ , the battery is good.
  - If  $A8 < A7$ , the battery requires replacement.
3. Calculate the signal-to-noise ratio:  
 $(A2 - \text{Mean of the buffer cells}) / (3 * \text{Standard deviation of buffer cells})$
4. Calculate the detection limit:  
 $A2 \text{ NIST measurement in attomoles} / \text{signal-to-noise ratio}$ 
  - If the reader is equipped with the low-noise PMT, the detection limit must be  $\leq 75 \text{ amol}$  to pass.
  - If the reader is equipped with the red-shifted PMT, the detection limit must be  $\leq 500 \text{ amol}$  to pass.

To determine which PMT is installed, check the label on the back of the reader. #49984 = low-noise PMT; #49721 = red-shifted PMT.

## Glowell Test

### Materials

- Glowell, PN GLO-466, formerly available from LUX BioTechnology, Ltd. ([www.luxbiotech.com](http://www.luxbiotech.com))
- Glowell Adapter Plate, available from BioTek, PN 7160006
- Gen5 protocol (see page 150)

### Procedure

1. If you have not already done so, insert the Glowell ("window" side up) into well D8 of the Adapter Plate.

2. If you have not already done so, create the Gen5 protocol as described on pages Glowell Test and Glowell Test.
3. Create an experiment based on the **F-LumTest\_Glowell.prt** or **M-LumTest\_Glowell.prt** protocol. Read the plate and then save the experiment.
4. Calculate and evaluate results as described under **Results Analysis** below.

### Plate Layout

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

### Glowell Results Analysis

A manual ATP correlation process determined that 0.021pW Radiant Flux is equivalent to approximately 1800 attomoles of ATP.

1. Locate these items on the Glowell's Calibration Certificate: Calibration Date, Radiant Flux (pW), Measurement Uncertainty of the Radiant Flux.
2. Calculate the number of days between the Calibration Date and the date the test was performed.
3. Correct the Glowell's Radiant Flux value for deterioration over time:  
Radiant Flux \*  $e^{(-0.0001536 \times \text{number of days since calibration})}$
4. Convert the Corrected Radiant Flux value to attomoles (see Note above):  
(Corrected Radiant Flux / 0.021) \* 1800
5. Calculate an error factor for the Corrected Radiant Flux (amol):  
(Corrected Radiant Flux in amol \* Measurement Uncertainty) / 100

6. Calculate the min/max criteria for the Corrected Radiant Flux (amol):  
MIN: Corrected Radiant Flux in amol - Error Factor  
MAX: Corrected Radiant Flux in amol + Error Factor
7. Calculate the Signal-to-Noise Ratio:  
$$\frac{\text{Measurement value of the Glowell} - \text{Mean of Column 9}}{3 \times \text{Standard Deviation of Column 9}}$$
8. Calculate the Detection Limit:  
Corrected Radiant Flux in amol/Signal-to-Noise Ratio
9. Calculate the min/max criteria for the Detection Limit:  
MIN: MIN for Corrected Radiant Flux in amol/Signal-to-Noise Ratio  
MAX: MAX for Corrected Radiant Flux in amol/Signal-to-Noise Ratio
  - If the reader is equipped with the low-noise PMT, the detection limit must be  $\leq 75$  amol to pass.
  - If the reader is equipped with the red-shifted PMT, the detection limit must be  $\leq 500$  amol to pass.

To determine which PMT is installed, check the label on the back of the reader. #49984 = low-noise PMT; #49721 = red-shifted PMT.

## Gen5 Protocol Reading Parameters

The information in the following tables represents the recommended reading parameters.

### F-LumTest\_Harta.prt

Parameter	Default Setting
Plate Type:	Costar 96 black opaque (#3915)
Delay Step:	3 minutes
<b>Read Step 1:</b>	
Read Wells:	A2
Step Label:	Reference well A2
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard

<b>Parameter</b>	<b>Default Setting</b>
Filter Sets:	1 (filter cube)
Excitation:	Plug
Emission:	Hole
Optics Position:	None
Gain:	150
Read Height:	7.00 mm
<b>Read Step 2:</b>	
Read Wells:	E1..H4
Step Label:	Background
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Filter Sets:	1 (filter cube)
Excitation:	Plug
Emission:	Hole
Optics Position:	None
Gain:	150
Read Height:	4.00 mm
<b>Read Step 3:</b>	
Read Wells:	A7-A8
Step Label:	Battery Check
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:01.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard

Parameter	Default Setting
Filter Sets:	1 (filter cube)
Excitation:	Plug
Emission:	Hole
Optics Position:	None
Gain:	50
Read Height:	4.00 mm

**M-LumTest\_Harta.prt**

Parameter	Default Setting
Plate Type:	Costar 96 black opaque (#3915)
Delay Step:	3 minutes
<b>Read Step 1:</b>	
Read Wells:	A2
Step Label:	Reference well A2
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Gain:	150
Read Height:	1.00 mm
<b>Read Step 2:</b>	
Read Wells:	E1..H4
Step Label:	Background
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard



Parameter	Default Setting
Gain:	150
Read Height:	1.00 mm
<b>Read Step 3:</b>	
Read Wells:	A7–A8
Step Label:	Battery Check
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:01.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Gain:	150
Read Height:	1.00 mm

**F-LumTest\_Glowell.prt**

Parameter	Default Setting
Plate Type:	Costar 96-well black opaque (#3915)
Delay Step:	3 minutes
Read Wells:	A8..H11
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Filter Sets:	1 (filter cube)
Excitation:	Plug
Emission:	Hole
Optics Position:	None
Gain:	150
Read Height:	1.00 mm

**M-LumTest\_Glowell.prt**

Parameter	Default Setting
Plate Type:	Costar 96-well black opaque (#3915)
Delay Step:	3 minutes
Read Wells:	A8..H11
Detection Method:	Luminescence
Read Type:	Endpoint
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Gain:	150
Read Height:	1.00 mm

**Luminescence Troubleshooting**

If either test fails, try the following suggestions. If the test(s) continues to fail, print the results and contact BioTek's Technical Assistance Center.

- 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.
- If the test continues to fail, the optical probe(s) may need to be cleaned. Contact BioTek's Technical Assistance Center for instructions.

**Glowell only:**

- Is the plate properly inserted into the adapter? The "window" side should be facing up. If necessary, clean the Glowell according to the manufacturer's instructions.
- Is the adapter plate clean? If dust has collected in the wells, try cleaning the plate using compressed air or an aerosol duster.
- Is the test failing because the standard deviation of the empty background ("buffer") wells is 0 (resulting in a division-by-zero error in the spreadsheet)? If yes, try pipetting 100  $\mu$ L of deionized water into all wells of Columns 9, 10, and 11 (the background wells).

## Dispense Module Tests

*This section applies only to models with the dispenser.*

BioTek has developed a set of tests that you can perform to ensure that the dispense module performs to specification initially and over time. We recommend performing these tests before first use (e.g., during the Initial OQ), 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 depends on the per-well volume dispensed: 2.0% for 80  $\mu\text{L}$ , 5.0% for 20  $\mu\text{L}$ , and 20.0% for 5  $\mu\text{L}$ . 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  $\mu\text{L}$  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  $\mu\text{L}$  and 5  $\mu\text{L}$  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  $\mu\text{L}$ , 20  $\mu\text{L}$ , and 5  $\mu\text{L}$ ) to four columns, the %CV (coefficient of variation) of 32 absorbance readings is calculated. Pass/Fail criteria depends on the per-well volume dispensed: 2.0% for 80  $\mu\text{L}$ , 7.0% for 20  $\mu\text{L}$ , and 10.0% for 5  $\mu\text{L}$ . 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.

### Failures

If any tests fail, prime the fluid lines and rerun the test(s).

If the test(s) fail again, the injector may require cleaning. See **Preventive Maintenance**.

If tests continue to fail, contact BioTek's Technical Assistance Center.

### Required Materials

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  $\pm 1.0\% \pm 0.010$  OD or better

and a repeatability specification of  $\pm 1.0\% \pm 0.005$  OD or better.

The Cytation3 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  $\mu$ L 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 on page 162 Gen5 Test Protocols for Models with Absorbance Capabilities

### Alternate Test Solutions

80  $\mu$ L of test solution with 150  $\mu$ L 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**

If you have not already done so, create Gen5 protocols **Disp 1 Test.prt** and **Disp 2 Test.prt**. Instructions begin on page 162.

1. Prime both dispensers with 4000  $\mu$ L of deionized or distilled water.
2. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000  $\mu$ L. This prevents the water from diluting the dye.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000  $\mu$ L of the solution. When finished, remove the priming plate from the carrier.
4. In Gen5, create an experiment based on **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.



Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /well to columns 9–12.
  - f. Remove the plate and weigh it. Record the weight.
  - g. Manually pipette 200  $\mu\text{L}$  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  $\mu\text{L}$ ” read at 405/750 nm, and the “20 and 5  $\mu\text{L}$ ” 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 **Disp 2 Test.prt**.
12. See page Results Analysis for instructions on analyzing the results.

When all tests are complete, prime both dispensers with at least 5000  $\mu\text{L}$  of deionized water to flush out the green dye solution.

## Results Analysis

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  $\mu\text{L}$ , 20  $\mu\text{L}$ , 5  $\mu\text{L}$ ), 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

Expected Weights for 32 wells: 80  $\mu$ L (2.560 g), 20  $\mu$ L (0.640 g), 5  $\mu$ L (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 $\mu$ L	$\leq$ 2.0%	$\leq$ 2.0%
20 $\mu$ L	$\leq$ 7.0%	$\leq$ 5.0%
5 $\mu$ L	$\leq$ 10.0%	$\leq$ 20.0%

### Procedure for Models without Absorbance Capabilities

The Product Qualification CD contains Gen5 protocols for use with this test. When running this procedure, you'll create experiments based on these protocols.

#### Disp 1 Test No Read.prt and 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.

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 Cytation3 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**

**Disp 1 Test Other Reader.prt** and **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:

	<b>80 <math>\mu</math>L Read</b>	<b>20 &amp; 5 <math>\mu</math>L Read</b>
<b>Primary Wavelength</b>	405 nm	630 nm
<b>Reference Wavelength</b>	750 nm	750 nm
<b>Plate Columns</b>	1-4	5-12

1. If you have not already done so, create the necessary Gen5 protocols as described on page 162.
2. Prime both dispensers with 4000  $\mu$ L of deionized or distilled water.
3. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000  $\mu$ L. This prevents the water from diluting the dye.
4. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000  $\mu$ L of the solution. When finished, remove the priming plate from the carrier.
5. In Gen5, create an experiment based on **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.



Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /well to columns 9–12.
  - f. Remove the plate and weigh it. Record the weight.
  - g. Manually pipette 200  $\mu\text{L}$  of deionized or distilled water into all 12 columns, on top of the green test dye solution.
10. Close the experiment without saving it.

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 158.

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 **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  $\mu\text{L}$ " read at 405/750 nm.
  - b. Perform the "20 and 5  $\mu\text{L}$ " 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  $\mu\text{L}$ , 20  $\mu\text{L}$ , and 5  $\mu\text{L}$  dispense weights recorded during step 8. 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 5–14 using **Disp 2 Test No Read.prt** and **Disp 2 Test Other Reader**.
20. See page Results Analysis for instructions on analyzing the results.

When all tests are complete, prime both dispensers with at least 5000  $\mu\text{L}$  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 Cytation3 (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 **Disp 1 Test.prt**.
5. Repeat steps 2–4 above to create **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  $\mu\text{L}$  dye to columns 1–4
- Dispense 20  $\mu\text{L}$  dye to columns 5–8
- Dispense 5  $\mu\text{L}$  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.

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.

<b>Gen5 Procedure Steps</b>		
<b>#</b>	<b>Step Type</b>	<b>Details</b>
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 200 uL/well of DI water into all 12 columns. Place the plate back on the carrier. Click OK to perform the Read steps.

Gen5 Procedure Steps		
#	Step Type	Details
7	Shake	Linear at 567 cpm (3 mm) for 15 seconds
8	Read	Step label: "80 ul Read_Dispatch 1" (or _Disp 2) 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_Dispatch 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.

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  $\mu$ L Read step at **405** nm.
4. Set the Data In for DS2 to the 80  $\mu$ L 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  $\mu$ L Read step at **630** nm
  - DS2 set to the 20 and 5  $\mu$ L 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. When you are finished, the Data Reduction Steps list shows two Delta OD transformations:
12. Click **OK** to close the Data Reduction dialog.

## Gen5 Test Protocols for Models without Absorbance Capabilities

The test procedure on page 130 dispenses three volumes of fluid to a microplate and then reads 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 supposed by Gen5, you will create one additional protocol to perform the Read step.

## Create the Dispense Protocols

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 protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 $\mu$ L Dispense 80 $\mu$ L at rate 275 $\mu$ L/sec
2	Plate Out,In	Suggested comment: Weigh the plate (80 $\mu$ L 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 $\mu$ L Dispense 20 $\mu$ L at rate 250 $\mu$ L/sec
4	Plate Out,In	Suggested comment: Weigh the plate (20 $\mu$ L 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 $\mu$ L Dispense 5 $\mu$ L at rate 225 $\mu$ L/sec

#	Step Type	Details
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 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 **Disp 1 Test.prt** and **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 **Disp Test Other Reader.prt**.



After running the experiment, view the Statistics for each Delta OD Data Set to view the calculations



# Cytation3 Dispense Accuracy & Precision Tests - Dispenser #1

80 $\mu$ L Dispense Delta ODs @405/750 nm				
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

80  $\mu$ L weight:  g  
 Expected weight: 2.5600 g

Accuracy % Error:  %  
 Must be  $\leq$  2.0%  P  F

Standard Deviation:   
 Mean:

%CV:  %  
 Must be  $\leq$  2.0%  P  F

20 $\mu$ L Dispense Delta ODs @630/750 nm				
	5	6	7	8

20  $\mu$ L weight:  g  
 Expected weight: 0.6400 g

Accuracy % Error:  %  
 Must be  $\leq$  5.0%  P  F

Standard Deviation:   
 Mean:

%CV:  %  
 Must be  $\leq$  7.0%  P  F

5 $\mu$ L Dispense Delta ODs @630/750 nm				
	9	10	11	12
A				
B				
C				
D				
E				
F				
G				
H				

5  $\mu$ L weight:  g  
 Expected weight: 0.1600 g

Accuracy % Error:  %  
 Must be  $\leq$  20.0%  P  F

Standard Deviation:   
 Mean:

%CV:  %  
 Must be  $\leq$  10.0%  P  F

Reader Model: \_\_\_\_\_  
 Reader S/N: \_\_\_\_\_  
 Reading Date: \_\_\_\_\_  
 Comments: \_\_\_\_\_

Tested By: \_\_\_\_\_  
 Signature: \_\_\_\_\_

Reviewed/  
 Approved By: \_\_\_\_\_  
 Signature: \_\_\_\_\_

## Cytation3 Dispense Accuracy & Precision Tests - Dispenser #2

80 µL Dispense Delta ODS @405/750 nm				
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

20 µL Dispense Delta ODS @630/750 nm				
	5	6	7	8

5 µL Dispense Delta ODS @630/750 nm				
	9	10	11	12
A				
B				
C				
D				
E				
F				
G				
H				

80 µL weight:  g  
 Expected weight: 2.5600 g

20 µL weight:  g  
 Expected weight: 0.6400 g

5 µL weight:  g  
 Expected weight: 0.1600 g

**Accuracy % Error:**  %  
 Must be <= 2.0%     P     F

**Accuracy % Error:**  %  
 Must be <= 5.0%     P     F

**Accuracy % Error:**  %  
 Must be <= 20.0%     P     F

**Standard Deviation:**   
 Mean:

**Standard Deviation:**   
 Mean:

**Standard Deviation:**   
 Mean:

**%CV:**  %  
 Must be <= 2.0%     P     F

**%CV:**  %  
 Must be <= 7.0%     P     F

**%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 Cytation3.

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## General Specifications

Microplates	
The Cytation3 accommodates standard 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry, Take3 and Take3 Trio Micro-Volume Plates, microplate slides, and T25 cell culture flasks. Maximum Plate Height: 1.0"	
Hardware and Environmental	
Light Source	
Absorbance, Fluorescence (FI), monochromator-based:	Xenon flash light source, 20W maximum average power (not user-changeable)
Fluorescence (FI/FP), filter-based:	Xenon flash light source, 5W maximum average power (not user-changeable)
TRF (filter-based):	Xenon flash light source, 5W maximum average power (not user-changeable)
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 If installing a stacker with the Cytation3, see "Select an Appropriate Location" in Chapter 2, <b>Installation</b> .
Weight:	With all modules installed, without power supply or dispense module attached, < 80 lbs. (36.3 kg)
Environment:	Power-up temperature 18° to 30°C <b>Note:</b> Performance measurements, including detection limits, were verified up to 25°C.
Humidity:	10% to 85% relative humidity (non-condensing)
Power Supply:	24-volt external power supply compatible with 100–240 V~; +/-10% @50–60 Hz
Power Consumption:	130W maximum
Incubation:	Temperature control ranges from 4°C over ambient to 45°C. Temperature variation ± 0.5°C across the plate @ 37°C, tested with Innovation Instruments, Inc. temperature test plate Top and bottom incubation controlled via software-adjustable gradient.

Plate Shaking	Linear: Amplitude: 1 mm to 6 mm in 1-mm steps Frequency*: ~18 Hz to ~6 Hz
	Orbital Slow: Amplitude: 1 mm to 6 mm in 1-mm steps Frequency*: ~10 Hz to ~3 Hz
	Orbital Fast: Amplitude: 1 mm to 6 mm in 1-mm steps Frequency*: ~14 Hz to ~5 Hz
	Double Orbital Slow: Amplitude: 1 mm to 6 mm in 1-mm steps Frequency*: ~10 Hz to ~3 Hz
	Double Orbital Fast: Amplitude: 1 mm to 6 mm in 1-mm steps Frequency*: ~14 Hz to ~5 Hz

\* Frequency is based on the amplitude selected.

## Dispense/Read Specifications

<b>Maximum Delay between End of Dispense and Beginning of Read 96/384-well plates, default probe heights</b>	
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

<b>Dispense/Read, for models with the dual-reagent dispense module</b>	
Plate Type	Both 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 $\mu$ L with a 5–20 $\mu$ L tip prime
Reagent Dead Volume	< 1100 $\mu$ L, with dead volume recovery function (back flush)
Injection Speeds	225, 250, 275, 300 $\mu$ L/second
Accuracy	$\pm$ 1 $\mu$ L or 2.0%, whichever is greater
Precision	< 2.0% for volumes of 50–200 $\mu$ L < 4.0% for volumes of 25–49 $\mu$ L < 7.0% for volumes of 10–24 $\mu$ L < 10.0% for volumes of 5–9 $\mu$ L

## Absorbance Specifications

<b>Optics</b>	
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	0.001 OD
Increment	1 nm
Wavelength Accuracy	$\pm$ 2 nm
Wavelength Precision	$\pm$ 0.2 nm
Minimum kinetic interval (450 nm)	< 20 seconds, sweep mode, 96-well microplate
<b>Plate In/Plate Out Speed</b>	
< 35 seconds, 450 nm, sweep mode, 96-well microplate	



<b>Accuracy, Linearity, Repeatability</b>	
Specifications apply from 250–999 nm, 200 $\mu$ L (96-well microplates)	
<b>Accuracy (tested with certified neutral density glass)</b>	
96-well plate, normal read speed	
0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms	
2–2.5 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 OD: +/-1% +/-0.010 OD	
<b>Linearity (by liquid dilution)</b>	
96-well plate, normal read speed	
0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms	
2–2.5 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 OD: +/-1% +/-0.010 OD	
<b>Repeatability (tested with certified neutral density glass/measured by one standard deviation: 8 measurements per data point)</b>	
96-well and 384-well plate, normal read speed	
0–2 OD: +/-1% +/-0.005 OD, delay after plate movement = 100 ms	
2–2.5 OD: +/-3% +/-0.005 OD, delay after plate movement = 100 ms	
96-well and 384-well plate, sweep read speed	
0–1 OD: +/-2% +/-0.010 OD	
<b>Take3 Plate</b>	
Detection Limit, 260 nm dsDNA	< 5 ng/ $\mu$ L

## Fluorescence Specifications (Mono-Based)

The Cytation3 measures fluorescence with monochromators from the top and bottom of 6- to 384-well plates. All detection limit (DL) requirements are measured by the “two-point” method, which gives the limit of detection at a signal-to-noise ratio of one where noise is defined as three times the standard deviation of the background wells.

<b>Monochromator-Based Fluorescence</b>	
Excitation range	250–700 with low-noise PMT 250–900 nm with red-shifted PMT
Emission range	250–700 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	<= 18 nm (excitation and emission)
Minimum kinetic interval	< 20 seconds, sweep mode, 96-well microplate
<b>Plate In/Plate Out Speed</b>	
< 35 seconds for filter set, sweep mode, 96-well microplate	
<b>Sensitivity</b>	
<p><i>Sodium Fluorescein in phosphate buffered saline (PBS)</i> DL &lt;= 20 pM top or bottom read, 5 pM typical Excitation 485 nm, Emission 528 nm</p> <p><i>Methylumbelliferone (MUB) in carbonate-bicarbonate buffer (CBB)</i> DL &lt;= 0.16 ng/mL (0.91 nM) top read Excitation 360 nm, Emission 460 nm</p> <p><i>Propidium Iodide (PI) in PBS</i> DL &lt;= 62.5 ng/mL bottom read Excitation 485 nm, Emission 645 nm</p>	

## Fluorescence Specifications (Filter-Based)

The Cytation3 measures fluorescence with filters from the top of 6- to 384-well plates.

<b>Plate In/Plate Out Speed</b>	
< 35 seconds for filter set, sweep mode, 96-well microplate	
<b>Fluorescence Intensity</b>	
DL $\leq$ 10 pM (3 pM typical) solution of Sodium Fluorescein in PBS Excitation 485/20, Emission 528/20, 510 nm mirror	
DL $\leq$ 0.16 ng/mL (0.91 nM typical) solution of Methylumbelliferone in CBB, Excitation 360/40, Emission 460/40, 400 nm mirror	
<b>Time-Resolved Fluorescence</b>	
DL Europium $\leq$ 250 fM (100 fM typical)	
Excitation 360/40 nm, Emission 620/40 nm, 400 nm mirror	
Integration time	20 to 2000 $\mu$ s
Delay	0 to 2000 $\mu$ s
Granularity	1- $\mu$ s step
<b>Fluorescence Polarization</b>	
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 Cytation3 measures luminescence from the top of 6- to 384-well plates. The following requirements apply to 96-well plates with 200  $\mu$ L/well, at room temperature.

Production testing is done using a Lux BioTechnology Glowell, which is a Gaseous Tritium Light Source. Approximately 0.021 pW of photons from the Glowell for a 10-second integration time give an equivalent, background-subtracted signal as 1800 amoles of ATP in a Promega ENLITEN ATP assay in a Synergy 2 instrument.

<b>Luminescence</b>	
DL	$\leq 75$ amol/well, 30 amol typical with low-noise PMT $\leq 500$ amol/well with red-shifted PMT
Integration Time	10 seconds
Gain	150
Blank Wells	16

## Imaging Specifications

The Cytation3 imaging specifications are based on using an NIH 3T3 plate: 10,000 wells per cell, GFP stain, Costar 3603 black-sided, plastic-bottom plate.

<b>Read Speed</b>	
At 20x, Autofocus on, 96-well plate, 1 image per well	< 10 minutes

## Appendix B

# Error Codes

This appendix lists and describes Cytation3 error codes that may appear in Gen5.

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## Error Codes Overview

When a problem occurs during operation with the Cytation3, an error code appears in Gen5. Error codes typically contain four characters, such as “4168,” and in most cases are accompanied by descriptive text, such as “PMT overload error.” With many errors, the instrument will beep repeatedly; press the carrier eject 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). Some problems can be solved only by trained BioTek service personnel. This appendix lists the most common and easily resolved error codes that you may encounter.

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 does not conclude successfully, record the error code and contact BioTek’s Technical Assistance Center.

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**.



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.

### Contact Info: BioTek Service/TAC

Use this appendix to diagnose problems and solve them if possible. If you need further assistance, contact BioTek’s Technical Assistance Center.

Phone: 800-242-4685 (toll free in the U.S.)

802-655-4740 (outside the U.S.)

Fax: 802-654-0638

E-Mail: [tac@biotek.com](mailto:tac@biotek.com)

For errors that are displayed during operation of the Cytation3 with the stacker, refer to the *BioStack3 Operator’s Manual*.

## 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 BioTek's Technical Assistance Center. The Gen5 Help system also provides troubleshooting tips.

Code	Description and Possible Remedy
2353	<p><b>Filter block not found on filter/mirror slide</b></p> <p>Verify that the filter block is correctly installed and that it matches the Gen5 optics library.</p>
2B0x	<p><b>Dispenser syringe 1 or 2 (respectively) did not home</b></p> <p>x=1-3</p> <p>Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. (Refer to the <b>Installation</b> chapter for instructions.) Restart the reader.</p>
2B0A	<p><b>Priming plate not detected</b></p> <p>Place priming plate on the carrier.</p>
2B04	<p><b>Dispenser syringe 1 or 2 (respectively) failed position verify</b></p> <p>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 <b>Installation</b> chapter for instructions.)</p>
37x0/47x0 38x0/48x0 39xy/49xy	<p><b>Noise Test Errors</b> <b>Offset Test Errors</b> <b>Dark Range Errors</b></p> <p>x=0, 1; y=0-6</p> <p>This series of System Test errors may indicate too much light inside the chamber. Make sure the plate carrier door and the front 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.</p>
4Fxy	<p><b>Fluorescence signal out of range.</b></p> <p>x=0, 1; y=0-6</p> <p>Too low of a reading indicates a light signal problem. Ensure that Gen5 Fluor/Lum wavelengths table matches the actual filter installed in the filter cube.</p>

Code	Description and Possible Remedy
4xxx	<p><b>PMT overload well error at &lt;well #xxx&gt;</b></p> <p>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.</p> <p><b>To identify the well:</b></p> <p>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):</p> <ol style="list-style-type: none"> <li>1. Convert the ASCII hex string to a decimal equivalent. Ex: "057" indicates 57 hex, yielding a well code of 87 decimal.</li> <li>2. Row = (well code) / (columns in plate), rounded up to a whole number. Ex: 87/12 = 7.25, indicating row 8 (or H).</li> <li>3. Column = (well code) - ((row-1) * (columns in plate)). Ex: 87 - ((8 - 1) * 12) = column 3.</li> </ol> <p>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, <i>not</i> the actual well where the error occurred.</p>
4Exy	<p><b>Detector saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).</b></p> <p>x=0, 1; y=0-6</p> <p>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.</p> <p>Try lowering the gain in your Read step(s).</p> <p>For models with the dispense module, the internal chamber may require cleaning (contact BioTek TAC).</p> <p>If a <b>4E18</b> error is detected during monochromator-based fluorescence, the luminescence probe may be picking up stray light. Try installing a plug in the filter cube. Restart the reader.</p>



Code	Description and Possible Remedy
4Fxy	<p><b>Fluorescence signal out of range</b></p> <p>x=0,1; y=0-6</p> <p>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.</p> <p>Verify that the microplate door is fully closing and the instrument cover is properly installed and sealed.</p> <p>Try lowering the gain in your Read step(s).</p> <p>The reading chamber may be contaminated by a spill that is fluorescing; see the Preventive Maintenance chapter.</p>
5003 5103	<p><b>Filter cube did not home</b></p> <p>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.</p>
5403	<p><b>Filter cube failed positional verify</b></p> <p>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.</p>
55xy	<p><b>&lt;Motor&gt; not homed successfully</b></p> <p>xy=axis</p> <p>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 from moving normally. Restart the reader.</p>

Code	Description and Possible Remedy
570x	<p><b>Axis obstruction error</b></p> <p>This error indicates that a moving part is being obstructed. Verify that:</p> <ul style="list-style-type: none"> <li>• the tip priming trough, microplate, plate lid, or other object has not become dislodged in the reading chamber.</li> <li>• the Plate Type selection in the Gen5 procedure is correct for the plate in use, and the Plate Height measurement is correct.</li> <li>• the filter cube is correctly installed.</li> <li>• nothing is preventing the dispenser syringes from moving.</li> </ul> <p>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 include a small matrix, or select wells in a different row or column.</p>
5A0x	<p><b>Plate carrier hit obstruction and lost steps</b></p> <p>x=0, 1</p> <p>Verify that the microplate is properly and securely seated in the carrier and nothing is obstructing carrier movement inside the reading chamber.</p> <p>Make sure the Plate Type defined in the Gen5 Protocol matches the plate you are using.</p> <p>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, create a new plate type in Gen5 with the correct Plate Height.</p>
5B00	<p><b>Plate carrier needs to be ejected from the reading chamber</b></p> <p>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.</p> <p>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.</p> <p>To resolve the error, eject the carrier prior to running the experiment.</p>

Code	Description and Possible Remedy
110C	<p><b>Side door interlock signal deactivated while LED is on or during an attempt to turn on the LED</b></p> <p>Verify the side door is closed.</p> <p>Verify the magnet has not become dislodged from the door.</p>
110D	<p><b>LED intensity at reference diode measures more than 5% below the specified target</b></p> <p>Run a System Test to see if the error is generated again.</p> <p>Check to see if the small aperture hole on the top of the imaging filter cube is blocked.</p> <p>Ensure the LED cube and imaging filter cube are present, plugged in, or not defective.</p> <p>Verify the imaging filters and are clean and match the configuration defined in Gen5.</p>
110E	<p><b>LED intensity at reference diode measures more than 5% above the specified target</b></p> <p>Run a System Test to see if the error is generated again.</p> <p>Ensure that the LED cube and imaging filter cube are not defective.</p> <p>Verify that the imaging filters are not delaminating or do not match the configuration defined in Gen5.</p>
110F	<p><b>Current DAC adjusted to more than double the starting value within the first 20 control cycles</b></p> <p>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.</p> <p>Verify that the imaging filter cube and fluorescence filter cube configuration is correct.</p> <p>Ensure that the LED cube and imaging filter cube are not defective.</p>

Code	Description and Possible Remedy
1110	<p><b>With current DAC adjusted to maximum output, LED intensity at reference diode measures more than 5% below the specified target</b></p> <p>Run a System Test to see if the error is generated again.</p> <p>Check to see if the small aperture hole on the top of the imaging filter cube is blocked.</p> <p>Ensure the LED cubes and imaging filter cubes are present, plugged in, or not defective.</p> <p>Verify the imaging filters are clean and match the configuration defined in Gen5.</p>
1185	<p><b>LED gain calibration signal is out of range</b></p> <p>This error occurs only during the System Test.</p> <p>Ensure the imaging filter cubes and fluorescence filter cube are present.</p> <p>Check the cable between the LED cube and the instrument's PCB to ensure it is not loose or defective.</p> <p>Verify that the light aperture on the top of the imaging filter cube is not blocked.</p> <p>Ensure that the LED cube and imaging filter cube are not defective.</p>
1186	<p><b>LED control timeout</b></p> <p>The LED output setpoint was not reached within 20 msec after turn on.</p> <p>Verify the LED cube and imaging filter cube settings. The XML file must match the LED cube and imaging filter cube contents.</p> <p>Run a System Test to reset LED gain calibrations.</p>
1187	<p><b>LED-on current feedback is out of range (low)</b></p> <p>Ensure the LED cube is present, plugged in, or not defective.</p> <p>Run a System Test to see if the error is generated again.</p>
1188	<p><b>LED-on current feedback is out of range (high)</b></p> <p>Run a System Test to see if the error is generated again.</p>
1189	<p><b>User-initiated positional calibration needed</b></p> <p>Run imager objective calibration in Gen5 setup.</p>

Code	Description and Possible Remedy
118A	<p><b>Carrier calibration needed</b></p> <p>Run imager objective calibration in Gen5 setup.</p>
500C 520C 550C	<p><b>Imager LED/Filter Slide error</b></p> <p>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.</p>
500D 520D 540D 550D 570D	<p><b>Imager Objective Changer error</b></p> <p>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.</p> <p>Ensure that the installed objective matches the objective configuration. If the objective installed is too tall, it may contact the bottom of the incubator.</p> <p>Verify that the z-axis motor is moving and that the objective is not binding on the incubator.</p>
500E 520E 550E 540E 550E 570E	<p><b>Z-Axis error</b></p> <p>Verify that the carrier shipping bracket is removed.</p>



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