

Hybrid Multi-Mode Microplate Reader

Synergy™ H4 Operator's Manual



Synergy™ H4

**Hybrid Multi-Mode Microplate Reader
Operator's Manual**

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Revision C
BioTek® Instruments, Inc.

Notices

BioTek® Instruments, Inc.

Highland Park, P.O. Box 998
Winooski, Vermont 05404-0998 USA

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Contact Information

❖ See also Product Support & Service on page 5.

Customer Service and Sales

Internet: www.bioteck.com
Phone: 888-451-5171 (toll free in the U.S.)
802-655-4740 (outside the U.S.)
Fax: 802-655-7941
E-Mail: customercare@bioteck.com

Service/TAC

Phone: 800-242-4685 (toll free in the U.S.)
802-655-4740 (outside the U.S.)
Fax: 802-654-0638
E-Mail: tac@bioteck.com

European Coordination Center/Authorized European Representative

BioTek® Instruments GmbH
Kocherwaldstrasse 34
D-74177 Bad Friedrichshall
Germany
Internet: www.bioteck.de
Phone: +49 (0) 7136 9680
Fax: +49 (0) 7136 968 111
E-Mail: info@bioteck.de

Revision History

Rev	Date	Changes
A	1/2010	Initial release
B	3/2010	<p><i>Preface:</i> Updated wording in regulatory information</p> <p><i>Chapter 1, Introduction:</i> Removed address from TAC section and added wording to contact TAC for an RMA number and the shipping address</p> <p><i>Chapter 5, Preventive Maintenance:</i> Added step to Run a Dispense Protocol (Optional) to select a Plate Type that matches the plate being used, per request from MPS</p> <p><i>Chapter 7, Instrument Qualification:</i> Updated the Harta Lum Plate layout to move the buffer wells further away from the lum test well; corrected calculation for Accuracy % Error in Dispense Module Test to read: $((\text{Actual Weight} - \text{Expected Weight}) / \text{Expected Weight}) * 100$</p> <p><i>Appendix A, Specifications:</i> Updated Luminescence specifications to include both low-noise and red-shifted PMTs</p>
C	5/2010	<p><i>Chapter 1, Introduction:</i> Added a note in the Optional Accessories list that Glowells were formerly available from LUX BioTechnology, Ltd.</p> <p><i>Chapter 7, Instrument Qualification:</i> Corrected the detection limit for mono fluorescence top/bottom pass/fail criteria to read $\leq 15\text{pM}$; reordered the Harta plate and Glowell Lum test information to put the Harta plate test first; formatted the detection limits for MUB, TRF, and FP to match those of mono- and filter-based SF tests.</p> <p><i>Appendix A, Specifications:</i> Corrected the Max Plate Height spec to 1.2"; added mono wavelength ranges; corrected the detection limit for mono fluorescence top/bottom pass/fail criteria to read $\leq 15\text{pM}$.</p>

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 Synergy H4 models are preceded by a notice in italics, for example: <i>Applies only to Synergy H4 models with injectors.</i>
	This icon calls attention to important information .

Intended Use Statement

The Synergy H4 is a 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 Synergy H4 can operate with standard robotic systems, such as the BioStack Microplate Stacker.
 - This product may be used for In Vitro Diagnostic, 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 & 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 and updates about the product(s) you have purchased.

You can register online through the Customer Resource Center (CRC) at www.bioteck.com or by calling 888-451-5171 or 802-655-4740.

Warnings



Operate the instrument on a flat surface away from excessive humidity. Bright 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 hazard warnings 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 two-prong plug adapter to connect primary power to the external power supply. Use of a two-prong 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! 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! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential 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.



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



Warning! Hot Surface. The tungsten lamp assembly is hot when the instrument is turned on. Turn off the reader and allow the lamp to cool for at least 15 minutes before attempting to replace it.

Warning! Unspecified Use. Failure to operate this 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 absorbance data. All information exported via computer control must be thoroughly analyzed by the operator.



Warning! Pinch Hazard. Do not reach in the side door during instrument operation. Doing so presents a potential pinch hazard.

Precautions

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



Caution: Service. The instrument should be serviced by BioTek-authorized personnel.

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 within the range of line voltages listed on it.

Caution: Shipping Panel/Hardware. The shipping panel, carrier shipping screw, mirror holder shipping bracket, and optic arm shipping block must be removed before operating the instrument. They must be reinstalled before shipping the instrument. See **Chapter 2, Installation**.

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: 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



Based on the testing described below and information contained herein, this instrument bears the CE mark.

❖ See the Declaration of Conformity for more information.

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 and EN 61326-2-6: 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 61000-4-11, Voltage Dips, Short Interruptions and Variations

Directive 73/23/EEC Low Voltage (Safety)

The system has been type-tested by an independent testing laboratory and was found to meet the requirements of EC Directive 73/23/EEC for Low Voltage. Verification of compliance was conducted to the limits and methods of the following:

EN 61010-1 (2001) 2nd Edition, "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."

EN 61010-2-81 (2003), "Requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."

EN 61010-101 (2002), "Particular requirements for in vitro diagnostic (IVD) medical equipment."

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

- Product registration with competent authorities
- Traceability to the U.S. National Institute of Standards and Technology (NIST)

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

- ❖ Changes or modifications to this instrument not expressly approved by the manufacturer could void the user's authority to operate the equipment.

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. Like all similar equipment, 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 interference, in which case the user will be required to correct the interference at their own expense.

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 de 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: 2004**

“Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements”

- **Canadian Standards Association CAN/CSA C22.2 No. 61010-1-04**

“Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements”

- EN 61010 Standards – See **CE Mark** list

Safety Symbols

Some of these symbols appear on the instrument or accessories:

	Alternating current Courant alternatif Wechselstrom Corriente alterna Corrente alternata		Both direct and alternating current Courant continu et courant alternatif Gleich - und Wechselstrom Corriente continua y corriente alterna Corrente continua e corrente alternata
	Direct current Courant continu Gleichstrom Corriente continua Corrente continua		Earth ground terminal Borne de terre Erde (Betriebserde) Borne de tierra Terra (di funzionamento)
	On (Supply) Marche (alimentation) Ein (Verbindung mit dem Netz) Conectado Chiuso		Protective conductor terminal Borne de terre de protection Schutzleiteranschluss Borne de tierra de protección Terra di protezione
	Off (Supply) Arrêt (alimentation) Aus (Trennung vom Netz) Desconectado Aperto (sconnessione dalla rete di alimentazione)		Caution (refer to accompanying documents) Attention (voir documents d'accompagnement) Achtung siehe Begleitpapiere Atención (vease los documentos incluidos) Attenzione, consultare la doc annessa
	Warning, risk of electric shock Attention, risque de choc électrique Gefährliche elektrische schlag Precaución, riesgo de sacudida eléctrica Attenzione, rischio di scossa elettrica	 	Warning, risk of crushing or pinching Attention, risque d'écrasement et pincement Warnen, Gefahr des Zerquetschens und Klemmen Precaución, riesgo del machacamiento y selección Attenzione, rischio di schiacciare ed intrappolarsi
	Warning, hot surface Attention, surface chaude Warnen, heiße Oberfläche Precaución, superficie caliente Attenzione, superficie calda		Warning, potential biohazards Attention, risques biologiques potentiels Warnung! Moegliche biologische Giftstoffe Atención, riesgos biológicos Attenzione, rischio biologico

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

Chapter 1

Introduction

This chapter introduces the Synergy H4 Multi-Mode Microplate Reader, describes its hardware and software features, and provides contact information for technical assistance.

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Product Description

The Synergy H4 is a multi-mode, single-channel microplate reader. Depending on the model, Synergy H4 detection modes include Fluorescence Intensity (FI), Fluorescence Polarization (FP), Time-Resolved Fluorescence (TRF), Luminescence, and UV-Visible Absorbance. The instrument is modular, and several upgrade options are available; contact BioTek Customer Care for more information.

The Synergy H4 uses a combination of a monochromator, filters, and dichroic mirrors. The reader is computer-controlled using Gen5 software for all operations, including data reduction and analysis. The Synergy H4 is robot accessible and compatible with the BioStack Microplate Stacker. Gen5 supports OLE automation to facilitate the Synergy H4's integration into an automated system.

The filter-based system uses two light sources for fluorescence determinations: a tungsten halogen lamp (part of the FI modules) and a xenon flash (part of the FP and TRF module), along with interference filters and dichroic mirrors for wavelength specificity and a photomultiplier tube (PMT) detector. The Synergy H4 modules with Fluorescence Polarization (FP) capability are equipped with polarizing filters.

The Synergy H4 monochromator system has both top and bottom probes for fluorescence measurements. The filter-based system is top-only.

Luminescence is measured by the low-noise PMT detector through an empty filter position in the Emission filter wheel. Filters can also be used if light filtering is necessary.

Absorbance measurements are made using the reader's monochromator optics. The xenon lamp allows for both UV and visible light absorbance measurements. The monochromator provides wavelength selection from 230-999 nm in 1-nm increments. Area and spectral scanning and pathlength correction are available read methods.

The Synergy H4 has a 4-Zone temperature control from 4°C over ambient to 65°C. Internal plate shaking is supported to ensure that reagents are properly mixed prior to reading.

The Synergy H4 supports the reading of 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with 128 x 86 mm geometry as well as the Take3 Multi-Volume Plate.

-
- ❖ 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.

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

Package Contents & Accessories

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

Item	Part #
<i>Synergy H4 Operator's Manual</i>	8031000
Power supply	7130560
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
Wrench	48576
Filter "plugs" (2) (also referred to as "dummy filters" or "blanks")	7082073
Spare filter retaining clips (2)	7082075
Plastic storage bag and Velcro strips	—
Models with injectors ("D" dispenser models), an external dispense module (packed separately), with the following accessories:	
Outlet tubes (2, plus 2 spare) from dispense module to instrument	7082120
Inlet tubes (2) from supply bottles to syringe drives	7082121
250-µL syringes (2)	7083000
Syringe thumbscrews (2)	19511
Priming plate	7132158
Injector tip priming trough	7132169
Dispense module communication cable	75107
Dispense module front cover	7082137
Dispense module box	7090568
Supply bottles (2, 30 mL)	7122609
Supply bottle holder assemblies (2)	7090564
Injector tip cleaning stylus and plastic storage bag	2872304

Optional Accessories

- ❖ Accessory availability and part numbers are subject to change. Please contact BioTek Customer Care if you have any questions or visit www.bioteck.com and use the Accessories search tool.

Item	Part #
7-filter Absorbance Test Plate for absorbance measurement testing	7260522
Synergy H4 Product Qualification (IQ-OQ-PQ) package	8030514
Take3 Multi-Volume Plate	TAKE3
PCR Tube Adapter Plates	6002072 6002076
BioCell Quartz Vessel	7272051
BioCell Adapter Plate	7270512
Replacement Shipping Materials	7130016
Glowell Adapter Plate (for Luminescence testing) Note: Glowells were formally available from LUX BioTechnology, Ltd. (www.luxbiotech.com), PN GLO-466	7160006
Harta Luminometer Reference Microplate and Adapter	8030015
Teflon tubes (Injector models)	7092123
Injectors (Injector models)	8032019
Additional Filters and Filter Wheels; contact BioTek for part numbers and availability	
The Synergy H4 is compatible with the BioStack Microplate Stacker. The BioStack 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.	
Solutions for Liquid Tests (see Chapter 7)	Part #
Absorbance Liquid Test Solutions: BioTek Wetting Agent Solution (PN 7773002) BioTek QC Check Solution No. 1 (25 mL) or BioTek QC Check Solution No. 1 (125 mL)	7773002 7120779 7120782
Dispense Module Liquid Test Solution: BioTek Green Test Dye	7773003
Fluorescence Liquid Test Solutions, if using Sodium Fluorescein: Sodium Fluorescein Powder Sodium Fluorescein Kit Fluorescence Polarization Kit TRF Europium Kit	98155 7160013 7160014 7160011
Fluorescence Liquid Test Solutions, if using Methylumbelliflone: 10-mg vial of Methylumbelliflone (MUB) Carbonate-Bicarbonate Buffer ("CBB") capsules MUB Kit	98156 98158 7160012

Product Support & 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.bioteck.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 onboard software part number and basecode version (available via Gen5 for the Synergy H4 by selecting **System > Reader Control > 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**)

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 properly (see 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 Synergy H4 and, if applicable, the external dispense module. Instructions are also included for preparing the reader and dispense module 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.bioteck.com or by contacting BioTek Customer Care.

Important Information



This chapter contains installation and setup tasks for a Synergy H4 reader that has **all** of the available modules (the "H4MLFPTAD" model). Your Synergy H4 model may be different. Perform the installation and setup tasks in the order presented, skipping those that do not apply to your reader's configuration.

Materials: You will need a slotted screwdriver and 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.



Remove the shipping hardware before turning on the instrument.

Re-install the shipping hardware and attach the shipping panel before repackaging the instrument for shipment.

1: Unpack and Inspect the Reader



The Synergy H4 should be removed from the box by two people. The instrument with all available modules weighs up to **80 pounds (36.3 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 of your reader immediately.

Perform these steps to unpack and inspect the reader, and then remove its protective shipping panel:

1. Set the outer shipping box close to the intended work surface. Open the outer shipping box. Remove the foam blocks to access the inner shipping box.
2. Open the inner shipping box. The reader is attached to a shipping panel, which has two metal handles for lifting.
3. With one person on each side, grasp the handles and carefully lift the reader out of the box. Place the reader on its back on the work surface, so the reader lies flat and the panel hangs over the edge of the surface.
4. Using a slotted screwdriver, remove the screws and washers that attach the panel to the reader. Carefully set the reader upright.
5. Place the panel with the screws and washers into the shipping box for storage. Place the packaging materials back into the shipping boxes for reuse if the reader needs to be shipped again.

2: Unpack and Inspect the Dispense Module



Save all packaging materials. If you need to ship the dispense module 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, module, and accessories for shipping damage. If the dispense module 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 dispense module. Refer to **Figure 14** and **Figure 15** on pages 28–29.

1. Open the outer shipping box. Remove the foam cap, inner shipping box, and accessories box.
2. **Using no sharp tools**, open the inner shipping box. Remove the two reagent bottle holders and shipping materials. Lift out the module and place it on a level surface.
3. Open the accessories box and remove its contents. The accessories should include the dispense module-related items listed under **Package Contents & Accessories** in Chapter 1.
4. Place all packaging materials into the shipping box for reuse if the dispense module needs to be shipped.

3: Select an Appropriate Location

Install the reader on a level surface in an area where ambient temperatures between 18°C (64°F) and 40°C (104°F) can be maintained.

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.

❖ If you will be installing the BioStack for operation with the Synergy H4, you may wish to seat the instruments in their aligning plates now. Refer to the *BioStack Operator's Manual* for more information.

4: Remove the Shipping Hardware



1. Locate the two screws that hold the reader's shroud (cover) in place; one on each side of the reader, in the lower-rear corners. Remove the two screws and slide off the shroud.

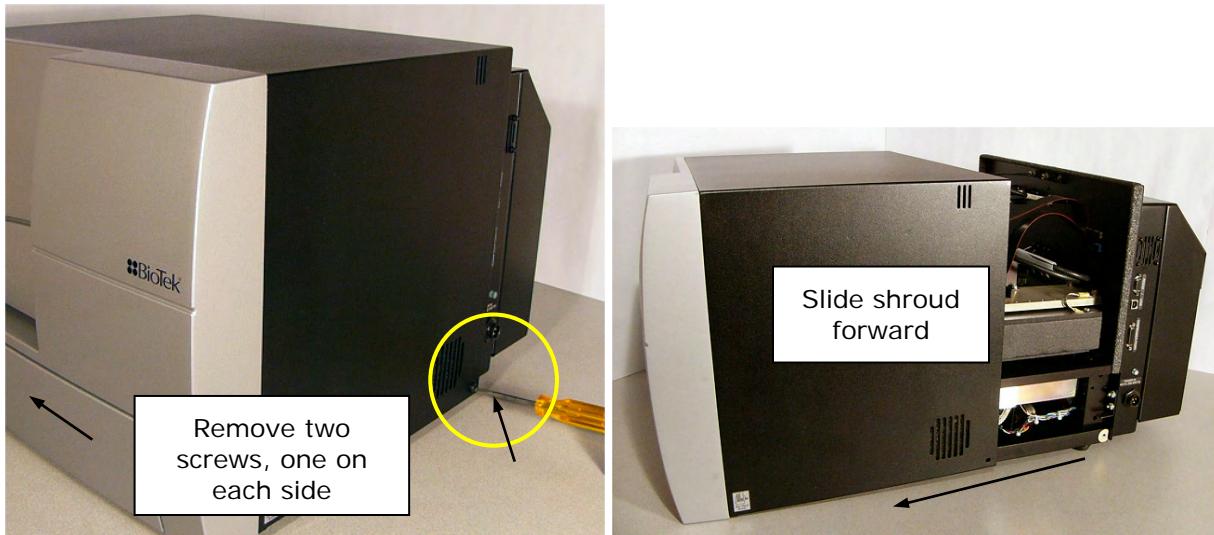


Figure 1: Removing the reader's shroud (cover)

2. Locate the shipping hardware, as shown in **Figure 2**.

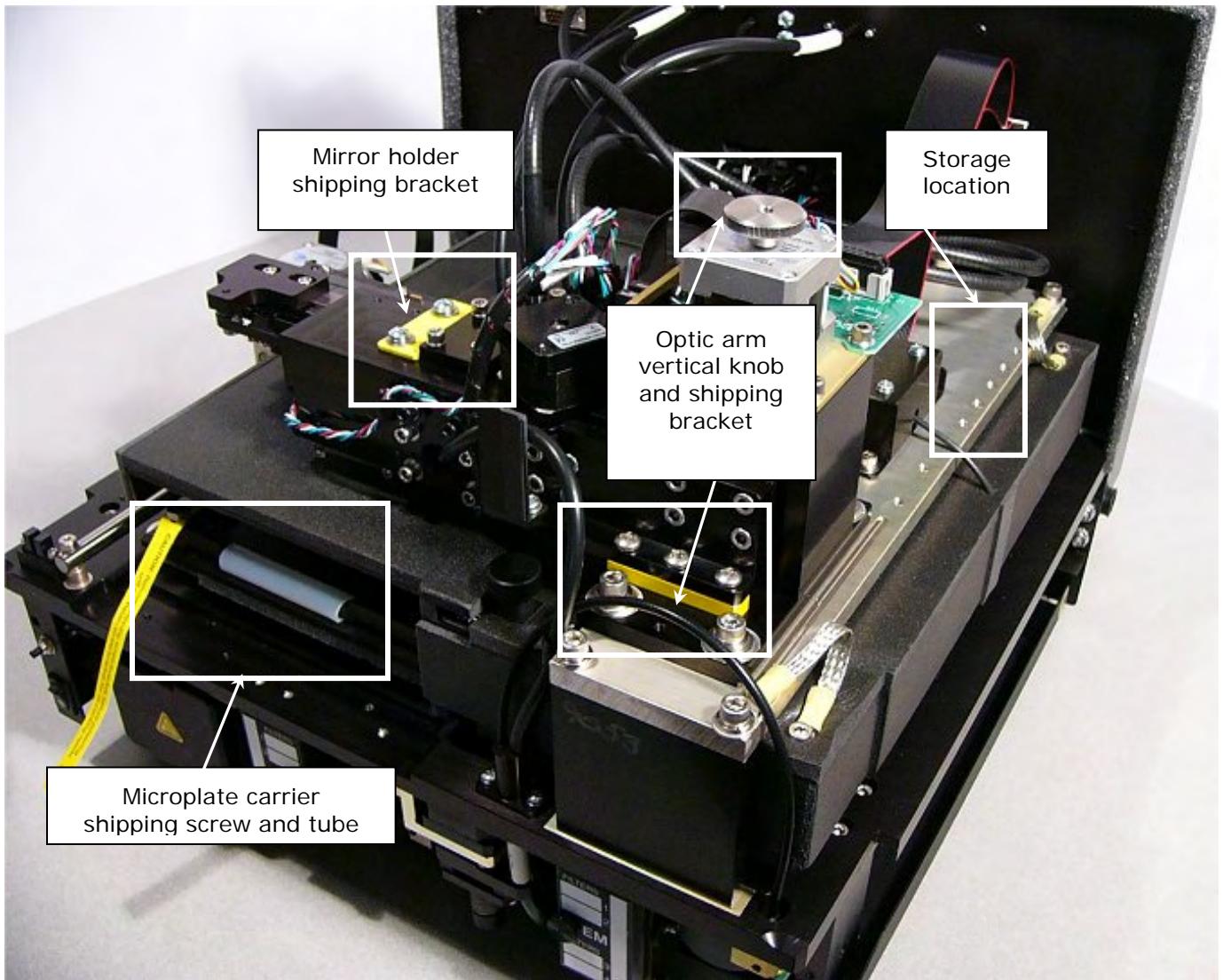


Figure 2: Internal compartment, with shipping hardware

3. As shown in **Figure 3** on the next page:

- Use the supplied wrench to remove the microplate carrier shipping screw (with its o-ring). Store the screw on the base plate as shown in **Figure 5** on page 14.
- Pull the flexible carrier shipping tube off of the carrier. Store the tube in the plastic storage pocket. Use the supplied Velcro strips to attach the pocket to the back of the reader for storage.

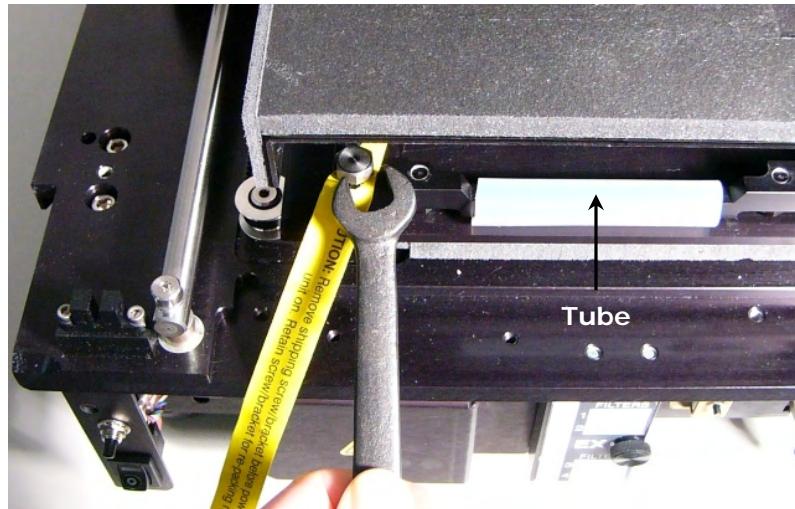


Figure 3: Remove microplate carrier shipping screw and tube

4. As shown below, remove three screws (with washers) that hold the optic arm shipping block in place. Turn the knob at the top of the motor clockwise to raise the optic arm. Slide the block out from under the arm.

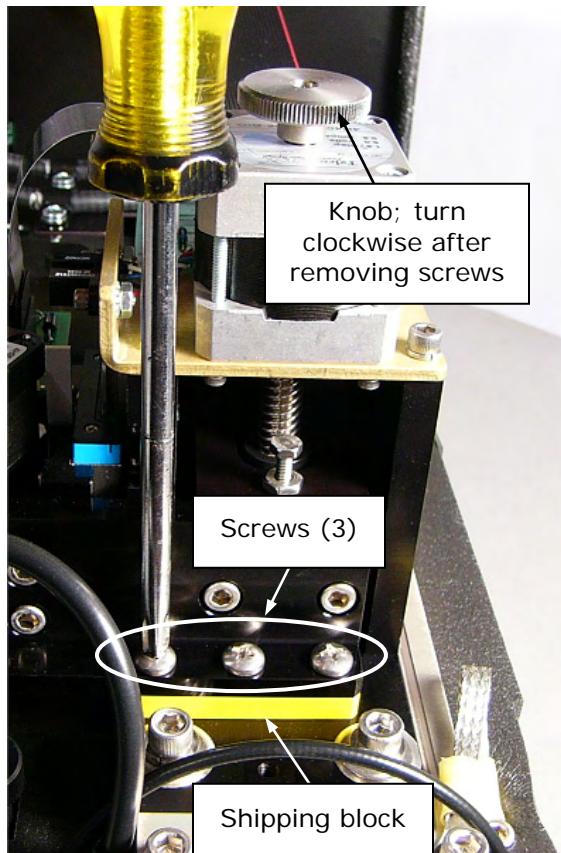


Figure 4: Remove three screws, turn the knob clockwise, remove the shipping block

5. Store the optic arm shipping block and its three screws (with washers) on the base plate.

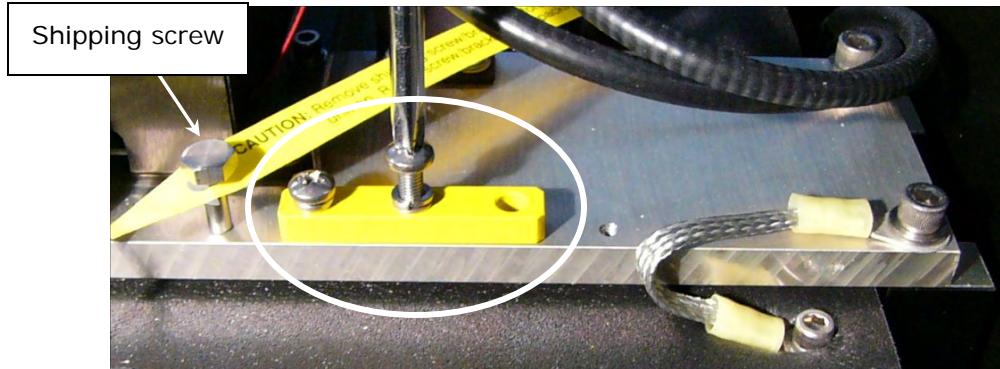


Figure 5: Carrier shipping screw and optic arm shipping bracket stored on the base plate

6. Remove the two screws holding the mirror holder shipping bracket in place. Store the screws and shipping bracket on the base plate shown in **Figure 5**.

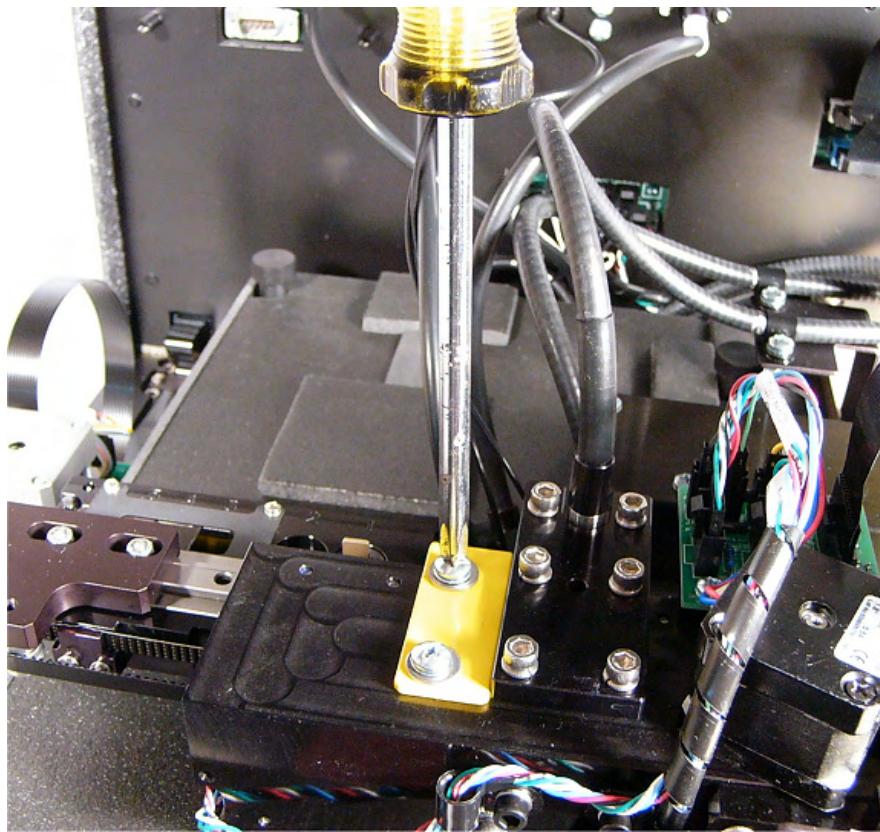


Figure 6: Removing the mirror holder shipping bracket

7. Slide the shroud onto the reader and replace the two side screws (**Figure 1**).

5: 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.

Perform these steps to install the power supply:

1. Locate the power inlet on the side of the reader; it is labeled "POWER IN."
2. Examine the power supply's plug. It has a small groove that will line up with a tab inside the power inlet. The plug is encircled by a securing ring.
3. Insert the plug into the power inlet. Align the groove with the tab and twist the ring clockwise until the plug is firmly seated.
4. Plug the power supply's cord into an appropriate power receptacle.



Figure 7: The external power supply (left) and the reader's power inlet (right)

6: Install the Dispense Module

1. Place the dispense module on top of the reader.

❖ Do not place the module next to the reader.



Figure 8: The dispense module on top of the reader

2. On the rear panel of the reader, identify the SYRINGE 1 and SYRINGE 2 tubing ports. Remove the nylon screws from both ports.
3. Open two of the plastic bags containing the outlet tubes (labeled as PN 7082120). Remove the clear plastic shrouds from the tubes. Put the other two bags in a safe place; they are spares.
4. Place the nylon screws and the plastic shrouds in the tool storage bag. Use the supplied Velcro strips to attach the bag to the rear panel of the dispense module.
5. Remove the two inlet tubes from their plastic canisters.
6. Identify the two syringe valves on the dispense module (see **Figure 9**). Each is labeled with a left-pointing arrow.

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

7. Screw the fitting of one inlet tube into the right side of the Syringe 1 valve.
8. Screw one end of one outlet tube into the left side of the Syringe 1 valve.
9. Screw the other end of the outlet tube into the SYRINGE 1 port on the rear of the reader.
10. Repeat these steps to attach the inlet and outlet tubing for Syringe 2.
11. Seat the outlet tubes in the clip to the left of the Syringe 2 valve.

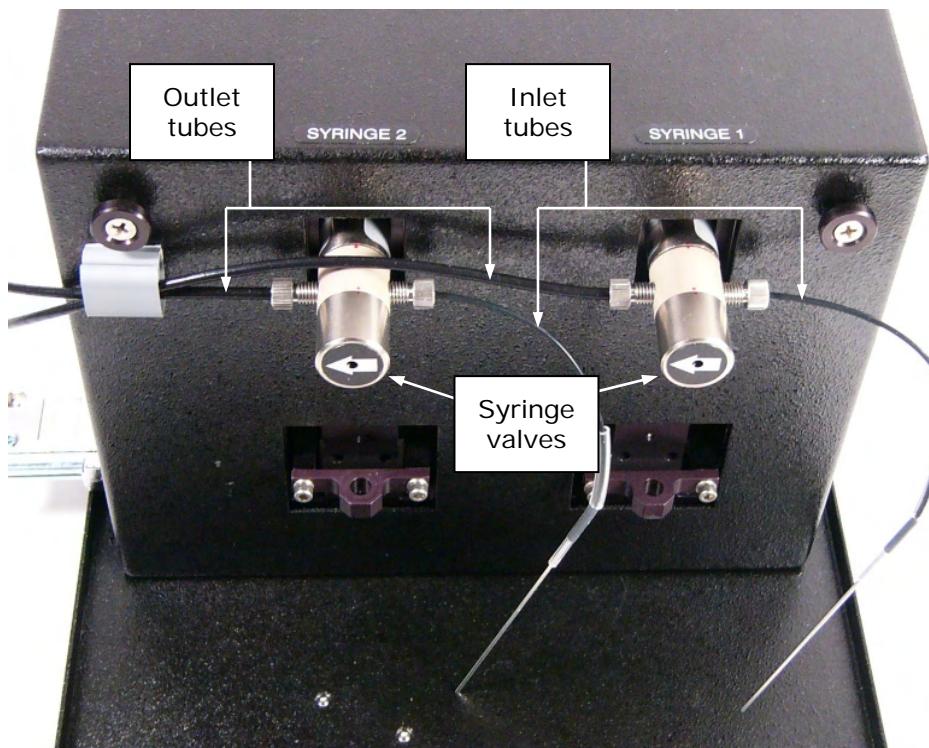


Figure 9: The dispense module's outlet and inlet tubes, and syringe valves

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, referring to **Figure 10**.
 - 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.

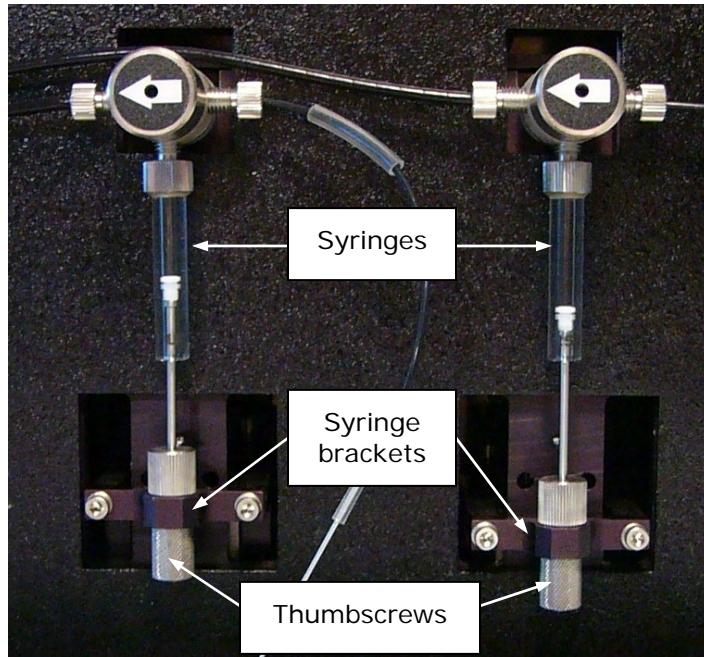


Figure 10: The dispense module; close-up view of the syringes

14. Locate the dispense module cable. Plug one end into the port on the left side of the dispense module. Plug the other end into the “Dispenser Port” on the right side of the reader.
15. Locate the injector tip-cleaning stylus, packaged in a small cylinder. Attach the cylinder to the back of the dispense module for storage.

7: Connect the Host Computer

The Synergy H4 is equipped with two communication ports: Serial (RS-232) and USB. Both ports are located on the right side of the reader.

- Two communication cables are included in the accessories box. Determine which cable is supported by the host computer.
- Connect one end to the appropriate port on the reader and the other end to the appropriate port on the host computer.

8: Install Gen5 on the Host Computer



The Synergy H4 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.

9: Turn on the Power Supply and Reader

1. If Gen5 is open, close it now.
2. The power supply has its own power switch. Locate the switch and turn the power supply on.
3. The reader's power switch is located on the lower-left corner of the front panel. Turn the reader on.

The reader performs a System Test. When the test is completed, the reader extends the microplate carrier.

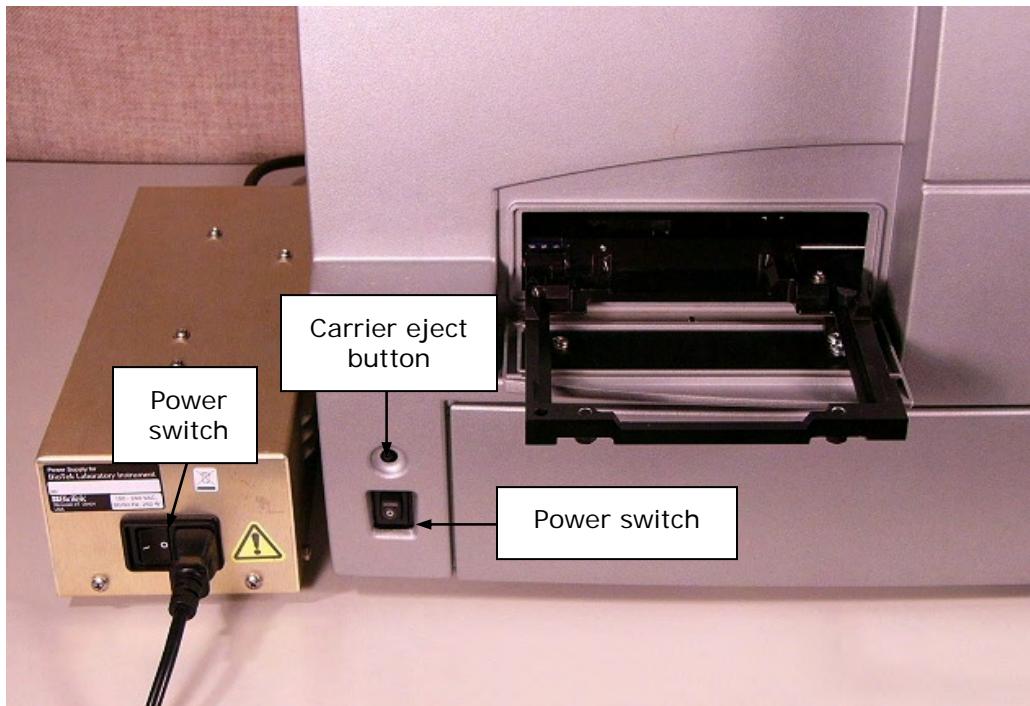


Figure 11: The reader with its microplate carrier extended.

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

10: Establish Communication

1. Refer to the instructions that shipped with the USB Driver Software CD to install the necessary drivers.
2. Start Gen5 and log in if prompted. The default System Administrator password is **admin**.
3. When the Welcome screen appears, select **System Menu**.
4. Select **System > Reader Configuration** and click **Add**.
5. Set the Reader Type to **Synergy H4**.
6. Select **Plug & Play**.
 - ❖ A Synergy H4 must be connected to the computer and turned on to appear in the Available Plug & Play Readers list.
7. Click **Test Comm**. Gen5 attempts to communicate with the reader. If the communication attempt is successful, 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?
- 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.

11: 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:
 - In Gen5, select **System > Reader Control > Synergy H4**.
 - 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 the **Synergy H4** and click **OK**.
 3. When the test is completed, a dialog requesting additional information appears. Enter the information and click **OK**.
 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 Installation records.
 - The 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.
5. Turn off the incubator:
 - Select **System > Reader Control > Synergy H4**.
 - Click the **Pre-Heating** tab and click **Off**.
 - Return to Gen5's main view.
 6. **Models with injectors:**
Keep Gen5 open and proceed to the next section, **Test the Injector System**.
All other models:
The installation and setup process is complete. Close Gen5 and proceed to **Operational/Performance Qualification** on page 23.

12: Test the Injector System

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

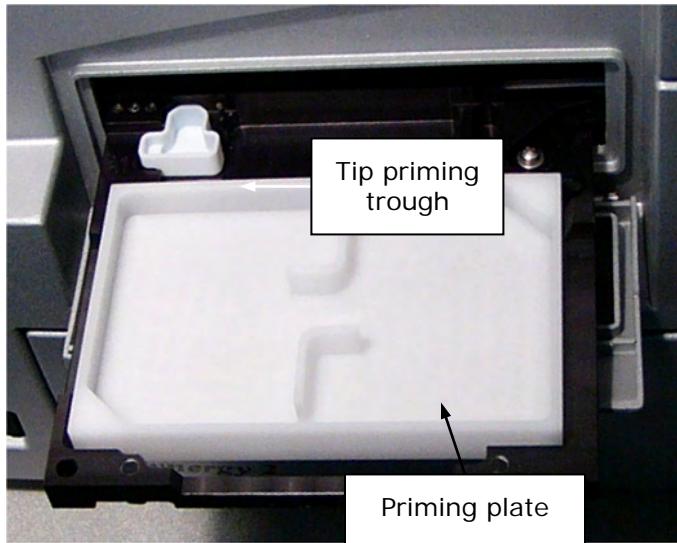


Figure 12: The tip priming trough and priming plate installed on the carrier

4. Fill the two reagent bottles with distilled or deionized water. Place the bottles in their holders, and place the holders directly in front of the syringes. Insert the inlet tubes into the bottles.

5. In Gen5, select **System > Reader Control > Synergy H4 (Com<#>)**.

6. Click the **Dispenser** tab.

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

The syringe should move down and up repeatedly, drawing fluid from the bottle. The fluid should pump through the tubing and dispense into the priming plate. Examine the fittings; no leaks should be detected. If leaks are detected, tighten all fittings and repeat the prime. If leaks are still detected, contact BioTek's Technical Assistance Center.

8. When the prime finishes, set Volume to **2000 µL** 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. See **Operational/Performance Qualification** next.

Operational/Performance Qualification

Your Synergy H4 Multi-Detection Microplate Reader 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 the Synergy H4.

- ❖ A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Synergy H4 is available for purchase (PN 8030514). Contact your local BioTek dealer for more information.

Repackaging and Shipping Instructions

Important! Please read all of the information provided below before preparing the Synergy H4 for shipment.



If the reader and/or dispense module 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 **Chapter 6, As Needed Maintenance** for decontamination instructions.

Remove the microplate and tip prime trough (if equipped) from the carrier before shipment. Spilled fluids can contaminate the optics and damage the instrument.



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.

Replace the shipping hardware before repackaging the reader. Please contact BioTek if you have misplaced any of these items, and order PN 7130016:

- Carrier shipping tube
- Carrier shipping screw/o-ring
- Optic arm shipping block/screws (3)

If you need to ship the Synergy H4 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.

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.

1. Contact BioTek's Technical Assistance Center for an RMA (Return Materials Authorization) number before returning equipment for service.
2. Decontaminate the reader and, if attached, the dispense module, according to the instructions provided in **Chapter 6**.
3. If you will also be shipping the dispense module, perform these steps now:
 - ❖ If you have not already done so as a part of decontamination, purge the dispense module and fluid lines.
 - With the reader on, start Gen5 and select **System > Reader Control > Synergy H4 (Com<#>)**.
 - Perform this step twice, for both dispensers: Click the **Dispenser** tab and set the dispenser number (1 or 2). Click **Maintenance**. The syringe bracket will lower. 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.
 - Fully detach the dispense module from the reader. Replace the two nylon screws into the Syringe 1 and 2 tubing ports on the rear of the reader. (The screws are stored in the plastic bag attached to the back of the module.) Set the module aside for the moment.
 - 4. Remove the tip priming trough and store it in the accessories bag.

5. If you have not already done so, retract the microplate carrier and then turn off and unplug the reader.
6. Remove the shroud and replace the shipping hardware (see page 11). Replace the shroud when finished.
7. Carefully tip the reader onto its back on the work surface. Attach the shipping panel to the bottom of the reader using the four flat-head screws and washers. Ensure the handles are oriented as shown in **Figure 13**.
8. Locate the inner shipping box. Using two people, grasp the handles on the shipping panel and carefully lower the reader into the inner shipping box.
9. Slide the cardboard sleeve straight down into place around the reader. Place the cover on the inner shipping box. Seal the box with tape.
10. Locate the outer shipping box. Place four foam blocks in the four bottom corners of the box. Using two people, lower the packed inner shipping box into the outer shipping box.
11. Place four foam corner blocks around the inner shipping box. Close and seal the outer box with tape.
12. When finished, write the RMA number on the outside of the box and ship the box to BioTek (see page 5 for the address).

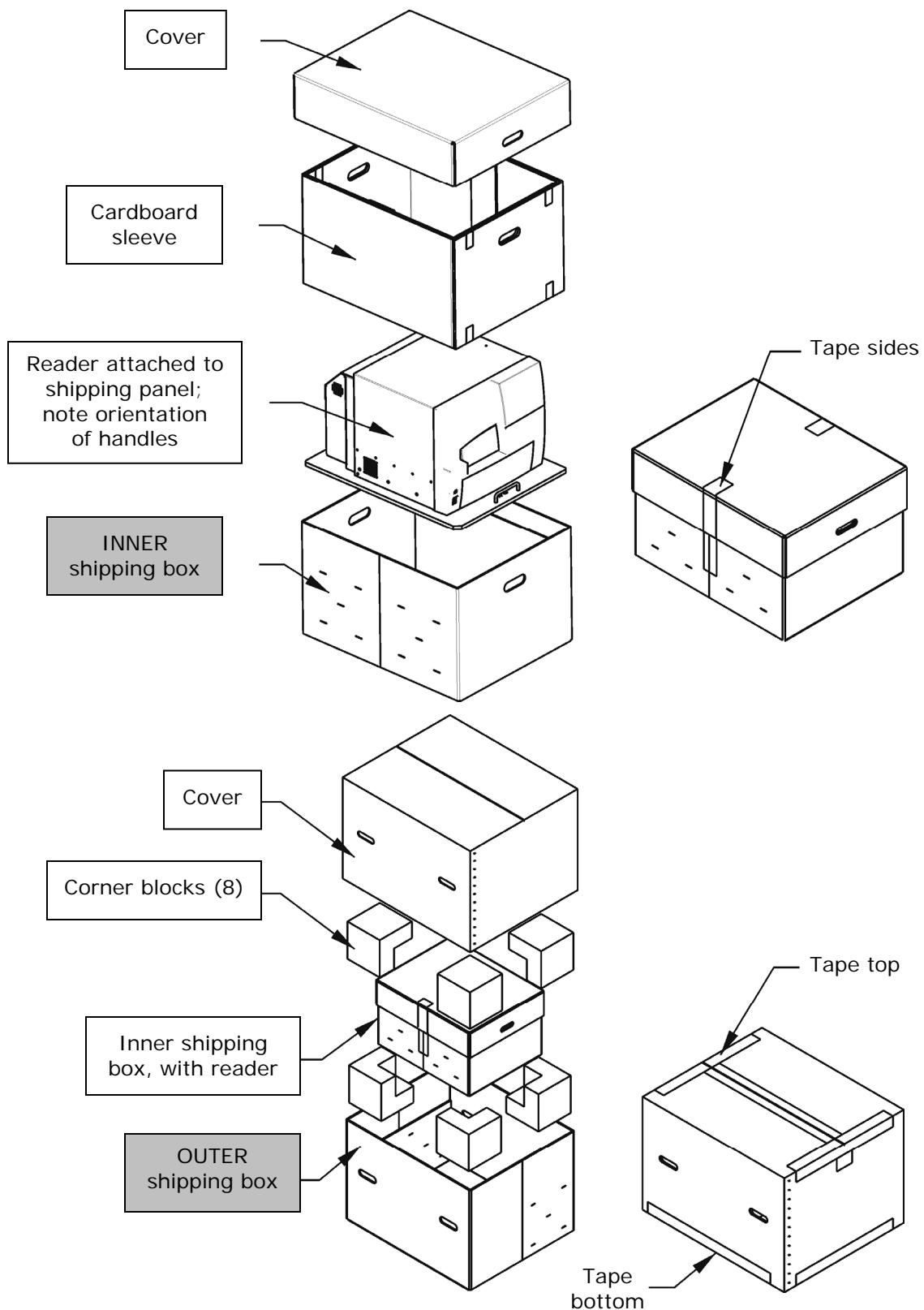


Figure 13: Packing the INNER and OUTER shipping boxes

To prepare the accessories for shipment:

1. Place the operator's manual and power supply into the compartments in the accessories box.
2. Place the remaining accessories on top of the manual.
3. Place the protective foam insert on top of the accessories.
4. Close the box and seal it with tape.
5. If you're shipping the accessories to BioTek, write the RMA number on the outside of the box. See page 5 for the shipping address.

To prepare the dispense module for shipment:

1. If you have not already done so:
 - Contact BioTek's Technical Assistance Center for an RMA (Return Materials Authorization) number before returning equipment for service.
 - Decontaminate the module according to the instructions in Chapter 6. Be sure to purge the dispense module of all fluid when finished.
 - Remove the two syringes (see step 3 on page 24) and store them in their original boxes.
 - Detach the dispense module outlet tubes and communication cable from the reader. Replace the two nylon screws into the Syringe 1 and 2 tubing ports on the rear of the reader.

❖ Refer to the figures on the next pages when performing these steps.

2. Remove the two inlet tubes from the syringe valves and store them in their plastic canisters.
3. 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.
4. Place the dispense module inside the inner shipping box. Slide the cardboard shipping insert down around the module. Pack the reagent bottle holders in bubble wrap and place them on top of the module. Seal the box with tape.
5. Locate the original accessories shipping box and foam end caps. Place the bottom foam end cap into the box.
6. Place the syringes, the inlet tubes, and the outlet tubes inside the bottom foam end cap. Place the dispense module shroud on top of the accessories.
7. Cover the accessories with the top foam end cap, place the dispense module cable inside the top of the end cap, and seal the box with tape.
8. Locate the original outer shipping box and foam end caps. Insert the bottom foam end cap. Lower the dispense module box into the end cap.
9. Insert the accessories box next to the dispense module box.

10. Insert the top foam end cap. Close and seal the outer box with tape.
11. Write the RMA number on the outside of the box. Ship the box to BioTek (see page 5 for the address).

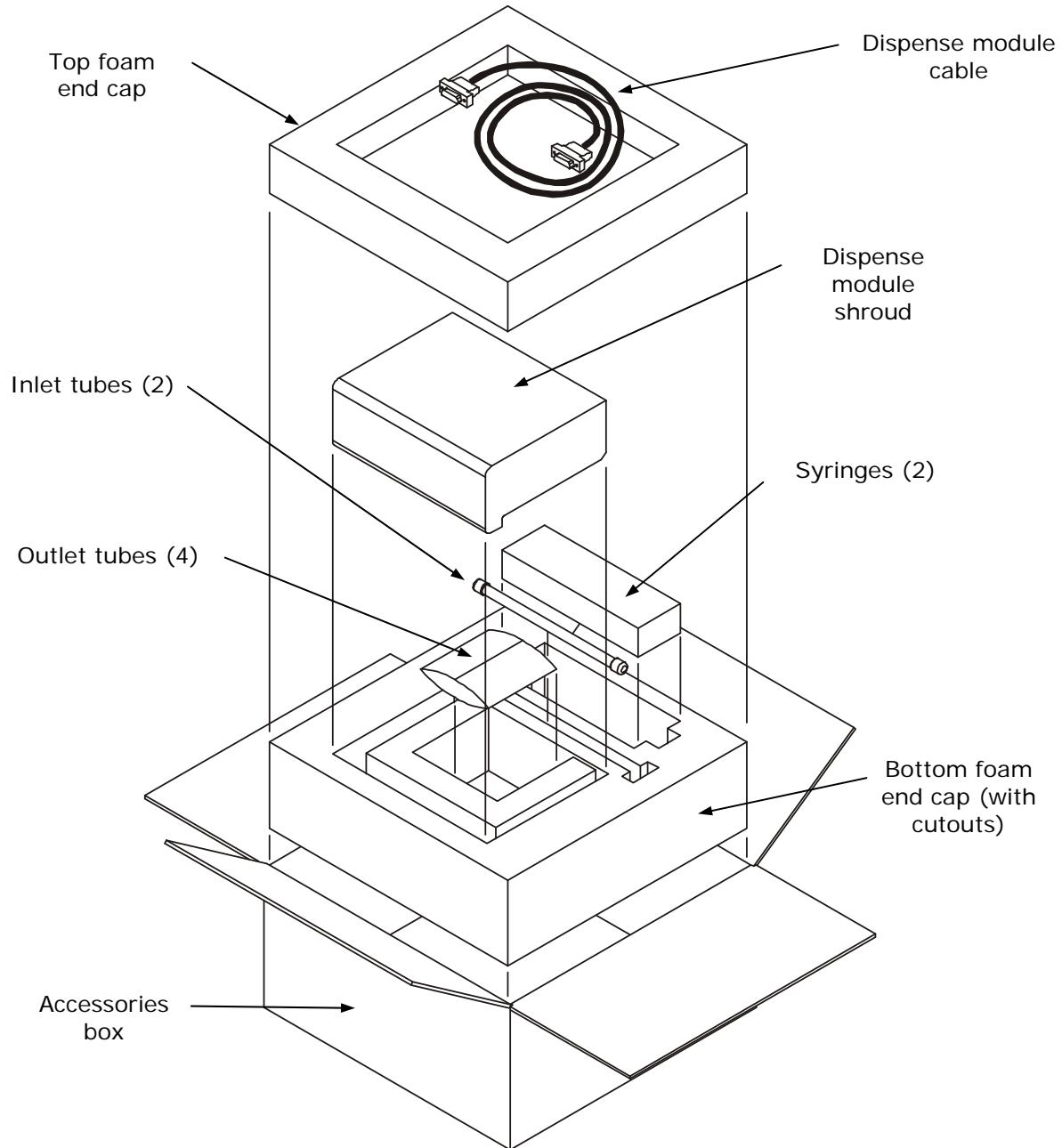


Figure 14: Dispense module accessories

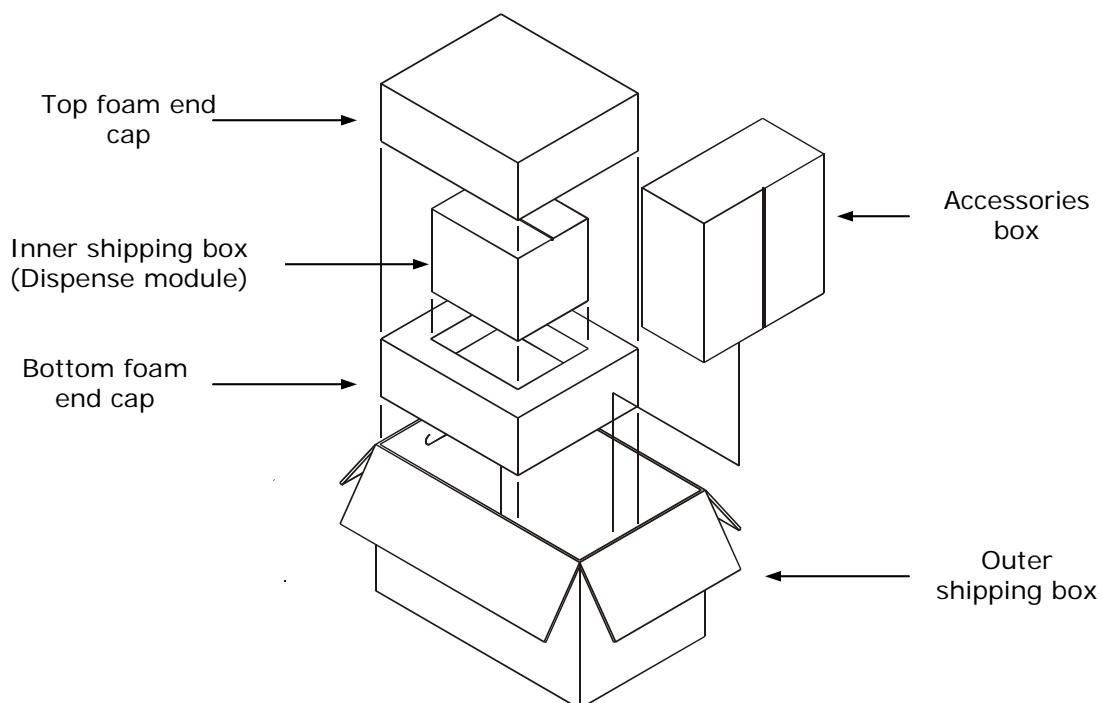
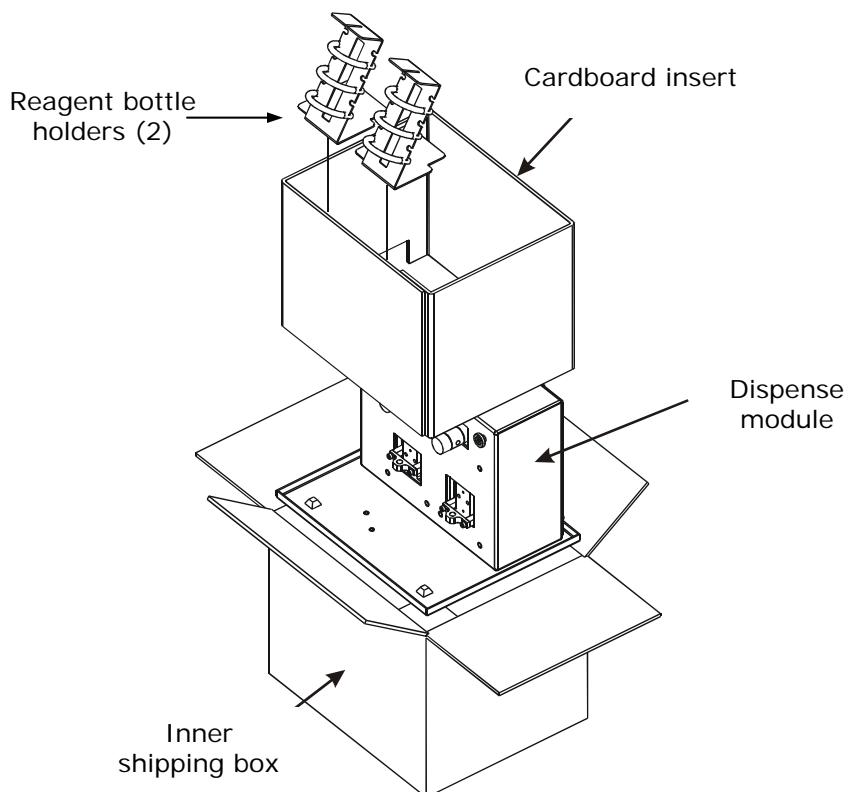


Figure 15: The contents of the inner (top) and outer (bottom) shipping boxes

Chapter 3

Getting Started

This chapter describes some of the Synergy H4'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 Synergy H4 is a multi-mode microplate reader, with a design that allows you to initially purchase only the modules 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 Dispense module, Gen5 will not provide the option to add a Dispense step to your assay protocol.

The module letters form the part number for each Synergy H4 model; for example, a reader with all modules installed is an H4MLFPTAD. This is indicated on a label on the reader.

Identifier	Module Description
H4	Synergy H4 base model. Includes temperature control to 65°C, plate shaking, and Gen5 software
M	Top and Bottom Monochromator-based Fluorescence
L*	Luminescence
F	Top Filter-based Fluorescence
PT*	Fluorescence Polarization/Time-Resolved Fluorescence
A*	Absorbance
D*	Dispense

* Optional modules that can be added on to the base Synergy H4 model.

External Components

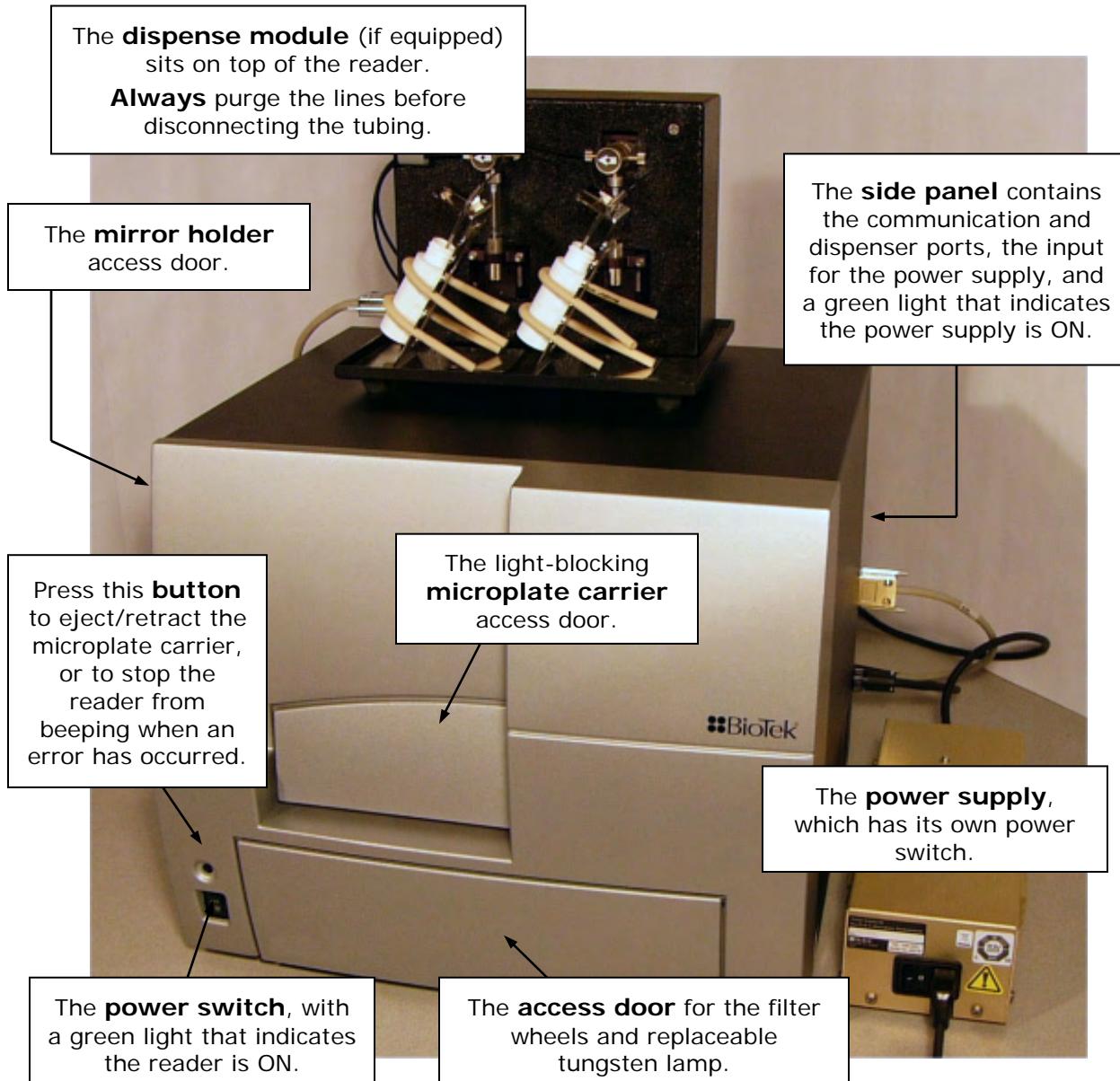


Figure 1: External components

Internal Components

- ❖ As discussed on page 32, not all of these components exist in all Synergy H4 models.

Component	What you need to know about it	Page
Tungsten Lamp	The tungsten lamp is accessible via the hinged door on the front of the instrument. A diagram showing the location of the lamp assembly and the orientation of the excitation and emission filter wheels is printed on the inside of the hinged door.	34
Emission and Excitation Filter Wheels	The filters and filter wheels can be changed to accommodate your assays, and filters (if used) should be cleaned periodically. The Synergy H4 supports Long Pass, Short Pass, and band pass filter types.	34
Mirrors	The mirror holder is accessible via a door on the left side of the reader. Do not put your hand in the side door while the reader is running; doing so presents a potential pinch hazard.	36
Injector System	The syringes may require replacement over time. The tubing and internal reading chamber may require cleaning over time. Applies to models with the Dispense module.	37

Tungsten Lamp

*Find instructions for changing the tungsten bulb in the **As Needed Maintenance** chapter.*

	The tungsten lamp assembly is hot when the instrument is turned on. Turn off the reader and allow the lamp to cool down before attempting to replace it.
---	--

The tungsten lamp assembly is accessible via a hinged door on the front of the instrument. To open the door, press on its lower-left and -right corners until the door opens downward. The lamp is on the left, behind a light shield with a hot surface warning label.

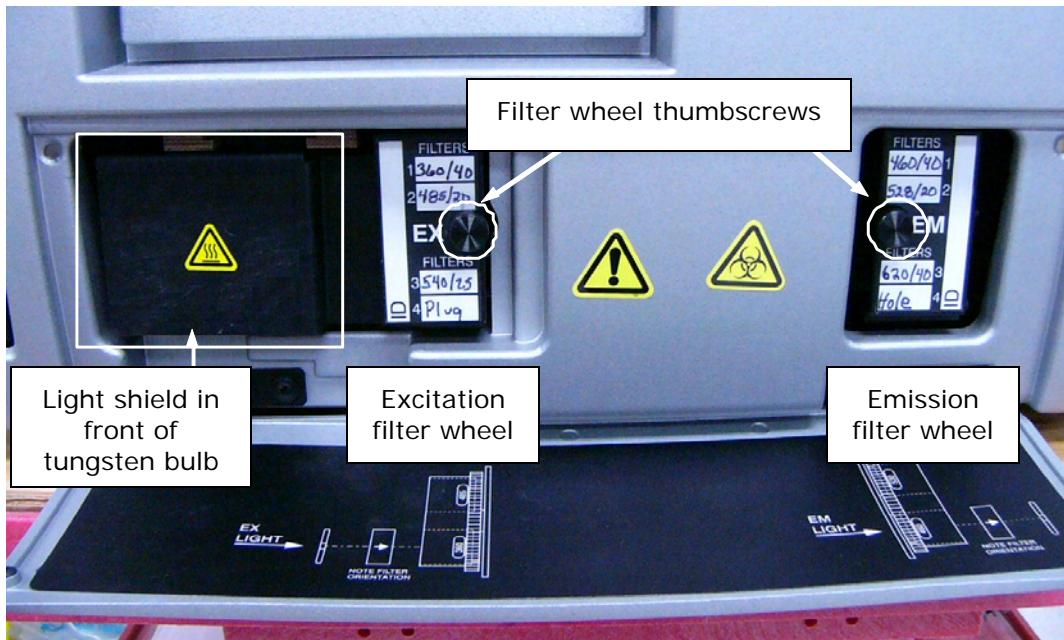


Figure 2: Open the front door of the reader



Keep the front door closed during operation. The intense broad spectrum light of the tungsten bulb can cause eye damage. The light shield in front of the bulb mitigates the risk.

Excitation and Emission Filters

The Synergy H4 is equipped with Excitation and Emission filter wheels for obtaining fluorescence and luminescence measurements. Each filter wheel is labeled **EX** or **EM**, and can contain up to four filters and/or black “plugs.” Each filter and plug is held securely in place with a C-clip filter retainer. The filter wheels are accessed through a hinged door on the front of the instrument (see Figure 2).

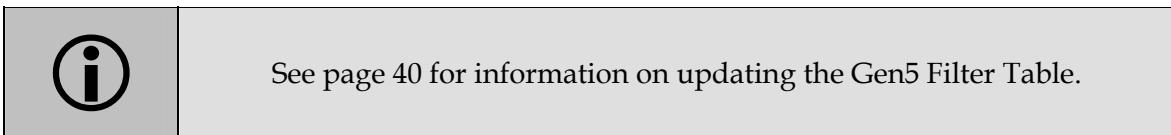
The Synergy H4 supports several filters types:

- **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.

Gen5 keeps track of each wheel’s contents and communicates this information to the instrument during operation. If you change either filter wheel, you must update Gen5’s filter table (**System > Reader Configuration**).

- Select **Band Pass**, **Long Pass**, or **Short Pass**, as appropriate for each filter type.
- Select **PLUG** to indicate the presence of a plug.
- Select **HOLE** to indicate an empty location.

Learn how to change the filter wheels in **Chapter 4, Filter and Mirrors**.



Configuring the System for Luminescence Measurements

- If your tests require that the light emitted from the samples remain unfiltered, the Emission filter wheel should have an empty location in it.
- If you made any changes to either filter wheel, you must update the Gen5 Filter Table. Select **PLUG** to indicate the presence of a plug and **HOLE** to indicate an empty location.
- When selecting a filter set for a read in Gen5, selecting **HOLE** indicates the empty location in the Emission filter wheel.

Mirrors

Learn more about mirrors, including how to change them, in the **Filters and Mirrors** chapter.

When taking fluorescence (FI, FP, or TRF) measurements from the top, the Synergy H4 uses mirrors to direct the excitation and emission light paths.

- ❖ Do not open the side door to access the mirror holder during instrument operation. Doing so may result in invalid data.

The mirrors are stored in a mirror holder, which is a removable rectangular box located inside the reader. (Additional mirror holders and mirrors can be purchased as an accessory.) The mirror holder and the mirrors are user-changeable. That is, you can replace the entire holder with a different one; this is the BioTek recommended option. Alternatively, you can install different mirrors in the mirror holder. Contact BioTek for more information on purchasing additional mirrors and holders.

The Synergy H4's mirror holder stores up to three mirrors. There are two possible mirror types:

- A **50%** mirror is a glass slide with silver polka dots. It works with any wavelength in the range of 200 to 850 nm.

- A **dichroic** mirror is wavelength specific: They require the excitation and emission filters to fall within specific ranges. Dichroic mirrors provide better sensitivity than 50% mirrors, but they are dye-specific.

Dichroic mirrors are available in two sizes:

- Half-size mirrors fit in positions 1 and 2 of the mirror holder. They can be used for any FI or TRF assay, but not FP.
- Full-size mirrors fit only in position 3 of the mirror holder. If the reader is equipped with the FP module, these mirrors can be used for all fluorescence detect modes (FI, TFF, and FP).

The mirror holder offers three reading positions, one for each mirror. For models with the Fluorescence Polarization (FP) module, the reader is also equipped with three polarizers. The polarizers are in position 3 of the mirror holder, so when you define an FP read in Gen5, it fixes the mirror (Optics Position) parameter to position 3. When running an experiment, Gen5 communicates with the reader to move the holder to the proper position based on the mirror defined in the Gen5 protocol.

Injector System

- ❖ The tubing and injectors should be cleaned at least every three months. See **Chapter 5, Preventive Maintenance**, for instructions.
- ❖ If a syringe is leaking, it may need to be replaced. See **Chapter 6, As Needed Maintenance**, for instructions.

External Dispense Module



Each dispense module is calibrated to perform with a specific Synergy H4 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 injectors located inside the instrument. Fluid is injected into one well at a time. The injectors support plate types from 6- to 384-well plates.

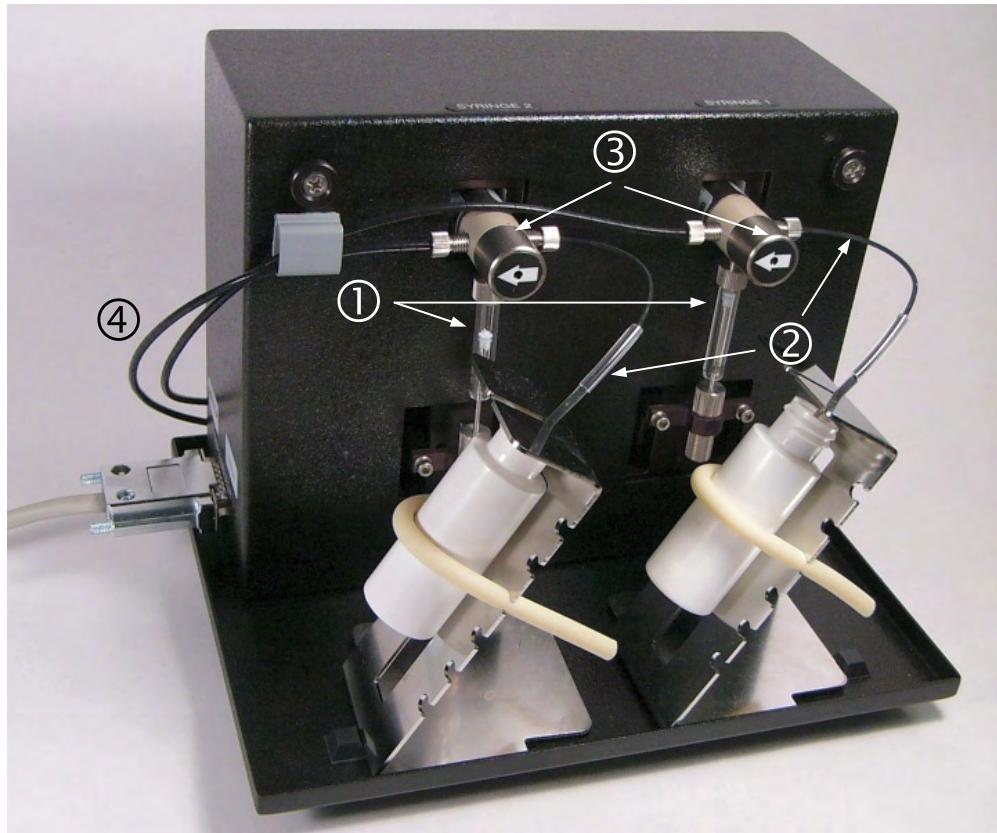


Figure 3: Dispense module components

- ① Two 250- μ 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 Valves switch the syringe flow from the inlet tubes to the outlet tubes.
- ④ Outlet tubes transport fluid from the syringes into the instrument, through the tubing ports on the Synergy H4's rear panel. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

Internal Tubing

Inside the Synergy H4, two Teflon tubes transport fluid from the tubing ports on the rear of the instrument to the two injectors. Both injectors dispense fluid into the same well.

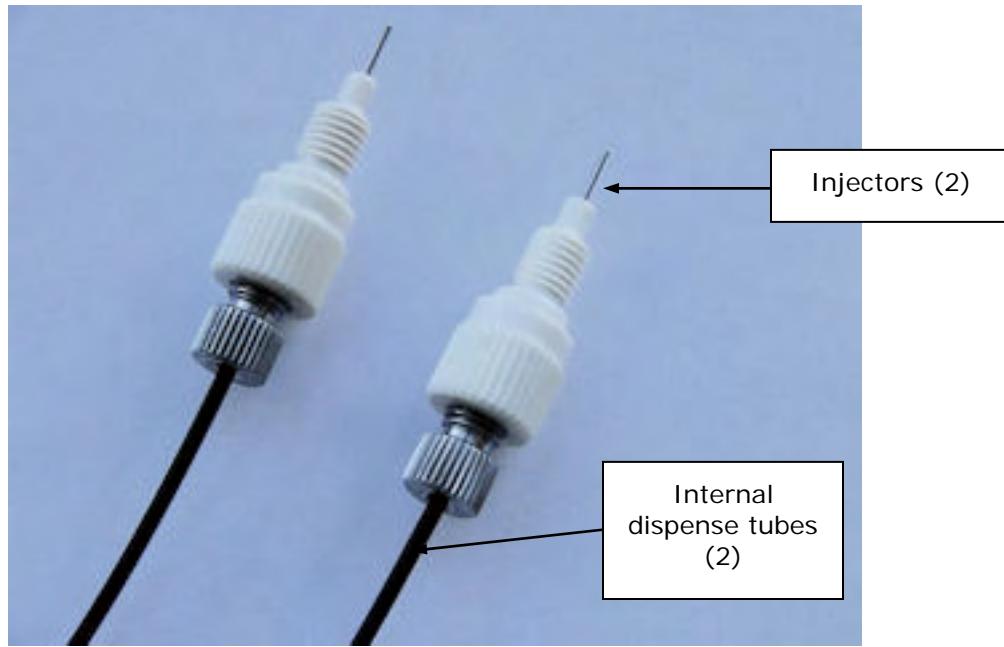


Figure 4: Close-up view of the injectors; see the **Preventive Maintenance** chapter for cleaning instructions (O-ring not shown)

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 the 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 (see page 43).

- ❖ 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 left-rear corner 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.

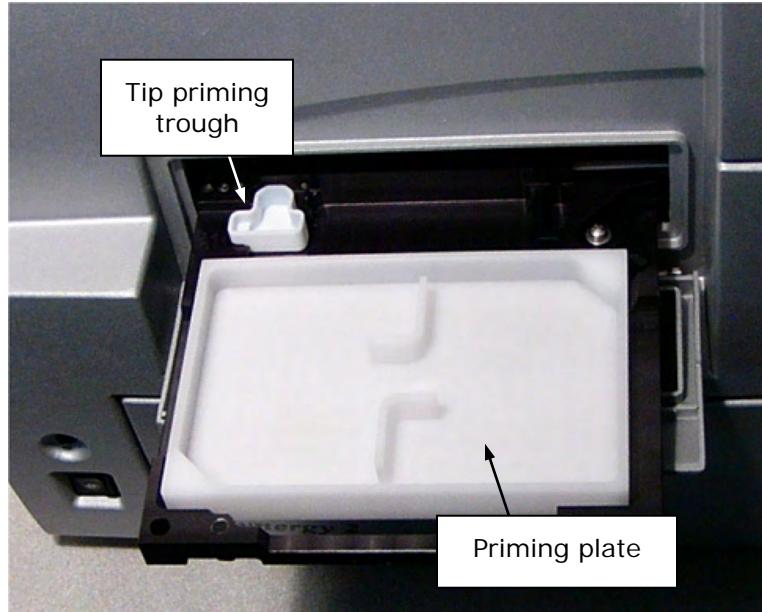


Figure 5: The priming trough and plate installed on the microplate carrier

Gen5 Software

BioTek Gen5 software supports all Synergy H4 reader models. Use Gen5 to control the reader and the dispense module (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 Excitation/Emission Filters

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

- Select **System > Reader Configuration**. Highlight the **Synergy H4** reader, and click **View/Modify**.
- Click **Setup** and then click the **Fluorescence/Luminescence** tab. Refer to the Gen5 Help system for additional instructions.

Define Mirrors

The reader's onboard software is configured with the mirror types and their characteristics. When Gen5 communicates with the reader, it "asks" for this information and then stores it in a Mirror Table on the computer. If you change any mirrors, you must define the changes in Gen5 and "send" the information to the reader.

- Select **System > Reader Configuration**. Highlight the **Synergy H4** reader, and click **View/Modify**.
- Click **Setup** and then click the **Mirrors** 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. Select **File > New Protocol**.
 2. Open the Procedure dialog. If prompted to select a reader, select the **Synergy H4** 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.
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 Builder, File Export Builder, or Power Export Builder options.
8. Select **File > Save** and give the protocol 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. Select **File > New Experiment**.
2. Select the desired protocol and click **OK**.
3. Highlight a plate in the menu tree and click **Read**. The Plate Reading dialog will appear.
4. Click **READ**. The door will open and the carrier will extend.
5. Place the plate on the carrier and click **OK** to begin the read.
6. When the read is complete, measurement values will appear in Gen5. Select the desired data set (e.g., "485, 528") from the Data drop-down list.
7. Select **File > Save** and give the experiment file an identifying name.

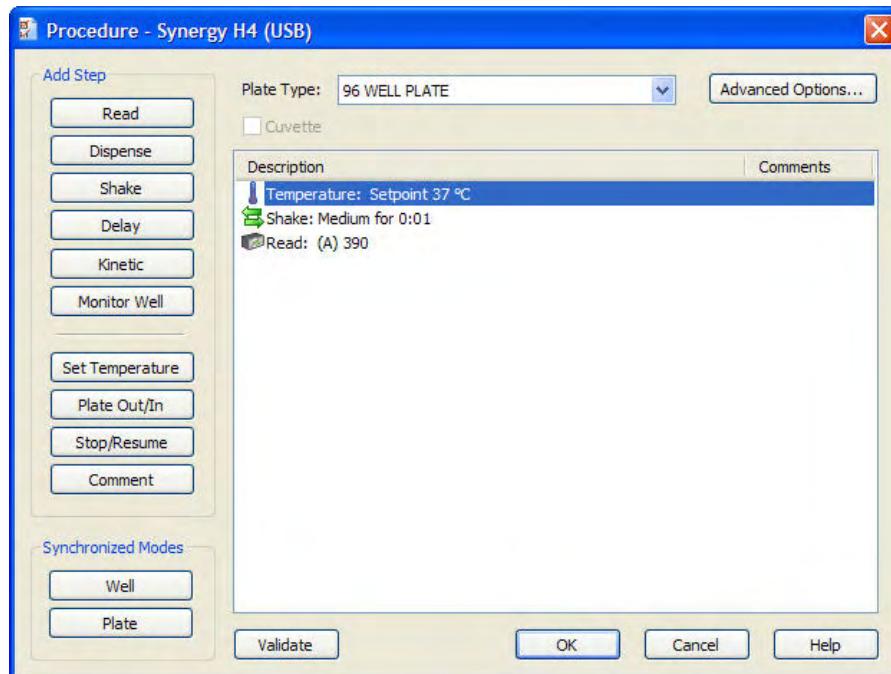


Figure 6: Defining the Procedure in a Gen5 Protocol

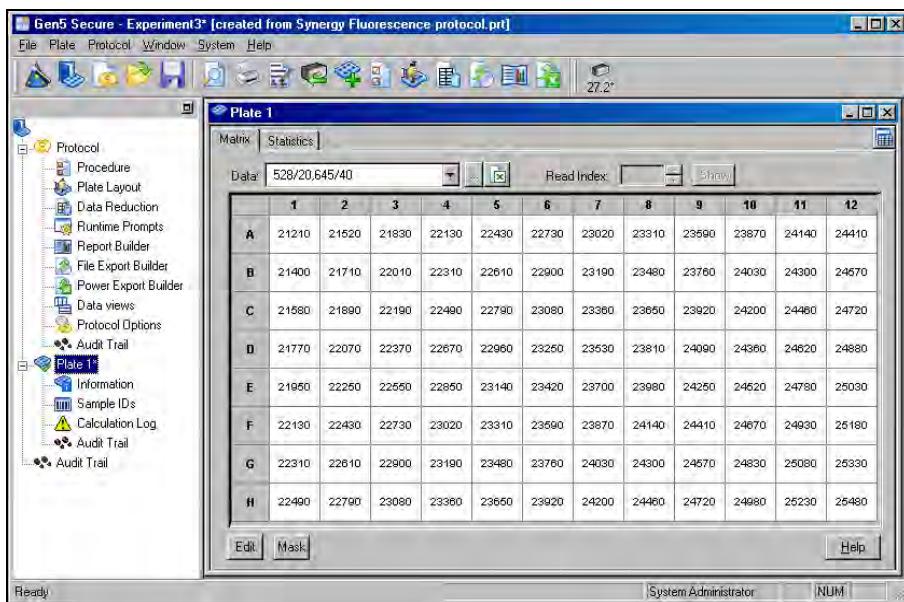


Figure 7: An Experiment (containing measurement data), based on a pre-defined protocol

Dispense Module Control

This section applies to models with injectors only.

Gen5 is used to perform several dispense module 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 path; see also “Flushing/Purging the Fluid Path” in **Preventive Maintenance**.

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 > Reader Control > Synergy H4 (Com<#>)** 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 µL.
6. Select a prime Rate, in µL/second.
7. Click **Prime** to start the process.

8. When finished, carefully remove the priming plate from the carrier and empty it.

❖ 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 > Reader Control > Synergy H4 (Com<#>)** 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 μL (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 Synergy H4 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 μL per well in a 96-well plate and 25 μL in a 384-well plate.
- Pipetting solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies. For best results, however, remove the air bubbles by degassing the plate in a vacuum chamber or spinning the plate in a centrifuge before reading.

- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Shake the microplate before reading to help bring it within acceptable limits. Use Tween 20, if possible (or some other wetting agent) to normalize the meniscus for absorbance measurements. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change. The meniscus shape will stabilize over time.

Luminescence Measurements

For highly sensitive Luminescence assays using white plates, add a Delay step to your Procedure to “dark adapt” the plates in the Synergy H4’s reading chamber before taking measurements.

Monochromator-Based Fluorescence Systems

For models equipped with the Time-Resolved Fluorescence module, TRF can be performed with the monochromator. The filter-based fluorescence system, however, 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 $\mu\text{L}/\text{well}$, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20 $\mu\text{L}/\text{well}$, we recommend a tip prime volume of 20 μL .

Chapter 4

Filters and Mirrors

Chapter 3, Getting Started provided an overview of the filters and mirrors installed in some Synergy H4 models. This chapter provides more detailed information on working with these components.

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Excitation/Emission Filters

Synergy H4 is equipped with **Excitation** and **Emission** filter wheels for obtaining fluorescence and luminescence measurements. The Excitation filter selects the band of light to which the sample will be exposed. The Emission filter selects the band of light with the maximum fluorescence signal of the sample, to be measured by the photomultiplier tube (PMT).

The filter wheels, shown in **Figure 1**, are accessed by opening the hinged door on the front of the reader.



Figure 1: Accessing the Excitation (EX) and Emission (EM) filter wheels

Each filter wheel is labeled as EX or EM, and can contain up to four filters and/or black plugs (also referred to as “dummy filters”). 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 (see **Figure 2**). Filters and plugs are secured with C-clip filter retainers.

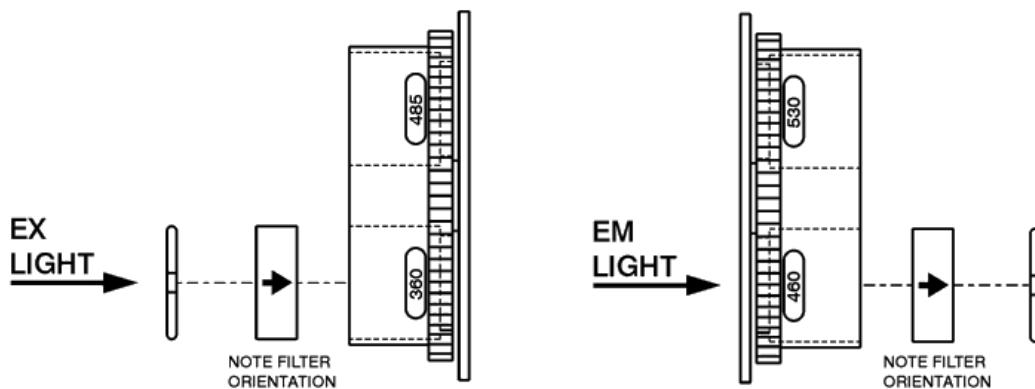


Figure 2: Excitation and Emission filter wheels, showing correct orientation of the filters

Change the Filter Wheels and Filters

Synergy H4's filter wheels are easily exchanged to meet your assay requirements. If you regularly need to change the filters on the reader, consider purchasing additional filter wheels from BioTek to make the process easier and faster.

As shown in **Figure 1**, labels on the front of the filter wheels can be marked with the central wavelength and band pass of each filter.

- ❖ Gen5 has a Filter Wheel Library feature that allows you to manage the contents of your filter wheels. Assign each wheel a unique identifier that matches its ID as defined in the Gen5 Filter Wheel Library.

When removing/replacing filter wheels:

- It is critical that the Gen5 Filter Table matches the actual filter locations in the EX and EM filter wheels. See "Define Excitation/Emission Filters" in the **Getting Started** chapter.
- The Excitation and Emission filter wheels are *not* interchangeable and are labeled EX = Excitation, EM = Emission.
- Filter direction within each filter wheel is important, and the direction differs depending on the filter wheel type. A diagram on the inside of the reader's front panel door indicates orientation; see **Figure 2**.
- Each filter is marked with an arrow indicating the correct direction of light through the filter. Filters are not specific to either excitation or emission.

To remove a filter wheel

1. Using your thumbs, push down on the bottom corners of the hinged door on the front of the reader to open the door.
2. Observe the two thumbscrews within the compartment. The left thumbscrew secures the Excitation filter wheel; the right secures the Emission filter wheel.
3. Remove the thumbscrew and slide the filter wheel's supporting metal bracket straight out of the compartment.

- ❖ The Emission filter wheel "springs" out when removed (a shutter behind the wheel closes quickly to protect the PMT).

To install a filter wheel

1. Ensure that all filters and/or plugs are inserted properly (see **Figure 2**).
2. Slide the filter wheel into its chamber.
3. Replace the thumbscrew and close the front door.

4. Use Gen5 to update the reader's internal software with the current filter wheel configuration; see "Define Excitation/Emission Filters" in the **Getting Started** chapter.



When removing or replacing a filter or C-clip filter retainer, do not use a sharp instrument. Use several layers of lens paper and your finger to remove and replace filters and clips. Using a sharp instrument, such as a flat screwdriver, will scratch the filter surface and make it unusable.

Do not touch the filters with your bare fingers.

To remove a filter or plug

1. Remove the filter wheel as instructed on the previous page.
2. Turn the filter wheel to align the desired filter with the hole in the supporting bracket.
3. Place the bracket on a flat surface, with the filter wheel facing down.
4. Prepare a multi-layered "cushion" of lens paper. Using your finger covered with the lens paper, gently push against the filter and its retainer until they pop out.

To replace a filter or plug

1. Hold the metal bracket with the filter wheel facing up.
2. Orient the filter or plug (see **Figure 2**): Observe the arrow on the filter indicating the light direction. Align the filter's wavelength number with the window in each filter holder, then drop it into the desired location.
 - ❖ Make note of the filter position number, 1–4.
3. Using your fingers, squeeze the sides of the C-clip retainer, and then insert it into the top of the hole containing the new filter. Cover your finger with several layers of lens paper, and then push down on all sides of the retainer until it sits flush against the filter.
4. Gently wipe both sides of the filter with lens paper.
5. When finished, install the filter wheel.

Clean the Filters

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

Filters Available from BioTek

Bandpass filters are available for purchase from BioTek. Please note that part numbers are subject to change, and new filters may become available. Custom filters are also available. Contact BioTek Customer Care with any questions.

Part Number	Wavelength	Main Application
7082259	284/10	Tryptophan excitation
7082248	310/20	Tyrosine emission, O-aminobenzoyl excitation
7082250	320/20	7-methoxycoumarin and Quanta Blu excitation
7082263	330/80	HTRF excitation
7082254	340/11	Fura-2 excitation
7082230	340/30	NADH excitation and tryptophan emission
7082220	360/40	MUB, caspace-3, europium chelate excitation
7082228	380/20	Fura-2 and EBFP excitation
7082242	400/10	
7082205	400/30	Porphyrin excitation, O- aminobenzoyl and 7-methoxycoumarin emission
7082206	420/50	CFP excitation and Quanta-Blu emission
7082227	440/30	Attophos excitation and caspace-3 emission
7082207	440/40	NADH emission
7082208	450/50	CBQCA excitation
7082222	460/40	NanoOrange excitation and EBFP and MUB emission
7082221	485/20	Fluorescein, EGFP excitation and CFP emission
7082209	485/40	Propidium Iodide excitation
7082256	500/27	YFP excitation
7082218	508/20	Fura-2 emission
7082246	516/20	EGFP emission
7082247	528/20	VIC excitation and Fluorescein and EGFP emission
7082223	530/25	5-Tamra excitation
7082249	540/25	Alexa Fluor 546, CY3, and rhod2 excitation and EYFP emission
7082253	540/35	Alamar Blu, Amplex red, RFP excitation
7082210	545/40	Rhodamine B excitation
7082215	560/15	Cell Titer Blue excitation
7082211	560/20	VIC emission
7082212	560/40	Attophos and CBQCA emission
7082264	570/100	AlphaScreen emission
7082245	575/15	ROX excitation and CY3 and 5-Tamra emission
7082244	580/50	NanoOrange and Attophos emission
7082225	590/20	Alexa Fluor 594 and Texas Red excitation and Cell Titer Blue emission
7082224	590/35	Rhod-2, Alexa Fluor 546, and CY3 emission
7082252	600/40	Alamar Blu, Amplex Red, RFP and porphyrin emission
7082265	620/10	HTRF / LANCE emission
7082251	620/15	ROX and Alexa Fluor 594 emission and Alexa Fluor 633 excitation
7082213	620/40	Rhodamine B, europium chelate emission, CY5 excitation
7082214	635/32	Texas Red emission
7082257	645/15	Alexa Fluor 633 emission
7082266	665/7.5	HTRF / LANCE emission
7082226	645/40	Texas Red and Propidium iodide emission
7082229	680/30	CY5 emission, AlphaScreen excitation

The fluorescence ratio associated with the HTRF readout is a correction method developed by CIS bio and covered by the US patent 5,527,684 and its foreign equivalents, for which CIS bio has granted a license to BioTek. Its application is strictly limited to the use of HTRF reagents and technology, excluding any other TR-FRET technologies.

Mirrors

- ❖ Do not open the mirror holder access door on the side of the instrument during operation. Doing so may affect measurements.

For filter-based, top-reading fluorescence analysis, the Synergy H4 uses mirrors to direct the excitation and emission light paths. Mirrors are required for fluorescence polarization (FP) measurements to direct light to the sample, because fibers cannot carry polarized light. Mirrors also provide increased sensitivity for fluorescence intensity (FI) and time-resolved fluorescence (TRF) measurements.

Mirrors are stored in a three-mirror holder (additional mirror holders and mirrors can be purchased as accessories). The holder and individual mirrors can be changed to meet your requirements. Labels on the mirror holder can be marked with the mirror types stored in the holder.

You can replace the entire holder with a different one; this is the BioTek recommended option. Alternatively, you can install different mirrors in the holder. Contact BioTek for more information on purchasing mirrors and holders.

For Synergy H4 models with the FP module, the reader is equipped with three polarizers:

- Excitation polarizer (visible-range or UV-range, see page 54)
- Emission polarizer, **parallel** to excitation polarizer
- Emission polarizer, **perpendicular** to excitation polarizer

Inside the reader, the mirror holder is labeled with five position numbers (see **Figure 3** on the next page), which translate to three possible measurement positions (see **Figure 4**).

The third measurement position is dedicated to FP, because it holds the polarizers. It is twice the size of positions 1 and 2, and it is numbered 3, 4, 5 in the reader. Gen5 recognizes only the three measurement positions. FI and TRF can be performed using position 3 as well. See **Figure 5** for a close-up view of measurement position 3.

When running an experiment, Gen5 communicates with the reader to move the holder to the proper position based on the mirror you define in a Read step in the Gen5 protocol.

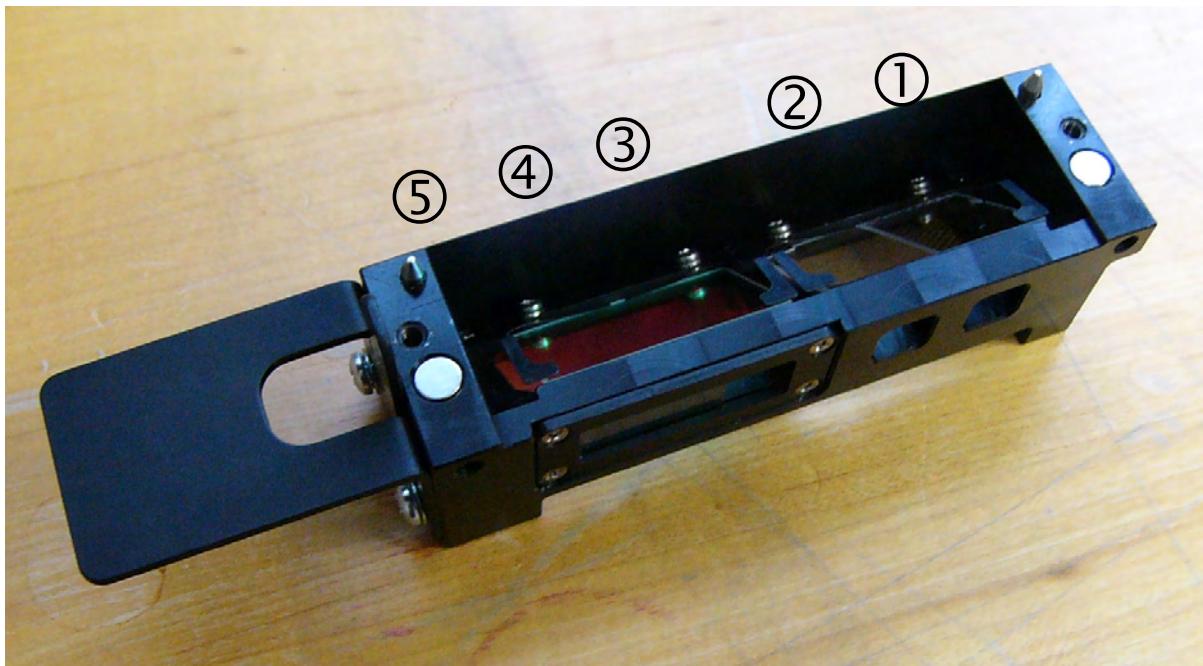


Figure 3: Mirror holder removed from the reader; the five position numbers are indicated

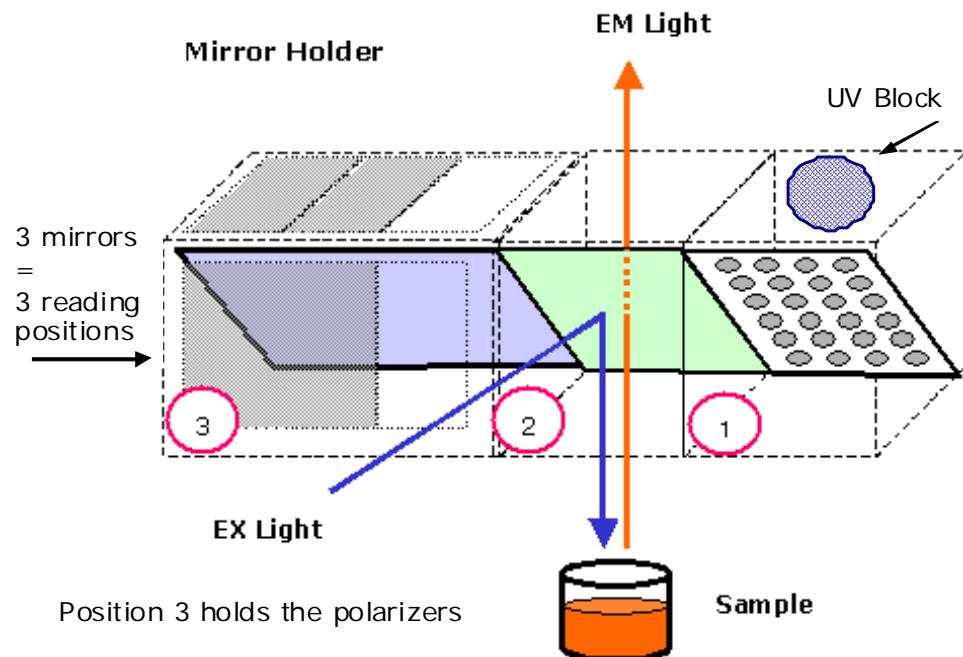


Figure 4: Mirror holder diagram; the three reading positions are indicated

- ❖ Position 1 in the mirror holder has an emission UV-blocking filter. It transmits light above 380 nm and blocks light below 360 nm. If you are using a 50% mirror and an excitation above 360 nm, for best results place the 50% mirror in position 1 (the default location). If emission of your assay is below 380 nm, do not use position 1 for these measurements; move the 50% mirror to position 2.

- ❖ **Models with the FP module:** The polarizers are always installed in positions 4 and 5, which translates to Mirror #3 in Gen5.

Position 3

This diagram shows Position 3 in the mirror holder. It holds the polarizer filters required for Fluorescence Polarization. It is the only mirror position that can be selected for an FP read. It can also be selected for FI and TRF, also, which use the open positions.

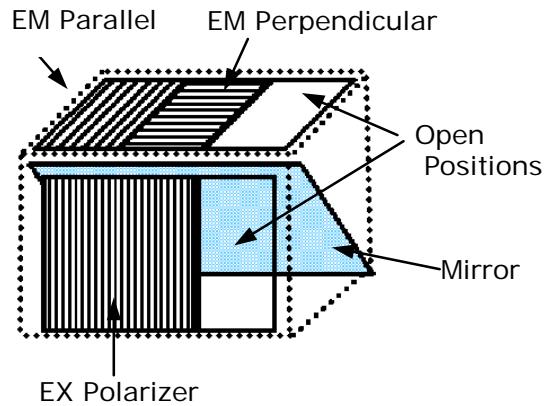


Figure 5: Mirror holder; Position 3 diagram

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 visible-range polarizer's bracket is etched with "400 nm". The emission (EM) polarizers are installed inside the reader, in the mirror holder's top plate.

The default mirror holder configuration is described here; your reader may be configured differently. Turn to page 60 for a list of mirrors available from BioTek.

Position #	Mirror #	Mirror Type	Polarizers	EX Range (nm)	EM Range (nm)	Use
1	1	50%	None	200–850	200–850	FI, TRF
2	2	400 nm Dichroic	None	320–390	410–800	FI, TRF
3	3	510 nm Dichroic	None	440–505	515–640	FI, TRF
4			EX (visible-range), parallel EM			
5			EX (visible-range), perpendicular EM			FP

Change the Mirror Holder and Mirrors

- ❖ **Do not touch the mirrors.** These optical elements are delicate and should be handled as carefully as possible. The glass and anti-reflective (AR) coated surfaces are damaged by any contact, especially by abrasive particles. Wear cloth gloves to reduce the risk of damaging the mirrors and polarizing filters. For cleaning instructions, see the **Preventive Maintenance** chapter.

Since dichroic mirrors are wavelength specific, it may be necessary to change a mirror before performing certain assays. BioTek offers additional mirrors and mirror holders as separate accessories.

Because mirrors and polarizing filters are easily damaged, if more than three unique mirrors are used in your lab, the preferred method is to use multiple mirror holders, which can be exchanged as needed.

Removing the mirror holder from the reader is required in either scenario. **Before** removing the mirror holder, take a moment to identify the reader's components that are described in the following procedures.

- ❖ On a new reader, the mirror holder is held in place by two shipping screws. These screws must be removed for easy replacement of the mirror holder. See **Chapter 2** for instructions on removing the screws.

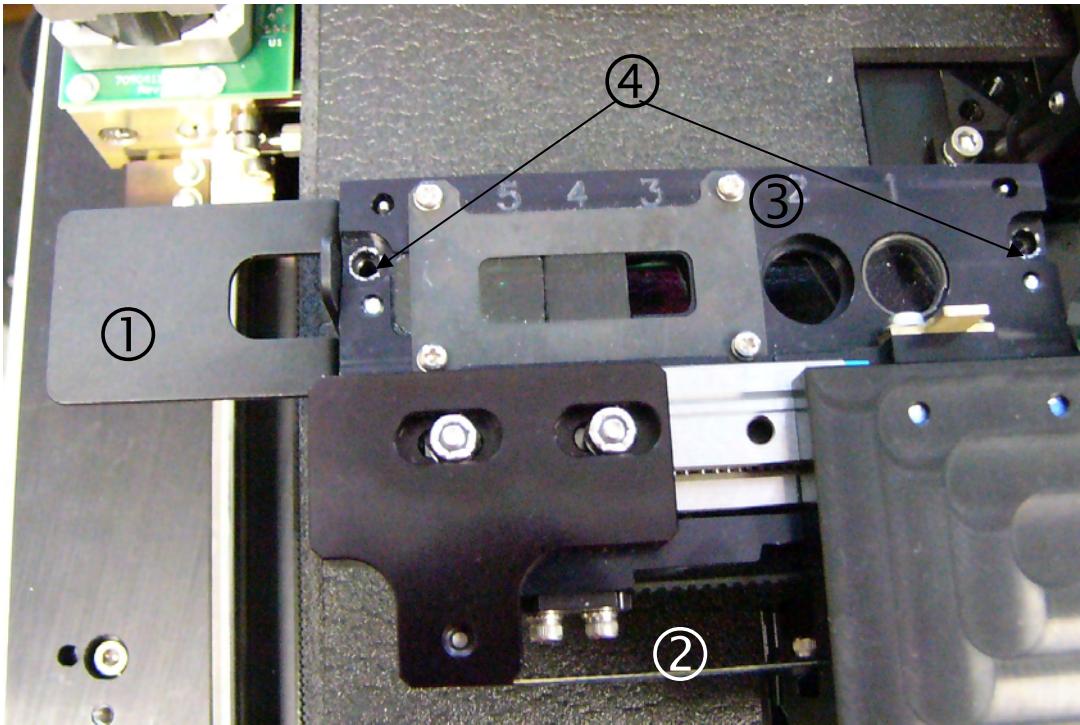


Figure 6: Mirror holder positioned for removal, as viewed from above the reader

1	Mirror holder handle
2	Belt drive
3	Top plate
4	Shipping screw holes

To remove a mirror holder

- ❖ You can remove the mirror holder from the Synergy H4 while the reader is turned on and no experiment is running. If a read is in process, wait for it to be completed before opening the side door.
- ❖ The screws holding the mirror holder in place for shipping must be removed before you can remove the holder through the access door. See the Remove the Shipping Hardware section of **Chapter 2, Installation**, for more information.
- ❖ A mirror holder must be configured in the Optics Library before you can perform this procedure.

1. Click **System > Optics Library > Set Reader**.

- ❖ The instrument must be connected and communicating to Gen5 to use the Set Reader command.

2. Select a mirror holder and click **Update Reader**. The reader moves the mirror holder to the “parked” position, from which you can easily remove it via the side door.
3. Open the door on the left side of the reader.
4. Reach into the reader, grasp the mirror holder’s handle, and pull gently down and out toward the door’s opening.
5. Install the new mirror holder in the reverse order, making sure it is properly engaged in the reader, then close the side door.
6. Click **OK**. The following message is displayed.



7. Click **OK**. The mirror holder name is updated with “(Match)” appended to the name, indicating that the mirror holder values for the specific holder have been downloaded to the reader.
- ❖ The reader does not verify the installed mirrors against the user’s mirror table.

To add a mirror holder to the Optics Library

1. Click **System > Optics Library > Mirror Holders**.
2. Click **Add** and enter a name for the mirror holder.
3. If the mirror values have already been entered via the Setup section of Gen5, click **Get current values** to populate the table. Otherwise, select the mirror type and enter the excitation and emission ranges.
4. Click **OK**.

To update the Gen5 Mirror Table

When you change the mirror holder, update Gen5 with the new mirror configuration:

- At the Welcome screen, click **System Menu**, then **System > Reader Configuration**.
- Double-click the **Synergy H4** reader to open the Reader Settings dialog.
- Click **Setup**.
- Select the **Mirrors** tab.

- For each Mirror position, 1, 2, 3, use the drop-down list to select the Type of mirror. For dichroic mirrors, enter the excitation and emission wavelength ranges. (Refer to the Gen5 Help for more information.)

-
- ❖ It is critical that the Gen5 Mirror Table reflects the actual location and characteristics of the mirrors in the reader.
 - ❖ If you accidentally touch the mirrors or polarizing filters, see the **Preventive Maintenance** chapter for cleaning instructions.

To change a mirror in the mirror holder

-
- ❖ Changing the mirrors within the holder is more difficult and risky than changing the entire mirror holder. BioTek recommends purchasing additional mirror holders that are configured at our factory to meet your requirements. Contact your BioTek representative or TAC for details.

Use these tools to change a mirror:

- Linen or cloth gloves
- Small Phillips screwdriver

-
- ❖ Touch the mirrors as little as possible; hold them by their edges only. The mirrors are easily damaged.

1. Remove the mirror holder from the reader.
2. The mirror holder has a light shield to protect the mirror. Use a Phillips screwdriver to remove the four screws and washers that attach this shield to the holder.
3. Use the Phillips screwdriver to remove the black metal bracket that holds the mirrors in place (see **Figure 7**). Set aside the screws. Lift the bracket away from the holder and set aside.
4. Grasping the mirror by its edges, remove it from the holder and store it properly.
5. Holding the replacement mirror by its edges, turn the mirror so its label is face-up and readable. Align it in this orientation when you insert it into the holder (see **Figure 7**).
6. Replace the metal bracket to secure the mirror. The bend in the bracket's arms should point away from the holder.
7. When you change the mirrors, update Gen5 with the new mirror configuration (see page 57).

-
- ❖ It is critical that the Gen5 Mirror Table reflects the actual location and characteristics of the mirrors in the reader.

- ❖ If you accidentally touch the mirrors or polarizing filters, see the **Preventive Maintenance** chapter for cleaning instructions.
- ❖ The light shield only fits the holder one way. Check its alignment when reattaching it to the mirror holder.

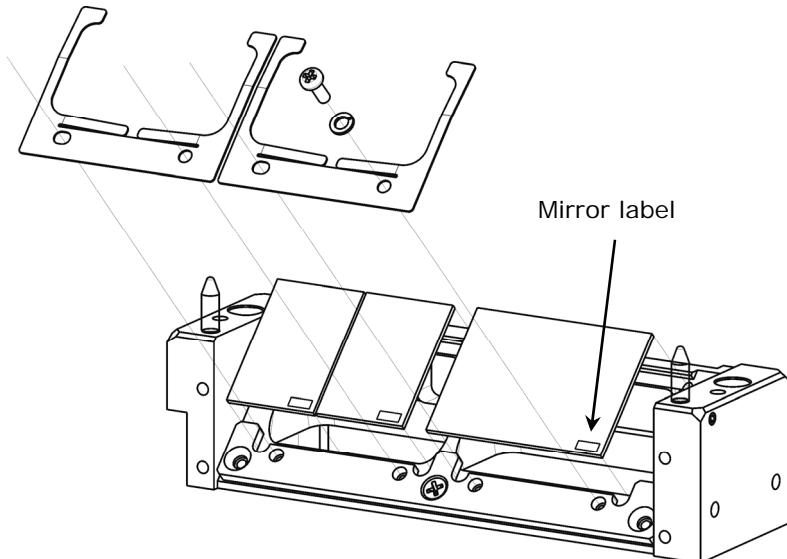


Figure 7: Mirror orientation; labels are face-up and readable

To reinstall the mirror holder:

Reverse the uninstall process to reinstall the mirror holder. The mirror holder is magnetized to make it easy to reinstall. Alignment pins help guide it back into place.

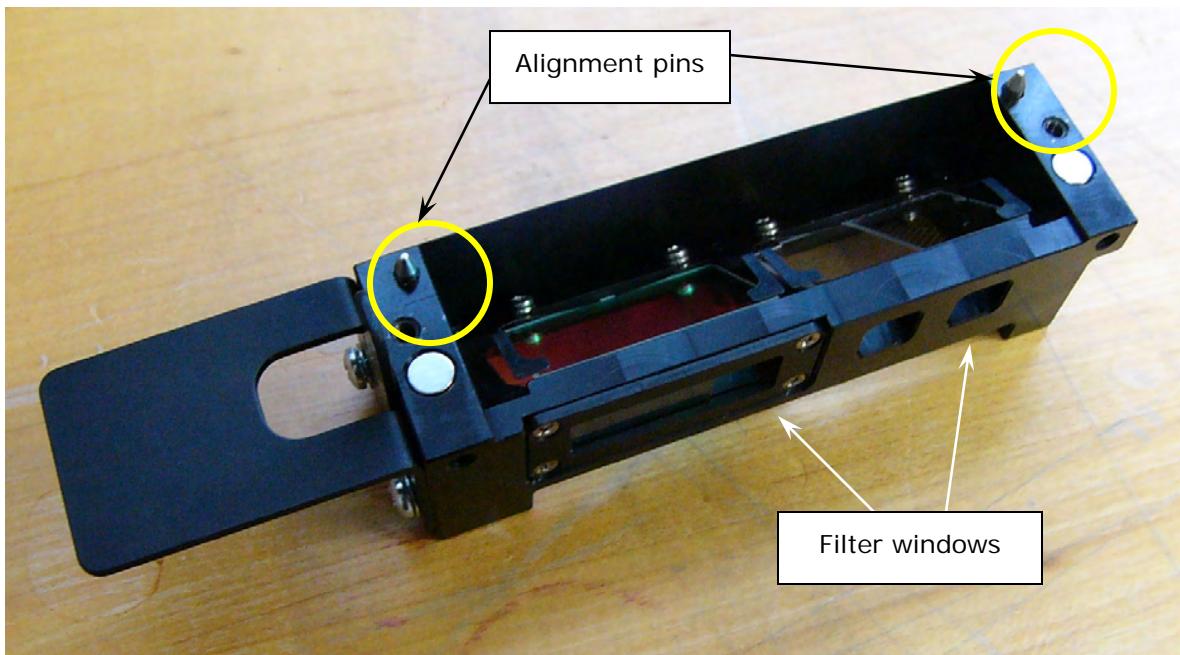


Figure 8: Mirror holder positioned for reinstallation

1. Hold the mirror holder with the alignment pins on top and the filter windows facing you. This is the holder's orientation inside the reader.
2. Inside the reader, put the mirror holder underneath its top plate, which has numbers for the mirror positions, as well as a polarizer in FP models. The holder's alignment pins will help guide it into place and because it is magnetized the holder will stay in place. (See photo on page 56.)
3. Reinstall the two Phillips-head screws and washers that secure it in the reader.

Clean the Mirrors

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

Mirrors Available from BioTek

Several mirrors are available for purchase from BioTek. Please note that part numbers are subject to change, and new mirrors may become available. Contact BioTek Customer Care with any questions.

- **Half-Size mirrors** fit into positions 1 and 2 of the mirror holder
- **Full-Size mirrors** fit into position 3.

Half-Size Part #	Full-Size Part #	Cut-off (nm)	Excitation Range	Emission Range	Main Applications
7132121	n/a	50%	200–850	200–850	All except FP
7138365	n/a	365	290–350	380–800	HTRF, MMP, Quanta Blu
7138400	7137400	400	320–390	410–800	MUB, Europium, Hoechst 33258
7138455	7137455	455	400–450	460–710	Attophos, CFP, Fluo-3
7138510	7137510	510	440–505	515–640	Fluorescein, Picogreen, FAM
7138525	7137525	525	475–520	530–670	Rhodamine 123, YFP
7138550	7137550	550	415–540	560–850	CY3, HEX, Rhodamine 6G
7138570	7137570	570	515–565	575–735	Alamar Blu, Amplex Red, TAMRA
7138595	7137595	595	540–590	600–770	ROX, Texas Red
7139635	n/a	635	640–780	400–630	AlphaScreen
7138660	7137660	660	580–655	665–850	CY5

Chapter 5

Preventive Maintenance

This chapter provides instructions for maintaining the Synergy H4 and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

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Preventive Maintenance

A general Preventive Maintenance regimen for all Synergy H4 models includes periodically cleaning all exposed surfaces and inspecting/cleaning the Emission and Excitation filters (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, internal 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.

Schedule

- ❖ The risk and performance factors associated with your assays may require performing some or all of the procedures more frequently than presented in the schedule.

Task	Page	Daily	Quarterly	As Needed
All models:				
Clean exposed surfaces	65			✓
Inspect/clean emission and excitation filters	65		✓	
Inspect/clean mirrors	66			<i>annually</i>
Decontamination	<i>see below</i>	<i>before shipment or storage</i>		
Models with injectors only:				
Flush/purge the fluid path	66	✓		
(Optional) Run Dispense protocol	68			✓
Empty/clean tip prime trough	69	✓		
Clean priming plate	70			✓
Clean internal components	70		✓	✓

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

Warnings and Precautions

Read the following before performing any maintenance procedures:

	Warning! Internal Voltage. Turn off and unplug the instrument for all maintenance and repair operations.
	Important! 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.

	<p>Important! 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.</p>
	<p>Warning! 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.</p>
	<p>Warning! 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.</p>
	<p>Caution! 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.</p>
	<p>Caution! Models with injectors. Before removing the reader's shroud to expose internal parts, purge the dispense module, turn off the instrument, and disconnect the fluid line, power cable, and PC cable.</p>
	<p>Warning! Pinch Hazard. Some areas of the reader can present pinch hazards when the instrument is operating. These areas are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.</p>

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

❖ **Models with injectors:** If the Tip Priming Trough overflows, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. If overflow is significant, you may have to remove the reader's shroud and the incubator housing to access the surface beneath the carrier.

- See page 71 for instructions on removing the shroud.
- See page 77 for instructions on removing the incubator housing.
- See page 80 for cleaning instructions.

Inspect/Clean Excitation and Emission Filters

Laboratory air is used to cool the lamp, and the filters 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. Pull down the hinged door on the front of the instrument. Observe the two thumbscrews within the compartment. The left thumbscrew secures the excitation (EX) filter wheel; the right secures the emission (EM) filter wheel. Remove each thumbscrew and slide the filter wheel straight out of the compartment.

❖ **Chapter 4** contains illustrations for identifying the filter wheels and their unique characteristics. It also contains instructions for replacing filters, if necessary.

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. Ensure that the filters remain in their current locations.
5. Use a magnifying glass to inspect the surface; remove any loose threads left from the cotton ball.
6. Replace the filter wheels in their respective positions and replace the thumbscrews. Close the hinged door.

Inspect/Clean Mirrors

We recommend inspecting/cleaning the mirrors and polarizing filters (if equipped) annually, especially if the mirror holder has been handled 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

- Cloth gloves
- Anhydrous reagent-grade ethanol
- Kimwipes
- Magnifying glass
- 100% pure cotton balls (for the polarizing filters)

Procedure

1. Turn off the reader and remove its shroud (see page 71 for instructions).
2. Perform the steps in **Chapter 4, Filters and Mirrors** to remove the mirror holder.
3. Use absorbent towels such as Kimwipes, **not** lens paper, and 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.
4. Wet the towel with an anhydrous reagent-grade ethanol.
5. Drag the trailing edge of the ethanol-soaked Kimwipe across the surface of the component, moving in a single direction. A minimal amount of pressure can be applied while wiping. However, too much pressure will damage the component.
6. Use the magnifying glass to inspect the surface; if debris is still visible, repeat with a new Kimwipe.
7. If equipped, clean the polarizing filters. Dampen a cotton ball with alcohol and gently stroke the surface of the filter to remove dust or fingerprints.
8. Reinstall the mirror holder and replace the shroud.

Flush/Purge the Fluid Path

Applies only to Synergy H4 models with injectors.

At the end of each day that the dispense module is in use, flush the fluid path using Gen5's 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 rear of 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. Select **System > Reader Control > Synergy H4 (Com<#>)**.
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 > Reader Control > Synergy H4 (Com<#>)**.
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.

Run a Dispense Protocol (Optional)

Applies only to Synergy H4 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:
 - Select **File > New Protocol** and then **Protocol > Procedure**.
 - Select a Plate Type that matches the plate being used.
 - Add a Dispense step with the following parameters:
 - Select **Dispenser 1**.
 - Set Tip Priming to **Before this dispense step** and Volume to **10 µL**.

- Set the Dispense Volume to **100 µL** (or an amount to match your assay protocol).
 - Adjust the Rate to support the dispensing volume.
 - Click **OK** to close the dialog and add the Dispense step to the procedure.
 - Add another Dispense step with the same parameters; select **Dispenser 2**.
 - Add a quick 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** and clear the **Use All Wells** checkbox. This action leaves only well A1 selected for the Read. Click **OK**.
 - Select any wavelength or define one Filter Set.
 - Click **OK** to close the dialog and add the Read step to the procedure.
 - Click **OK** to close the procedure.
 - Select **File > Save** and give the protocol an identifying name, such as "Dispense Observation."
2. Fill the reagent bottles with a DI H₂O-Tween solution (e.g., add 1 mL Tween 20 to 1000 mL of deionized water).
 3. Select **File > New Experiment** and select 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 as described in "Clean the Internal Components" beginning on page 70.

Empty/Clean the Tip Priming Trough

Applies only to Synergy H4 models with injectors.

The tip priming trough is a removable cup located in the left rear 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 its pocket in 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 Synergy H4 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 Internal Components

Applies only to Synergy H4 models with injectors.



For models **without** injectors, the internal chamber and probes are not customer-accessible. Contact BioTek's Technical Assistance Center with any questions about your particular model.

The Synergy H4's internal dispense tubes and injectors require routine cleaning, at least quarterly and possibly more frequently depending on the type of fluids dispensed.

Cleaning inside the reader is required whenever fluid has spilled inside the instrument and/or if an unusually high background signal has been flagged by the assay controls.

Start with **Remove the Reader's Shroud** and execute the procedures that meet your needs, in the order in which they are presented. Finish with **Reassemble the Components**.



The buildup of deposits left by the evaporation of spilled fluids within the read chamber can impact performance of the fluorescence, luminescence, and absorbance functions. Be sure to perform a System Test before and after maintenance so that any changes in performance can be noted.

Required Materials



Wear protective gloves and safety glasses when performing the procedures.

For all tasks:

- Protective gloves
- Safety glasses

For removing the shroud:

- Phillips screwdriver

For cleaning the internal dispense tubes and injectors, and for wiping the surface under the plate carrier:

- 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 Reader's Shroud



Before removing the shroud: Purge the dispense module (see page 66 for instructions), and then turn off and disconnect the reader from its power supply, the PC, and the dispense module.

1. If you have not already done so, purge the dispense module of fluid.
2. Disconnect power and all cables. Set the external dispense module aside.
3. Clear the work surface around the reader so you can easily access all sides of the instrument.
4. Remove two screws: one on each side of reader at the bottom-rear corner.

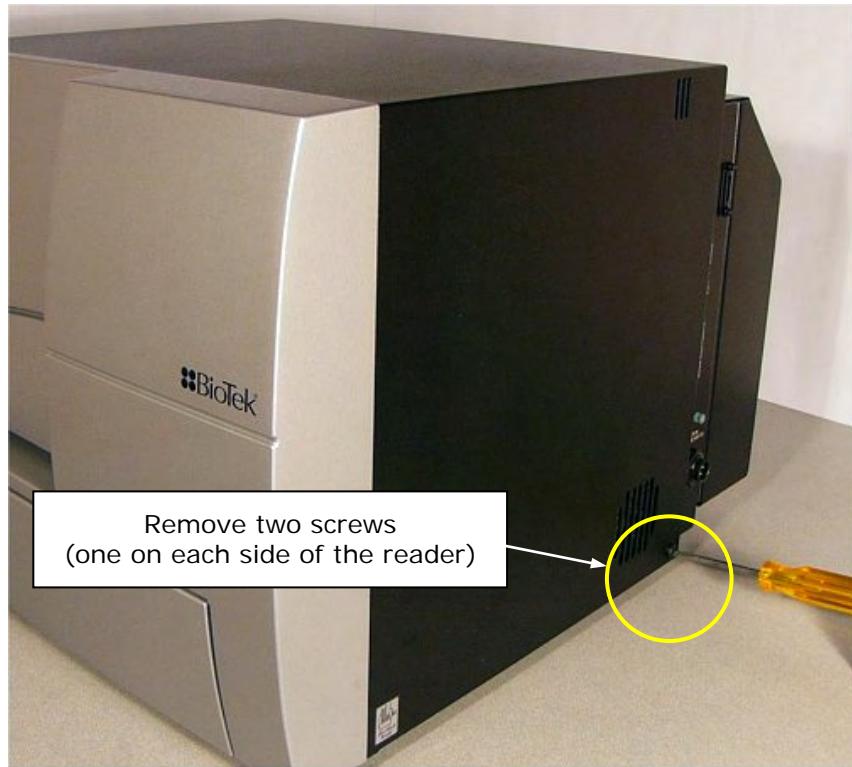


Figure 1: Screws in the lower-rear corners

5. Stand facing the front of the instrument. Grasp both sides of the shroud, slide it toward you, and pull it straight off the instrument. Set the shroud aside.



Figure 2: Removing the reader's shroud

- ❖ To reinstall the shroud, rest it on the table in front of and aligned with the reader, then gently glide it into place. Wheels roll along the reader's bottom track to properly position the shroud.

Clean the Shroud's Air Filters

If dust accumulates on the shroud's air filters:

- Use a vacuum cleaner to clean the filters in place, **or**
- Remove the grate holding the filters and soak the filters in mildly soapy water. Rinse well and reinstall.

- ❖ Clean the air filter on the underside of the reader as well.

Remove the Internal Dispense Tubes and Injector Holders

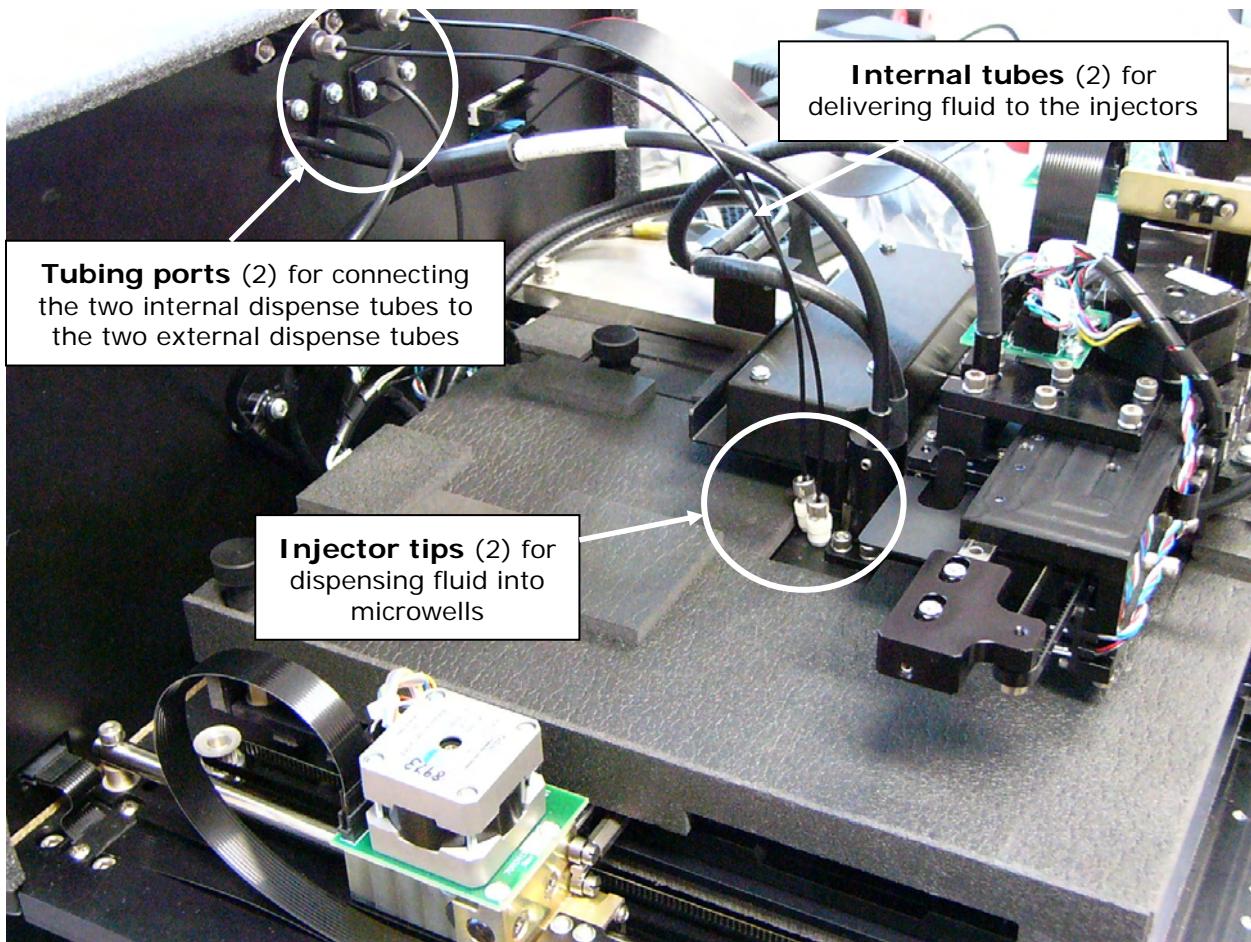


Figure 3: Internal components for the injection system

1. Locate the tubing ports on the reader's rear wall (**Figure 4**). Turn each tube's thumbscrew counterclockwise and gently pull the tube from the port.
2. Gently slide the mirror holder all the way to the right.

3. Locate the injector holders (**Figure 5**). Turn each tube's thumbscrew counterclockwise and gently pull each tube from its injector tip (**Figure 5**).
4. Turn each injector tip counterclockwise to disconnect them from the reader.

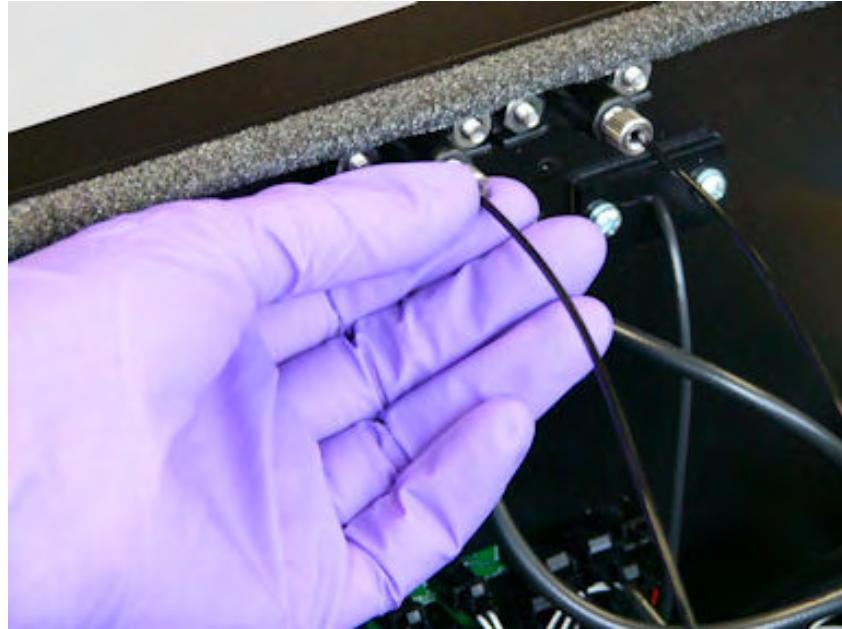


Figure 4: Disconnect the dispense tubes from the back wall of the reader

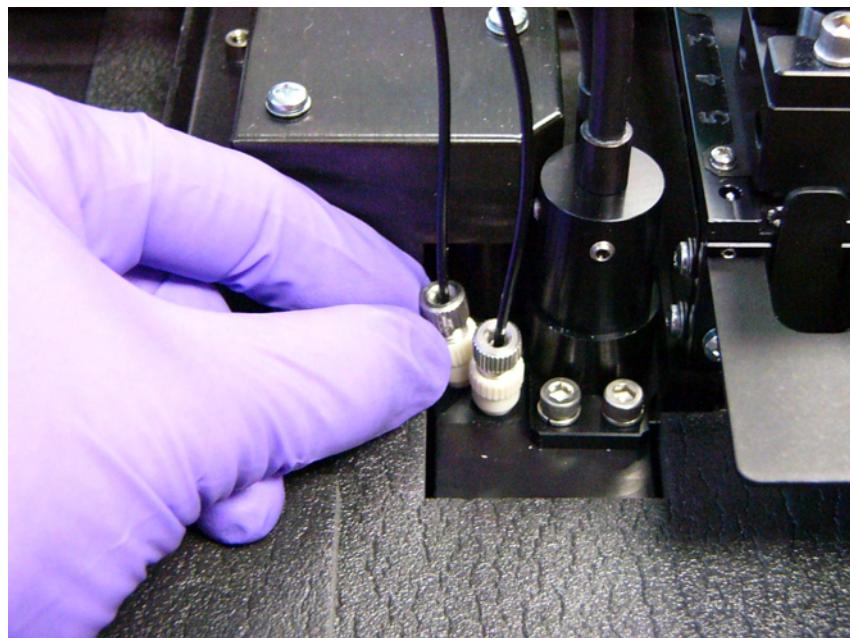


Figure 5: Disconnect the injector holder from the reader



Figure 6: Detach the tubes (2) from the injector holder

Clean the Dispense Tubes and Injectors

As discussed on page 62, 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 internal 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 injector tips:

- 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 or reader.)
 - 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.



Figure 7: Using the stylus to clean inside the injector tips

Clean Inside the Reader

Applies only to Synergy H4 models with injectors.

The internal surface and some components should be cleaned if reagent has spilled or if an unusually high background signal has been identified.

- We recommend performing this process in conjunction with the previously defined steps for cleaning the dispense tubes and injectors.
- The first step is to unplug the reader and remove its shroud (cover). If you have not already done this, turn to page 71 for instructions.
- For this procedure you will need:
 - Deionized or distilled water and mild detergent (optional)
 - 2 or more lint-free cotton cloths

Take a moment to identify the components discussed in this section. The internal dispense tubing and injector holder have already been removed in this image:

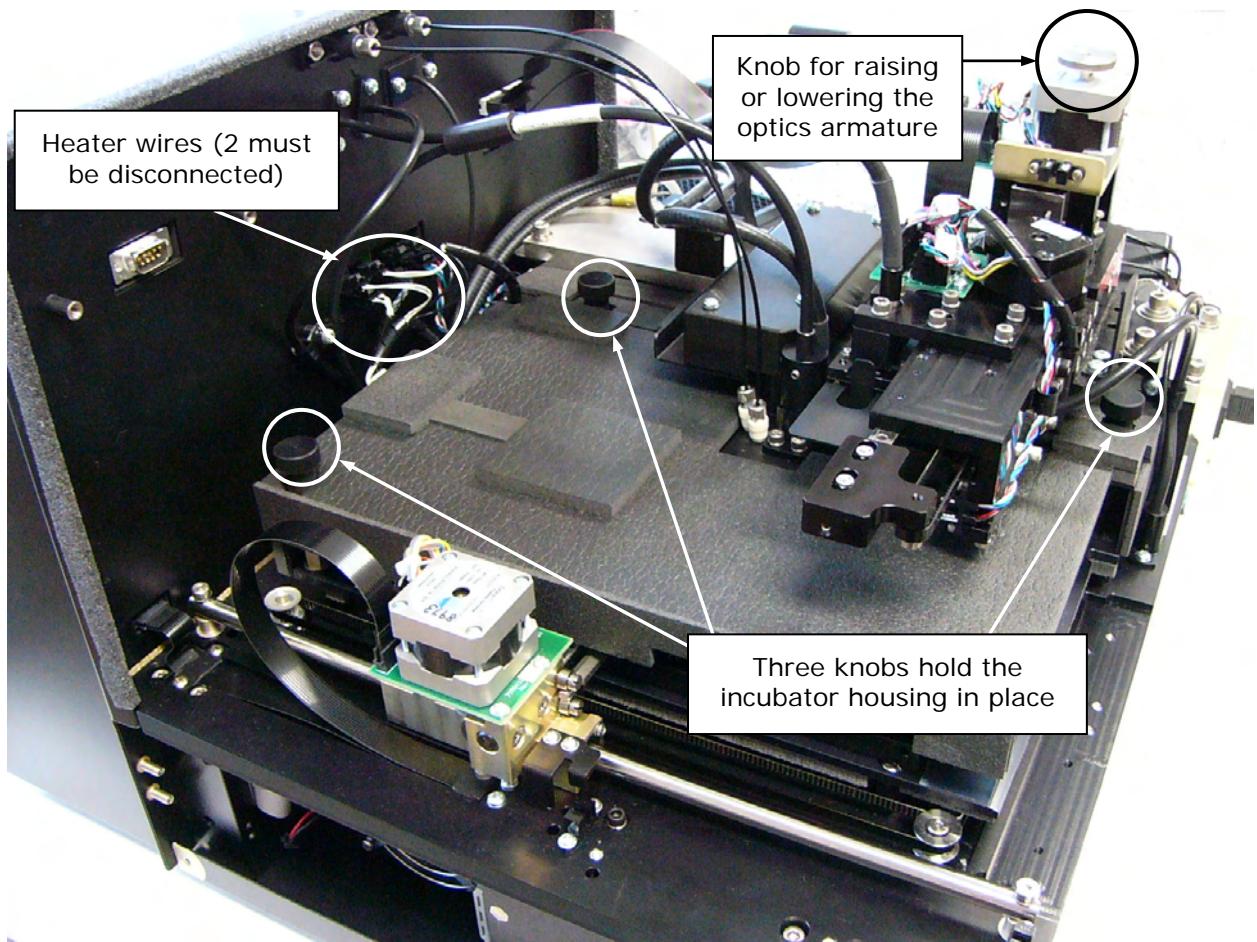


Figure 8: Internal components to be removed/adjusted for cleaning

Remove the Incubator Housing to Access Internal Components

1. Disconnect two of the heater wires located in the center of the reader's rear wall. The two wires appear to be in positions 1 and 3 of the top row of wires (see **Figure 10**). To disconnect them, depress the small tab and separate the connectors.

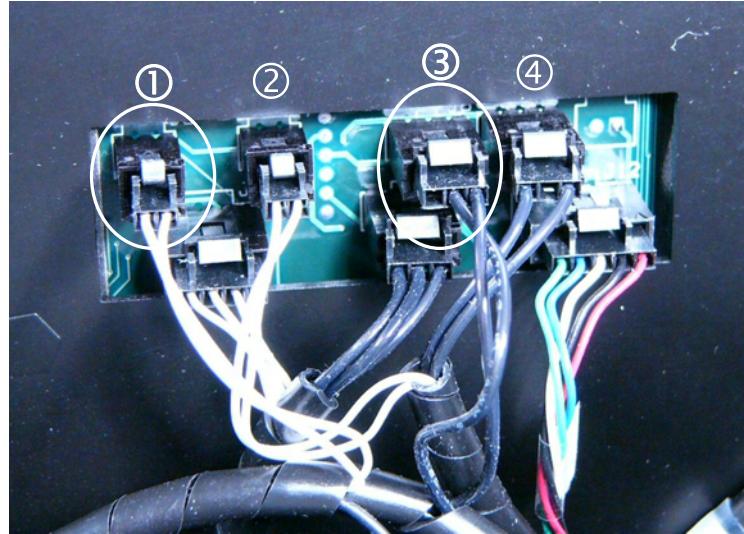


Figure 9: Heater wires on rear wall of reader: disconnect **1** and **3** in the top row

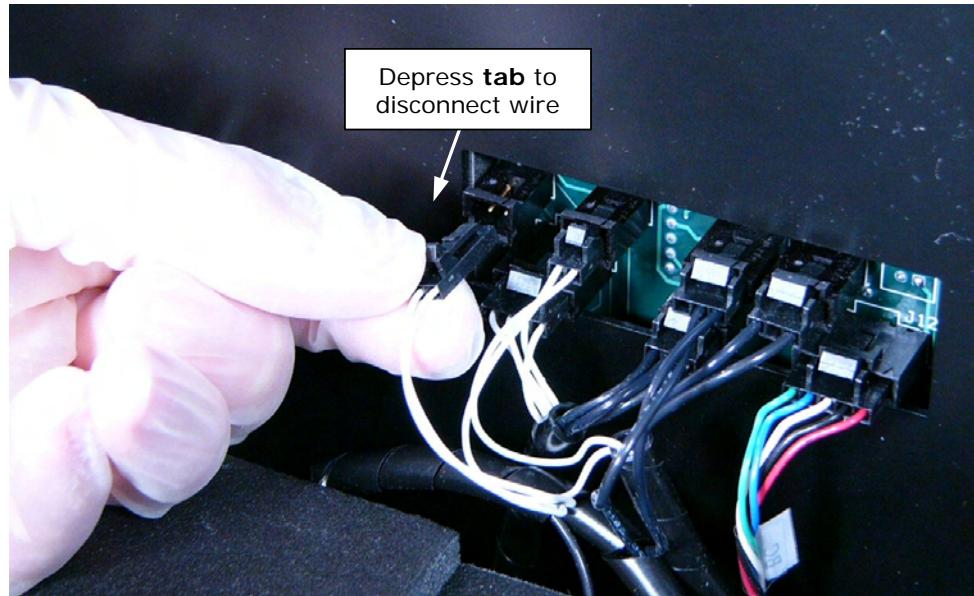


Figure 10: Disconnect the heater wires from the rear wall of the reader

2. Locate the three black knobs that hold the incubator housing in place. Remove them and set them aside (**Figure 11**).

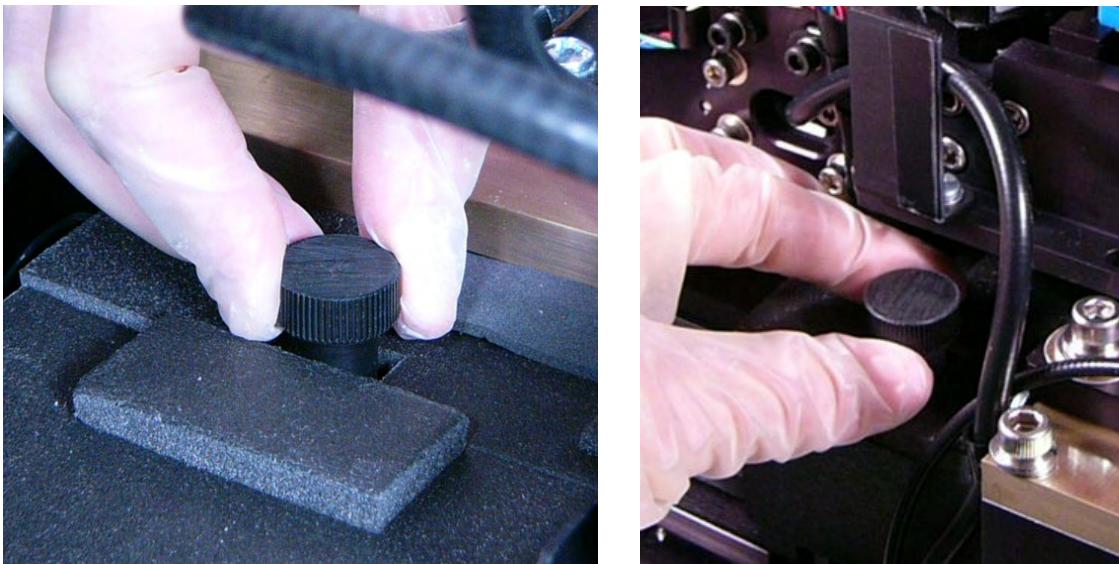


Figure 11: Remove the black knobs (3 total) holding the incubator housing

3. Turn the optic arm knob clockwise to raise the optics armature as high as it will go (**Figure 12**).

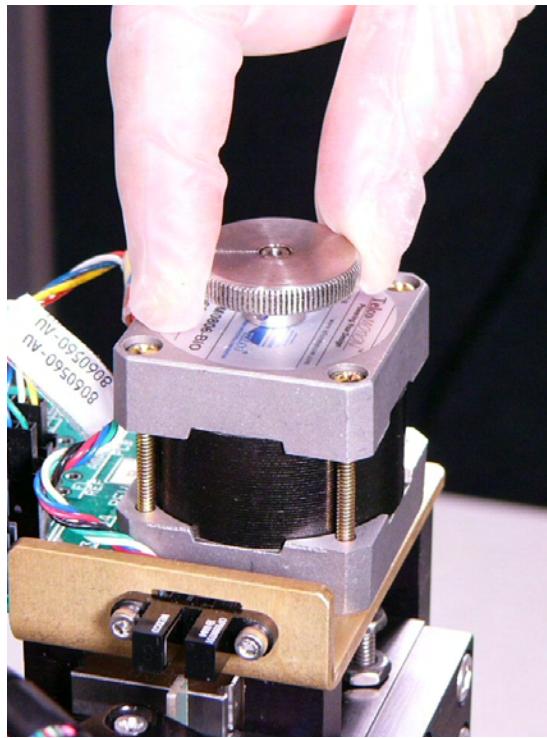


Figure 12: Raise the optics armature to ease access

4. Lift the left side of the incubator housing and carefully slide it out (**Figure 13**).

5. Turn over the incubator housing and clean the surface with water and mild detergent. Set it aside and let it dry completely.

- ❖ When replacing the incubator housing, the two “forks” on its right side should wrap around the holding screws. The forks should not slide under the fixed foam housing. See **Figure 16**.

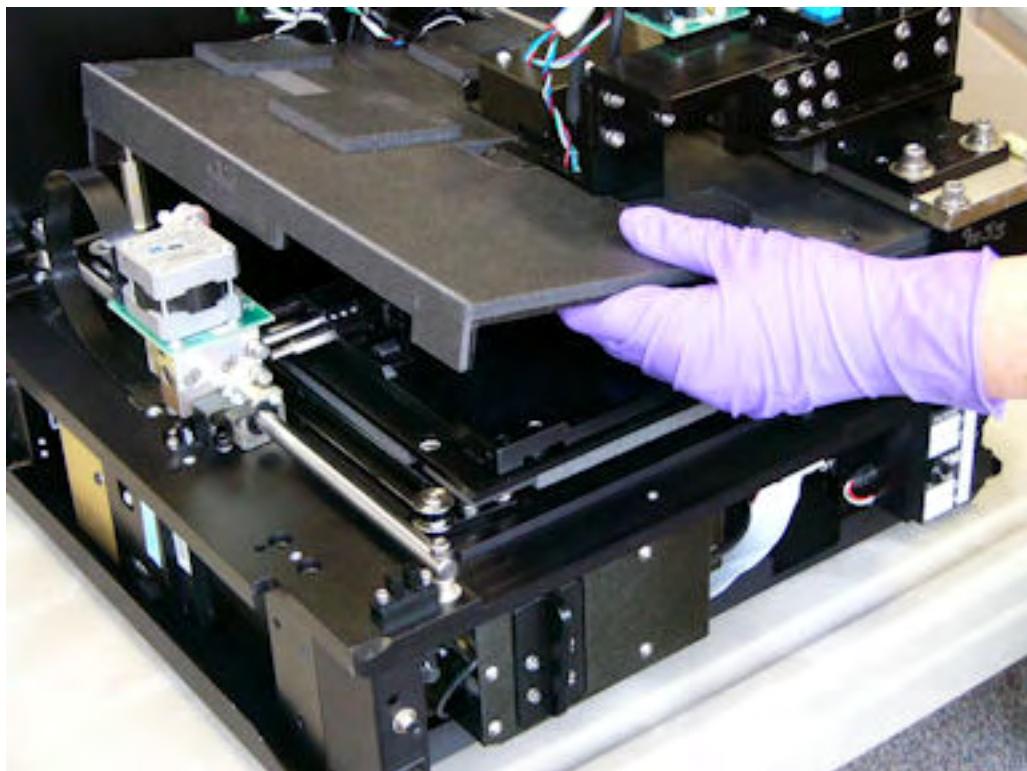


Figure 13: Remove the incubator housing

Clean the Reader’s Internal Surface

1. After you remove the incubator housing, slide the microplate carrier to the left to engage the support pin, and then toward the front of the reader.

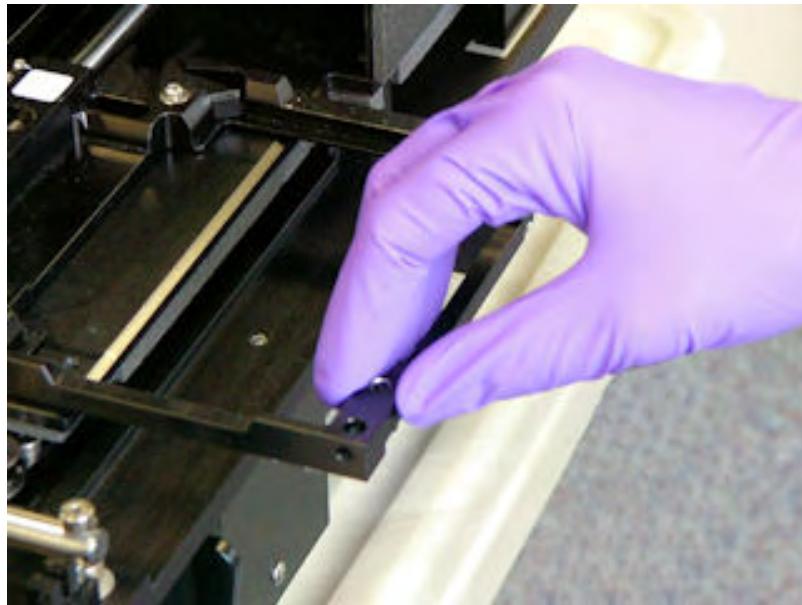


Figure 14: Sliding out microplate carrier

2. Moisten (**do not soak**) a clean cotton cloth with water, or with water and mild detergent. Wipe all sides of the plate carrier. Wipe the instrument's horizontal surface.

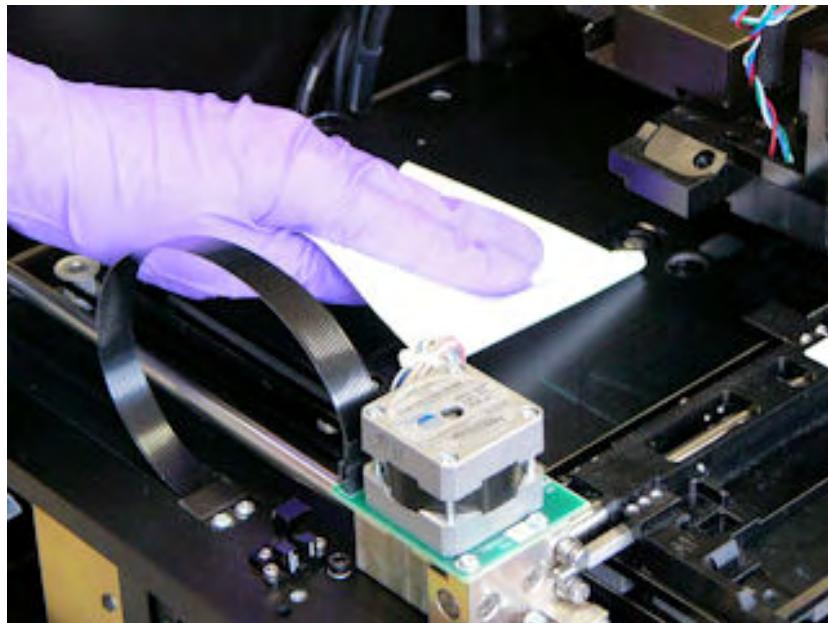


Figure 15: Cleaning the inside of the reader

If the injector holder has been removed from the reader, use the cloth to **gently** clean the underside of the optical armature around the injectors.

- ❖ Do not apply pressure—components can be easily damaged.

3. If detergent was used, wipe the surfaces with a cloth moistened with water.
4. Use a clean, dry, lint-free cloth to dry all wet surfaces.

Reassemble the Components

1. Slide the microplate carrier all the way into the instrument.
2. If the incubator housing was removed, reinstall it:
 - Observe the two forks on the right side of the housing; they must wrap around the holding screws on either side of the optics armature. Do not slide the forks **under** the fixed foam housing.



Figure 16: An incubator housing fork in the correct position

- Secure the incubator housing in place with the three black knobs.
 - Reconnect the two heater wires to the rear wall of the reader: the white wire fits into position 1 in the top row of wires; the black wire fits into position 3 (see Figure 9).
3. Reconnect the internal dispense tubes to the injector holders.
 4. Insert the injector holders into their sockets, and secure each by turning its thumbscrew. Make sure to insert injector 1 into the holder labeled "1" and injector 2 into the holder labeled "2"; if the injectors are inserted into the incorrect holders, a dispense could flood the instrument or contaminate the microplate.

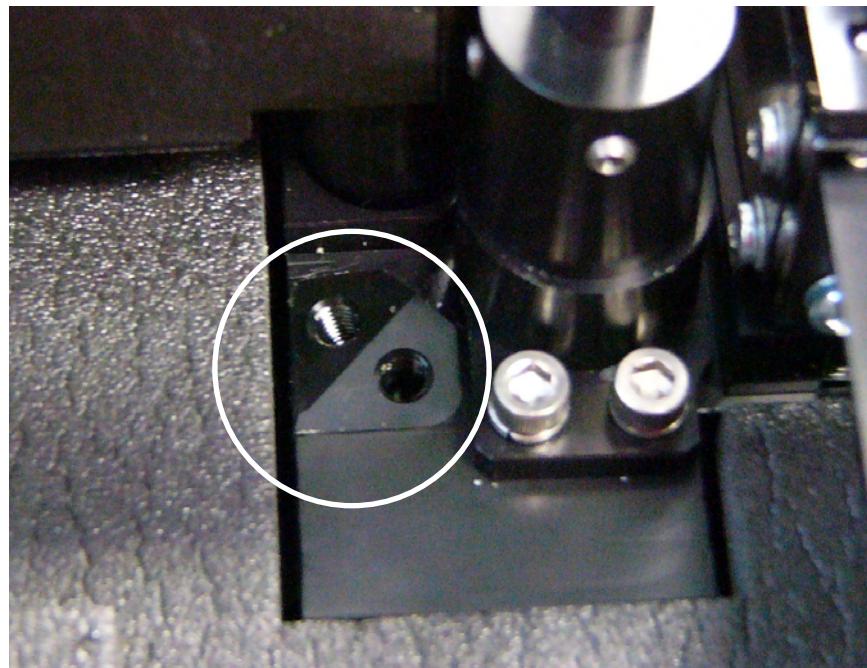


Figure 17: Injector holder sockets

5. Attach the two internal dispense tubes to the tubing ports on the rear wall of the reader. They can go into either port (see page 74).
6. Review the steps you just performed to make sure the components have been properly reassembled.
7. Slide the shroud onto the instrument (see page 72).
8. Replace the two screws to securely attach the shroud to the base (see page 73).

Verify Performance

After reassembling the instrument, perform the following to verify that the instrument is functioning properly:

- Plug the instrument in and turn it on; allow its start-up self-test to complete. Run a System Test using Gen5.
- Run any required OQ/PQ tests, such as the Dispense Module Test, to check for leaks.

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.

	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.
	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.
	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.

Required Materials

For all Synergy H4 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:

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

Procedure for Models without the Dispense Module

	<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>

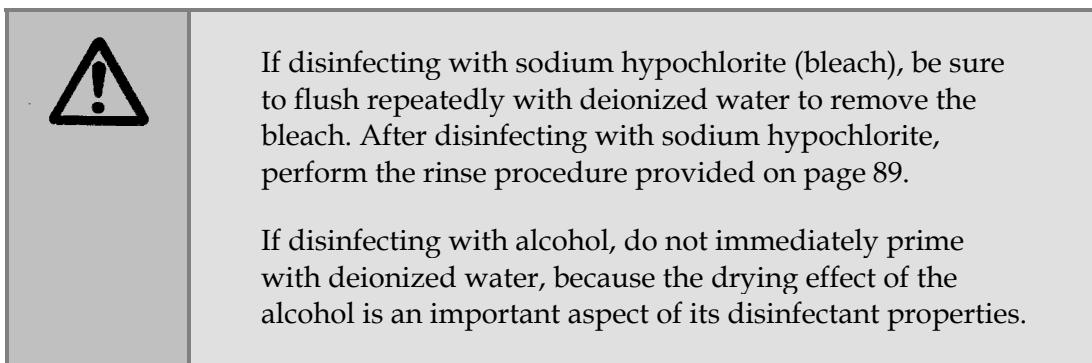
1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.
 - ❖ Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 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 the Dispense Module

- ❖ Perform the **Routine Procedure** when the Synergy H4 is functioning normally. If you are unable to perform a prime due to a system failure, perform the **Alternate Procedure** described on page 90.

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 89.

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 external dispense module.
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. Reassemble the instrument as necessary.
9. If the dispense module is installed, purge any fluid (see **Flush/Purge the Fluid Path in Chapter 5**) and detach the outlet tubes from the rear panel of the instrument. If it is not installed, attach only the dispense module's communication cable to the instrument. Remove the supply bottles and their holders.
10. Perform the decontamination procedures described below through page 90.

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 dispense module.
2. Place the SYRINGE 1 inlet tube in the beaker.
3. If you have not already done so, detach the dispense module's outlet tubes from the instrument's rear panel. Place the ends of the outlet tubes in an empty beaker and set the beaker next to the dispense module.
4. Launch Gen5, select **System > Reader 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 Reader 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 dispense module.

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 Reader Control dialog, select **Dispenser 1 or 2**, set the Volume to **5000 μ L**, and keep the default dispense Rate.
5. Run five prime cycles, for a total of 25,000 μ L.
6. Pause for 10 minutes and then run one prime cycle with 5000 μ 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 Internal Tubing and Injectors

Perform the procedures under “Clean the Internal Components” 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 Synergy H4 due to a system failure, decontaminate the instrument and the Dispense Module as follows:

1. Perform the procedures under “Clean the Internal Components” in **Chapter 5, Preventive Maintenance**. When finished, leave the shroud off the reader and proceed to step 2.
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 surfaces of the shroud
 - All surfaces of the plate carrier
 - The instrument's rear panel
 - The exposed surfaces of the dispense module, including the syringe valves
6. Remove the external tubing and the syringes from the dispense module 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 Dispense module.
10. Reassemble the instrument and dispense module as necessary.
11. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Replace the Tungsten Lamp

The tungsten bulb is expected to operate without replacement for a minimum of 1000 hours. The intensity of the bulb will slowly drop over time until the reader's System Test detects a low signal and displays an error message. In addition, error code 2901 may be displayed during normal operation. The lamp should be replaced at this time. Contact BioTek and order **part number 7080500**.

When the reader's front panel is opened, the tungsten lamp is located behind a light-blocking panel with the hot surface warning label. The lamp is secured by a bracket that also holds a condenser lens and a heat absorber. Two red cables extend from the back of the lamp to plug into the reader.



The tungsten lamp is hot when the instrument is on. Before replacing the lamp, turn off the reader and allow the lamp to cool for at least 15 minutes.



Do not touch the glass lenses! Fingerprints on the condenser lens or heat absorber may negatively affect performance.

1. Turn off and unplug the reader. Wait at least 15 minutes for the lamp to cool.
2. Remove the reader's shroud (cover).
3. Remove the EX (excitation) filter wheel and set aside.

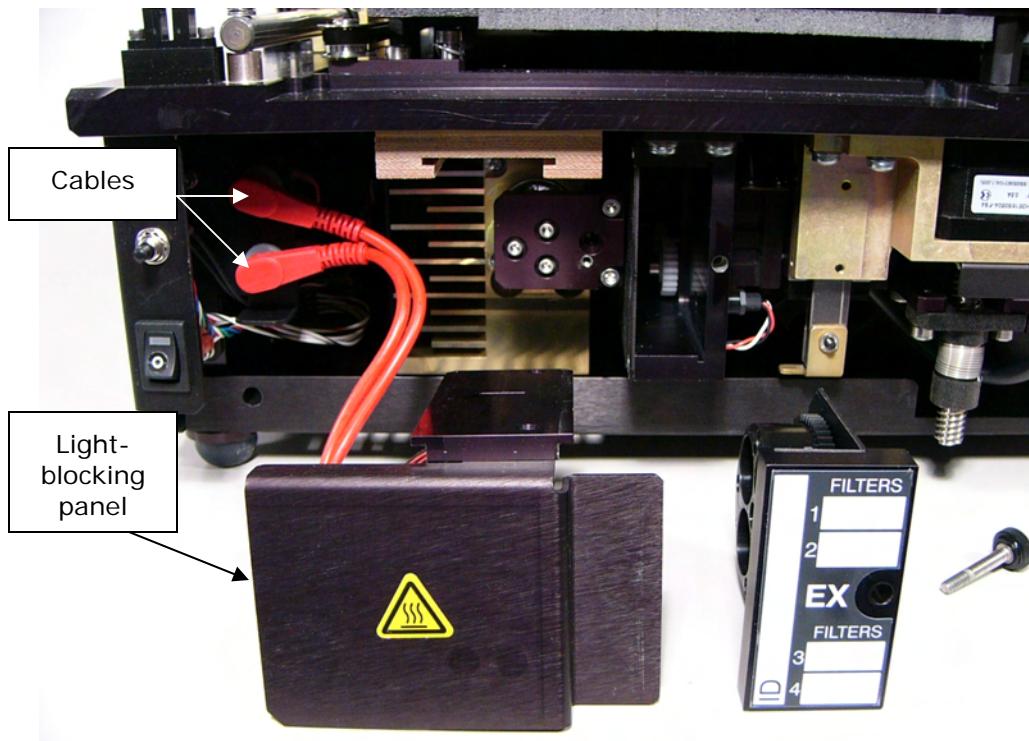


Figure 1: Excitation wheel removed for lamp replacement

4. Grasp the light-blocking panel and slide the assembly toward you, out of the reader.
5. Unplug the lamp's cables from the reader.

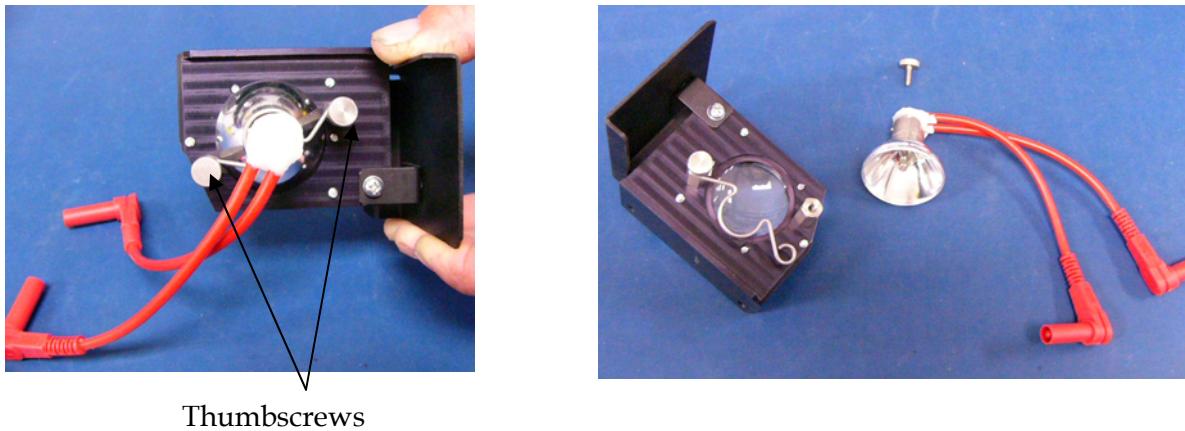


Figure 2: Lamp replacement

6. Gently, without touching any glass components, unscrew one of the thumbscrews holding the bulb in its wire bracket.
7. When the bracket is free, remove the old bulb.
8. Insert the new bulb, position the wire bracket, and tighten the thumbscrew.
9. Align the lamp assembly with the reader, preparing to slide its top metal plate into its slot, and plug in the red cables. The cables can go into either plug.
10. Slide the lamp assembly into place without touching any other internal component. You may need to move the cables slightly downward to make room for the lamp.
11. Reinstall the EX filter wheel and the reader's shroud.
12. Plug in and turn on the reader. If the system test passes the lamp replacement was successful. Otherwise, note any errors and consult **Appendix B, Error Codes**.

Dispense Module: 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 dispense module 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

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. In Gen5, select **System > Reader Control > Synergy H4 (Com<#>)** 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.



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

Replace the Syringe

After using Gen5 to move the syringe into its Maintenance Position (refer to **Figure 3**):

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 Dispense Module” in the **Installation** chapter.)

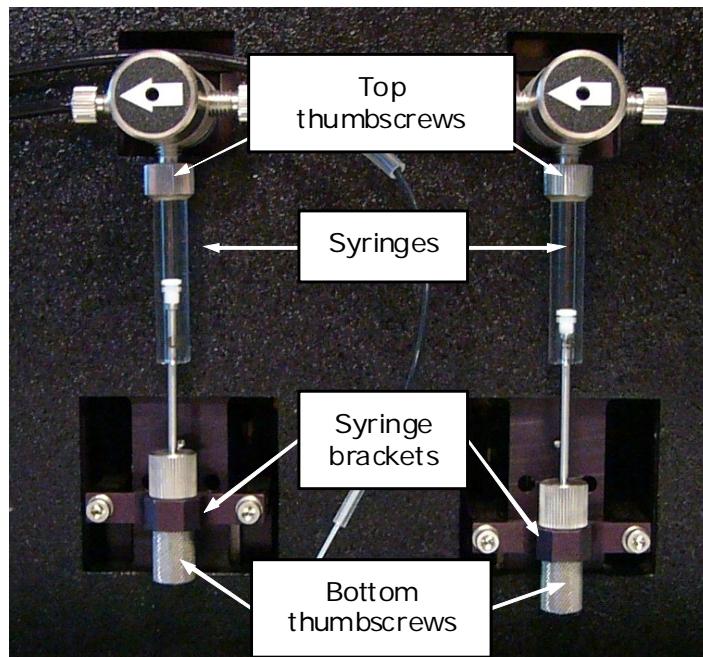


Figure 3: The dispense module; close-up view of the syringes.

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. In Gen5, select **System > Reader Control > Synergy H4 (Com<#>)**. Click the **Dispenser** tab and click **Initialize**.

Chapter 7

Instrument Qualification

This chapter contains procedures for qualifying the initial and ongoing performance of the Synergy H4 and the external dispense module (if equipped).

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Overview

This chapter contains BioTek Instruments' recommended Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) procedures for all models of the Synergy H4 Multi-Mode Microplate Reader.

Every Synergy H4 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 8030514) for the Synergy H4 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

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, 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 perform an actual assay prior to accepting the reader for routine use. If this is the case, you should not use the data obtained from the first assay run on the reader until you have confirmed that the package insert criteria have been met.
- 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, 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

The schedule below defines BioTek-recommended intervals for qualifying a Synergy H4 used two to five days a week. The actual frequency, however, may be adjusted depending on your usage of the instrument and its various modules. This schedule assumes the reader is properly maintained as outlined in the **Preventive Maintenance** chapter.

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
All models:				
Installation, setup, and configuration of the reader, dispense module (if equipped), the host computer, and Gen5 software	✓			
System Test	✓	✓	✓	
Models with the Absorbance module:				
Absorbance Plate Test		✓	✓	
Absorbance Liquid Test 1 or 2*		✓		✓
Absorbance Liquid Test 3 (optional)**		✓		✓
Models with the Fluorescence module:				
Corners, Sensitivity, Linearity Tests		✓	✓	
Fluorescence Polarization (FP) Tests		✓		✓
Time-Resolved Fluorescence (TRF) Test		✓		✓
Models with the Luminescence module:				
Luminescence Test		✓	✓	
Models with the Dispense module:				
Injection System Test	✓			
Dispense Accuracy and Precision Test		✓		✓

* Regarding Absorbance Liquid Tests 1 and 2:

- If you have an Absorbance Test Plate, run Liquid Test 1.
- If you do not have an Absorbance Test Plate, run Liquid Test 2.

** Liquid Test 3 is optional; it is provided for sites requiring verification at wavelengths lower than those attainable with the Absorbance Test Plate.

System Test

Each time the Synergy H4 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 LED on the power switch remains on.

If any test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek, the reader will beep repeatedly and the LED on the power switch will flash. 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, select **System > Reader 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 test 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.
 - A sample test report is shown on the next few pages.
 - 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.

- ❖ A sample test report for a Synergy H4 model with **all** modules installed is shown on the next few pages. Your instrument's test report may be different. If you have any questions about the report's contents or the test results, please print the report and then contact BioTek's Technical Assistance Center.

Gen5 System Test Report					
Reader:	Synergy H4 (Serial Number: 219880)				
Basecode:	P/N 8030200 (v0.04.6a)				
Date and Time:	1/4/2010 12:05:06 PM				
User:	Administrator				
Company:	BioTek				
Comments:					
Test Results					
Operator _____ ID: _____ Notes: _____ <hr style="border: 0.5px solid black;"/>					
SYSTEM SELF TEST					
8030200 Version 0.04.6a		219880	1110	1000	0000 0000
Voltage Reference Test		Min	Low	High	Max
Mono System Flash		727	905	1201	1594
Filter System Flash		838	1091	1263	
Motor Power		3725			
Tungsten Lamp		25			3320
ABSORBANCE					
Optics Test	Ref	Meas	Gain	Resets	
#1:230			2.56	1	
Light	13945	39851			
Dark	9887	9920			
Delta	4058	29931			
#2:352			1.68	8	
Light	12706	39983			
Dark	9875	9897			
Delta	2831	30086			
#3:620			2.98	4	
Light	12981	39897			
Dark	9873	9885			
Delta	3108	30012			
#4:790			1.50	4	
Light	13164	39685			
Dark	9875	9894			
Delta	3289	29791			

#5:860			2.23	4
Light	13222	39799		
Dark	9878	9904		
Delta	3344	29895		
#6:962			2.82	2
Light	13313	39958		
Dark	9878	9899		
Delta	3435	30059		
Noise Test				
Max	9888	9962		
Min	9886	9961		
Delta	2	1		
 FLUORESCENCE/LUMINESCENCE				
Monochromator PCB				
Bias current offset	-1.1	counts	PASS	
Offset voltage	1703	counts	PASS	
750V measurement	234.3	counts	PASS	
750V noise	125	counts		
750V offset	1721	counts		
500V measurement	14.9	counts		
500V noise	6	counts		
500V offset	1703	counts		
Reset offset	1726	counts		
Reference bias	0.1	counts	PASS	
Reference offset	10957	counts	PASS	
Reference noise	0.3	counts	PASS	
Filter PCB				
Bias current offset	-0.5	counts	PASS	
Offset voltage	1418	counts	PASS	
750V measurement	19.3	counts	PASS	
750V noise	4	counts		
750V offset	1420	counts		
500V measurement	4.0	counts		
500V noise	1	counts		
500V offset	1419	counts		
Reset offset	1449	counts		
Reference bias	0.3	counts	PASS	
Reference offset	10958	counts	PASS	
Reference noise	0.2	counts	PASS	
Filter System - Excitation Wheel				
	500V	650V	750V	
#1:360/40				
Gain	1.00	1.00	1.00	
Light	14622	16558	17322	
Dark	10958	10958	10958	
Delta	3664	5600	6364	
#2:485/20				
Gain	1.00	1.00	1.00	
Light	14079	15134	15792	
Dark	10958	10958	10958	
Delta	3121	4176	4834	
#3:HOLE				
#4:PLUG				

```

Filter System - Emission Wheel
#1:460/40
#2:528/20
#3: HOLE
#4: PLUG

Mono Fluorescence - Optics Test
Bottom Probe      1.3mm slit    3.0mm slit
Sensitivity:40   Ref   Meas     Ref   Meas
#1:300
    Light      14277  4808  18208  9826
    Dark       10957  1704  10957  1702
    Delta      3320   3104  7251   8124
    Max        3410   3160  7335   8219
    Min        3239   3033  7058   7948
    StdDev     53     43    85    81
#2:400
    Light      17617  16486 25466  35639
    Dark       10957  1704  10957  1696
    Delta      6660   14782 14509  33943
    Max        6713   14845 14708  34245
    Min        6607   14662 14235  33600
    StdDev     33     50    149   230
#3:425
    Light      20483  21300 31088  43813
    Dark       10957  1703  10957  1696
    Delta      9526   19597 20131  42117
    Max        9676   19827 20332  42380
    Min        9325   19313 19930  41937
    StdDev     95     144   130   145
#4:485
    Light      20769  17204 30879  36387
    Dark       10957  1703  10957  1696
    Delta      9812   15501 19922  34691
    Max        9904   15739 20138  34977
    Min        9702   15176 19500  34270
    StdDev     69     165   184   181
#5:535
    Light      19122  11708 28709  27915
    Dark       10957  1705  10957  1699
    Delta      8165   10003 17752  26216
    Max        8223   10094 17970  26440
    Min        8046   9902  17507  25943
    StdDev     52     59    146   139
#6:700
    Light      13503  2488  16512  3946
    Dark       10957  1702  10957  1706
    Delta      2546   786   5555  2240
    Max        2563   799   5594  2268
    Min        2515   771   5474  2215
    StdDev     14     9    40    16
Top Probe      1.3mm slit    3.0mm slit
Sensitivity:34   Ref   Meas     Ref   Meas
#1:300
    Light      13346  4824  16197  9587
    Dark       10957  1705  10957  1703
    Delta      2389   3119  5240   7884
    Max        2435   3160  5343   7988
    Min        2368   3070  5159   7805
    StdDev     20     24    53    65
#2:400
    Light      15619  18238 21187  36269
    Dark       10957  1699  10957  1697

```

Delta	4662	16539	10230	34572
Max	4737	16743	10317	34775
Min	4611	16418	10120	34241
StdDev	42	104	60	145
#3:425				
Light	17641	23238	25177	43574
Dark	10957	1702	10957	1694
Delta	6684	21536	14220	41880
Max	6804	21677	14378	42268
Min	6588	21363	14079	41551
StdDev	64	112	102	237
#4:485				
Light	17848	18921	25037	37134
Dark	10957	1702	10957	1697
Delta	6891	17219	14080	35437
Max	6938	17353	14178	35724
Min	6835	17045	13943	35076
StdDev	35	117	86	222
#5:535				
Light	16672	13087	23421	29405
Dark	10957	1704	10957	1700
Delta	5715	11383	12464	27705
Max	5767	11496	12545	27945
Min	5622	11198	12390	27382
StdDev	47	103	55	165
#6:700				
Light	12755	2585	14908	4206
Dark	10957	1702	10957	1703
Delta	1798	883	3951	2503
Max	1812	896	4002	2525
Min	1776	869	3879	2469
StdDev	13	10	34	17

CALIBRATION

Carrier - Top Filter Fluorescence

Upper Left	x=10588	y= 220
Lower Left	x=10588	y= 5748
Lower Right	x= 1900	y= 5744
Upper Right	x= 1892	y= 224
Delta 1	10588 -10588=	+0
Delta 2	1892 - 1900=	-8
Delta 3	224 - 220=	+4
Delta 4	5744 - 5748=	-4

Carrier - Top Filter Luminescence

Upper Left	x=11620	y= 3072
Lower Left	x=11616	y= 8600
Lower Right	x= 2928	y= 8600
Upper Right	x= 2928	y= 3072
Delta 1	11620 -11616=	+4
Delta 2	2928 - 2928=	+0
Delta 3	3072 - 3072=	+0
Delta 4	8600 - 8600=	+0

Carrier - Bottom Mono Fluorescence

Upper Left	x= 9720	y= 4588
Lower Left	x= 9716	y=10116
Lower Right	x= 1024	y=10116
Upper Right	x= 1020	y= 4596
Delta 1	9720 - 9716=	+4
Delta 2	1020 - 1024=	-4

```

Delta 3           4596 - 4588=   +8
Delta 4           10116 -10116=   +0

Carrier - Top Mono Fluorescence
Upper Left        x= 9952   y= 3136
Lower Left         x= 9952   y= 8668
Lower Right        x= 1264   y= 8668
Upper Right        x= 1260   y= 3140
Delta 1            9952 - 9952=   +0
Delta 2            1260 - 1264=   -4
Delta 3            3140 - 3136=   +4
Delta 4            8668 - 8668=   +0

Carrier - Plate Absorbance
Upper Left        x=11264   y= 4584
Lower Left         x=11264   y=10104
Lower Right        x= 2576   y=10108
Upper Right        x= 2572   y= 4588
Delta 1            11264 -11264=   +0
Delta 2            2572 - 2576=   -4
Delta 3            4588 - 4584=   +4
Delta 4            10108 -10104=   +4

Carrier - Injectors
Upper Left        x= 9700   y= 4552
Lower Left         x= 9700   y=10080
Lower Right        x= 1012   y=10076
Upper Right        x= 1004   y= 4556
Delta 1            9700 - 9700=   +0
Delta 2            1004 - 1012=   -8
Delta 3            4556 - 4552=   +4
Delta 4            10076 -10080=   -4

Carrier - Test Sensors
Middle Sensor      y=11960
Tested             11964
Delta              +4
Back Sensor        x=11564   y=10660
Tested             11564   10656
Delta              +0       -4

Probe Height       33.87 mm
Plate Sensor       432

Filter System
Lamp Reflector     896
Probe Changer      704
Mirror Slider       3976
Mirror 1            y= 6936   485/20
Mirror 2            y= 6940   360/40
Mirror 3            y= 6952   485/20
Probe Aperture      2264

Excitation Mono Probe Changer
Bottom Fluorescence -131
Top Fluorescence    642
Plate Absorbance    1424

Emission Mono Probe Changer
Bottom Fluorescence -140
Top Fluorescence    632

```

```

Excitation Monochromator
Bottom Fluorescence B=-0.00003255 C=+0.72189641
Top Fluorescence    B=-0.00001233 C=+0.21377032
Plate Absorbance   B=+0.00115939 C=-0.07525918
305LP Edge          -965.56
Tested              -965.47

Emission Monochromator
Bottom Fluorescence B=-0.00081430 C=+0.03456220
Top Fluorescence    B=-0.00079260 C=+0.61682516
629nm Peak          -22.01
Tested              -23.04

INCUBATION

Temperature Setpoint: 0.0      Current Average: 23.4      A/D Test: PASS
Zone 1: 23.5  Min: 23.4  Max: 23.5  Range: PASS  Thermistor: PASS
Zone 2: 23.3  Min: 23.3  Max: 23.3  Range: PASS  Thermistor: PASS
Zone 3: 23.3  Min: 23.3  Max: 23.3  Range: PASS  Thermistor: PASS
Zone 4: 23.4  Min: 23.3  Max: 23.4  Range: PASS  Thermistor: PASS

SYSTEM TEST PASS
0000

Dispenser 1: 005.0,010.0,020.0,040.0,080.0,200.0
Dispenser 2: 005.0,010.0,020.0,040.0,080.0,200.0

Mirror 1: 50% Mirror, 0200, 0850, 0200, 0850
Mirror 2: Dichroic , 0320, 0390, 0410, 0800
Mirror 3: Dichroic , 0440, 0505, 0515, 0700
Polarizers present.

Reviewed/Approved By: _____ Date: _____

```

Figure 1: Sample output for the Synergy H4 System Test

Absorbance Plate Test

This section applies to models with the Absorbance module only.

Description

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

- **Mechanical Alignment:** The Test Plate has precisely machined holes to confirm mechanical alignment. The amount of light that shines through these holes is an indication of whether the microplate carrier is properly aligned with the absorbance optical path. A reading of more than 0.015 OD

for any of the designated alignment holes indicates that the light is being “clipped” and the reader may be out of alignment.

- **Accuracy/Linearity:** The Test Plate contains neutral-density glass filters of known OD values at several wavelengths. Actual measurements are compared against the expected values provided in the Test Plate’s Standards Certificate. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is proven that the reader is accurate at these OD values, the reader is also considered to be linear.
- **Repeatability:** This test ensures the instrument meets its repeatability specification by reading each neutral-density filter on the Test Plate twice with the filter in the same location.
- **Wavelength Accuracy:** The Test Plate contains a glass filter in position C6 that is used to check the wavelength accuracy of the absorbance monochromator. The filter is scanned across a specified wavelength range in 1-nm increments. The wavelength of maximum absorbance is compared to the expected peak wavelength supplied on the Test Plate’s certificate.

Test Plate Certificates

To run this test on the Synergy H4, you’ll need BioTek’s 7-Filter Absorbance Test Plate (PN 7260522), with its accompanying certificates.

- The Standards Certificate contains standard OD values for the filters at several different wavelengths.
- The Peak Wavelength Certificate contains one or more “Peak Wavelength” values for the glass filter in position C6 on the plate. Each value has a valid test range associated with it. For example, a Peak Wavelength value may be 586 nm with a test range of 580 to 590 nm (or tolerance values of -6/+4).

<p>This test plate can be used for testing the reproducibility, linearity, and alignment of your BioTek autoreader. The following calibration data has been recorded by a N.I.S.T. traceable spectrophotometer.</p> <p style="text-align: center;">WAVELENGTH (nm)</p>								
Well	405nm	450nm	490nm	550nm	620nm	630nm	690nm	750nm
C1	0.147	0.140	0.135	0.130	0.136	0.136	0.127	0.134
E2	0.618	0.575	S A M P L E			0.568	0.485	0.434
G3	1.133	1.052				1.040	0.881	0.783
H6	1.701	1.578				.560	1.323	1.179
F5	2.279	2.024	1.976	1.956	1.893	1.865	1.537	1.272
D4	2.945	2.604	2.545	2.513	2.437	2.400	1.972	1.632
Set # 2453				Serial # 161259				

Figure 2: Sample Standards Certificate, showing OD/Wavelength combinations for each of six locations on the Absorbance Test Plate

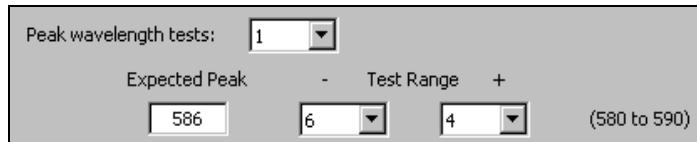
Before the Absorbance Plate Test can be performed, the standard OD values and the peak wavelength value(s) must be entered into Gen5. You'll enter and save these values once initially, and then update them each time the test plate is recertified by BioTek (typically annually).

Instructions for defining the Test Plate's characteristics and for running the test follow.

Define Absorbance Test Plate Parameters

1. Obtain the certificates that came with the Test Plate.
2. Start Gen5 and select **System > Diagnostics > Test Plates > Add/Modify Plates**.
3. Click **Add**. The Absorbance Test Plate dialog will appear.
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 are appropriate for your tests, carefully enter the OD values from the Standards Certificate 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 (1 to 4), based on the number of peak wavelength values provided on the Peak Wavelength Certificate.

8. Enter the Expected Peak value(s) from the Peak Wavelength Certificate. (If multiple values are given for a wavelength, use those in the 2.4 nm Spectral Bandpass table.) For each value, define the expected Test Range by selecting the minus/plus tolerance values. The range must span at least 8 nm.



- ❖ For certificates that have only one peak wavelength and a fixed wavelength range of 580 to 590 nm, enter the Expected Peak wavelength value and adjust the Test Range values so the range displayed in parentheses is 580 to 590 (as demonstrated above).

9. Review all of the values you entered, and then click **OK** to save the data.

The information you just entered will be available in 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. In Gen5, select **System > Diagnostics > Test Plates > Run**.
 2. If prompted, select the desired Test Plate and click **OK**.
 3. When the Absorbance Test Plate Options dialog appears, select **Perform Peak Wavelength Test** if it is not already selected.
 4. Highlight the wavelength(s) to be included in this test.
- ❖ You only need to select those wavelengths most appropriate for your use of the reader.
5. (Optional) Enter any Comments.
 6. Click **Start Test**.
 7. Place the Test Plate in the microplate carrier so that well A1 is in the left-rear corner of the carrier.
 8. Click **OK** to run the test.
 9. When the test is completed, the results report appears. Scroll through the report; every result should show "PASS". See page 113 for information on results and troubleshooting tips in the event of failures.
 - A sample test report is shown below.
 - 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.

Sample Report

Absorbance Test Plate Results						
Reader:	Synergy H4 (Serial Number: 219880)					
Basecode:	P/N 8030200 (v0.05.3a)					
Date and Time:	1/4/2010 8:32:59 AM					
Absorbance Plate:	7 Filter Test Plate (P/N 7260522) - S/N 179269					
Last Plate Certification:	November 2009					
Next Plate Certification Due:	November 2010					
User:	Administrator					
Comments:						
Peak Absorbance Results						
Well	C6					
Reference	586					
Tolerance	3					
Read	586					
Result	PASS					
Alignment Results						
Wells	A1	A12	H1	H12		
Read	0.001	0.002	0.002	0.002		
Tolerance	0.015	0.015	0.015	0.015		
Result	PASS	PASS	PASS	PASS		
Wavelength = 405 nm						
Accuracy Results						
Wells	C1	E2	G3	H6	F5	D4
Reference	0.140	0.632	1.200	1.744	2.076	2.679
Min Limit	0.117	0.599	1.156	1.689	1.973	#N/A
Max Limit	0.163	0.665	1.244	1.799	2.179	#N/A
Read 1	0.145	0.633	1.199	1.744	2.071	2.687
Result	PASS	PASS	PASS	PASS	PASS	#N/A
Repeatability Results						
Wells	C1	E2	G3	H6	F5	D4
Read 1	0.145	0.633	1.199	1.744	2.071	2.687
Min Limit	0.139	0.622	1.182	1.721	2.004	#N/A
Max Limit	0.152	0.644	1.216	1.766	2.138	#N/A
Read 2	0.145	0.633	1.199	1.744	2.072	2.687
Result	PASS	PASS	PASS	PASS	PASS	#N/A
Wavelength = 490 nm						
Accuracy Results						
Wells	C1	E2	G3	H6	F5	D4
Reference	0.140	0.592	1.122	1.631	1.829	2.336
Min Limit	0.117	0.560	1.080	1.578	1.772	2.223
Max Limit	0.163	0.624	1.164	1.684	1.886	2.449
Read 1	0.145	0.592	1.120	1.629	1.825	2.334
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.145	0.592	1.120	1.629	1.825	2.334
Min Limit	0.138	0.581	1.104	1.608	1.802	2.259
Max Limit	0.151	0.603	1.137	1.651	1.848	2.409
Read 2	0.145	0.592	1.120	1.629	1.825	2.334
Result	PASS	PASS	PASS	PASS	PASS	PASS

Wavelength = 630 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.156	0.598	1.131	1.648	1.746	2.200
Min Limit	0.133	0.566	1.088	1.595	1.691	2.092
Max Limit	0.179	0.630	1.174	1.701	1.801	2.308
Read 1	0.162	0.599	1.132	1.648	1.746	2.202
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.162	0.599	1.132	1.648	1.746	2.202
Min Limit	0.155	0.588	1.116	1.627	1.723	2.131
Max Limit	0.168	0.610	1.149	1.670	1.768	2.273
Read 2	0.162	0.599	1.132	1.648	1.746	2.203
Result	PASS	PASS	PASS	PASS	PASS	PASS

Wavelength = 750 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.145	0.484	0.905	1.316	1.254	1.506
Min Limit	0.122	0.454	0.867	1.270	1.209	1.456
Max Limit	0.168	0.514	0.943	1.362	1.299	1.556
Read 1	0.150	0.484	0.905	1.316	1.253	1.506
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.150	0.484	0.905	1.316	1.253	1.506
Min Limit	0.144	0.474	0.891	1.298	1.235	1.486
Max Limit	0.157	0.494	0.919	1.334	1.270	1.526
Read 2	0.150	0.484	0.905	1.316	1.253	1.506
Result	PASS	PASS	PASS	PASS	PASS	PASS

Reviewed/Approved By: _____ Date: _____

Figure 3: Sample output for the Synergy H4 Absorbance Plate Test.

Results & 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 ± 3 nm (± 2 nm instrument, ± 1 nm filter allowance). If the reader fails this test:

- Make sure the information entered into Gen5 matches the Test Plate's 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. A R Squared value of at least 0.990 is expected.

Absorbance Liquid Tests

This section applies to models with the Absorbance module only.

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 µL in a flat-bottom microwell.

Solution B

- Deionized water
 - FD&C Yellow No. 5 dye powder (typically 90% pure)
 - Tween 20 (polyoxyethylene (20) sorbitan monolaurate) **or** BioTek wetting agent (PN 7773002) (a 10% Tween solution)
 - Precision balance with capacity of 100 g minimum and readability of 0.001 g
 - Weigh boat
 - 1-liter volumetric flask
1. Weigh 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 μ 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 μ L of the concentrated solution (A or B) into the first column of wells in the microplate.
3. Pipette 200 μ 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 at Variable speed 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.

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 (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% (0.8004×0.010) 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 five 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 $\pm 1.0\% \pm 0.010$ OD, then the expected range for the mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD.
 $1.902 \times 0.010 + 0.010 = 0.029$; $1.902 - 0.029 = 1.873$; $1.902 + 0.029 = 1.931$.

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

Linearity and Repeatability Tests

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

❖ 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% (1.951×0.010) = 0.0195, which, when added to the 0.005 ($0.0195 + 0.005$) = 0.0245 OD, which is the allowable 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 OD 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 OD to 2.500 OD

Absorbance Liquid Test 3 (optional)

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

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

Buffer Solution

- Deionized water
 - Phosphate-Buffered Saline (PBS), pH 7.2–7.6, Sigma tablets, #P4417 (or equivalent)
 - β -NADH Powder (β -Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma bulk catalog number N 8129, or preweighed 10-mg vials, Sigma number N6785-10VL (or BioTek PN 98233). Store the powder according to the guidelines on its packaging.
1. Prepare a PBS solution from the Sigma tablets.
 2. In a beaker, mix 50 mL of the PBS solution with 10 mg of the β -NADH powder and mix thoroughly. This is the **100% Test Solution**.
 3. (Optional) Read a 150- μ 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 µL of the 100% Test Solution into all wells of columns 1 and 2
 - 150 µL of the 75% Test Solution into all wells of columns 3 and 4
 - 150 µL of the 50% Test Solution into all wells of column 5 and 6

Read the Plate

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

Analyze the Results

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 (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% (0.8004×0.010) 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.

Fluorescence Liquid Tests

This section applies to models with the Fluorescence module(s) only.

For Synergy H4 models with fluorescence capability, BioTek has developed a series of module-specific liquid tests for verifying performance.

Fluorescence Module	Applicable Liquid Test(s)
Filter-Based Fluorescence Intensity	Corners, Sensitivity, Linearity
Monochromator-Based Fluorescence Intensity	Sensitivity, Linearity
Fluorescence Polarization	"FP"
Time-Resolved Fluorescence	"TRF"

- **Corners Test:** Verifies that the plate carrier is properly aligned in relation to the fluorescence probes.
- **Sensitivity Test:** Verifies the fluorescence reading capability of the reader. The ability to detect specific compounds at low concentrations ensures that the monochromators, optical paths, and PMT are all in working order. This test verifies that the difference between the mean of wells with known lower limits of concentration of the substance under investigation is statistically distinguishable from the mean of wells with pure diluent.
- **Linearity Test:** Verifies that the system is linear, that is, signal changes proportionally with changes in concentration. Proving that the system is linear allows the Sensitivity Test to be run on two points instead of using serial dilutions.
- **FP Test:** Verifies the ability of the instrument to measure polarization of the solution properly. It verifies the polarizers are installed in the proper orientation, and the mechanism is in proper order.
- **TRF Test:** Verifies the performance of the xenon flash bulb and that the filters, optical path, and PMT are all in working order.



The tests presented in this section require specific microplates, solutions, wavelengths, mirrors, and filters. Your laboratory may require a deviation from some of these tests. For example, you may wish to use a different fluorescing solution, dichroic mirror, or microplate.

If deviation from the tests as presented in this section is required, the following steps should be taken the first time each test is run (e.g., during the Initial OQ):

- 1 Perform the tests exactly as described on the following pages.
- 2 Rerun the tests using your particular solutions, filters, mirrors, microplates, and so on. If results are comparable, then the results from these tests will be your baseline for future tests.
- 3 Document your new test procedure(s), and save all test results.

Required Materials

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

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 described starting on page 135

For the Filter-Based Fluorescence System:	
FI_T.prt	Corners, Sensitivity, and Linearity tests, using the Top optics
FI_MUB.prt	Alternative top optics test, using methylumbelliferone
FIFP_T.prt	Optional “combination” protocol to test Corners, Sensitivity, Linearity, and Fluorescence Polarization (top optics)
FP.prt	Fluorescence Polarization test
TRF.prt	Time-Resolved Fluorescence test
For the Monochromator-Based Fluorescence System:	
FI_H4_B_SF.prt	Corners, Sensitivity, and Linearity tests, using the Bottom optics
FI_H4_T_SF.prt	Corners, Sensitivity, and Linearity tests, using the Top optics
FI_H4_T_MUB.prt	Alternative top optics test, using methylumbelliferone

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.
- ❖ Methylumbelliferone can be used as an alternative or supplemental method for performing these tests for the top probe. See page 138.

- 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; if you are testing the monochromator-based system, ensure that the reader is initialized with this Excitation filter wheel 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) and 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 and Emission filter 528/20 nm installed
- 510-nm dichroic mirror and polarizers installed

Time-Resolved Fluorescence (TRF) Test

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

Test Solutions

Determine which tests you need to run for your reader model, and then prepare the necessary solutions. The ingredients for each test are listed under Required Materials starting on page 123.

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

Corners/Sensitivity/Linearity Tests

	<p>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.</p> <p>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.</p> <p>Wrap the vial containing the stock solution in foil to prevent exposure to light. Discard unused solution after seven days.</p> <p>Discard any open, unused buffer solution after seven days.</p>
---	---

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 µM	
110 µL of 50.2 µM SF	13.89 mL	400 nM	
3.5 mL of 400 nM SF	10.5 mL	100 nM	
0.46 mL of 100 nM SF	13.54 mL	3.3 nM	<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 on page 125, the recommended test solutions are available from Invitrogen Corporation and from BioTek. They do not require additional preparation.

Time-Resolved Fluorescence (TRF) Test

As described on page 125, the recommended test solutions are available from Invitrogen Corporation and from BioTek.

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

Procedure

1. If you have not already done so, create the Gen5 protocols as described starting on page 135.
2. If you have not already done so, prepare the solutions for the tests you plan to perform. See pages 125–127.
 - ❖ Refer to the pipette maps starting on page 128 for the remaining steps.
3. Perform the Corners/Sensitivity/Linearity tests using the Top optics of the filter-based fluorescence system:
 - Pipette the solutions for the Corners, Sensitivity, and Linearity Tests into a clean 96-well quartz or glass-bottom microplate.
 - Create an experiment based on the **FI_T.prt** protocol. Read the plate and then save the experiment.

4. If your reader is equipped with the Fluorescence Polarization module:
 - Pipette the solutions for the “FP” test into the same plate as used in step 3.
 - Create an experiment based on the **FP.prt** protocol. Read the plate and then save the experiment.
5. If your reader is equipped with the monochromator-based fluorescence system, perform the Sensitivity/Linearity tests for that system:
 - Using the same plate as was used in step 4, pipette 50 µL/well of buffer into wells C1-F5 and C10-F12.
 - Create experiments based on the **FI_H4_B_SF.prt** (for bottom optics) and **FI_H4_T_SF.prt** (for top optics) protocols. Read the plate and then save the experiments.
6. If your reader is equipped with the Time-Resolved Fluorescence module:
 - Pipette the solutions for the “TRP” test into a new 96-well solid white plate.
 - Create an experiment based on the **TRF.prt** protocol. Read the plate and then save the experiment.
7. Calculate and evaluate results as described under Results Analysis, starting on page 131.

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 a plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing a 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 bottom optics 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.
- 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 µL of the 3.3 nM SF solution into the “corner” wells.
- Pipette 200 µL 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 µL of the buffer into columns 2–5 and 10–12. Discard the tips.
- Pipette 150 µL of the 1 nM SF solution into column 1.
- Pipette 150 µL of the 1 nm SF solution into column 2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 2 and dispense into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 3 and dispense into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 4 and dispense into column 5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 5. Discard the solution and the tips.

	1	2	3	4	5	6	7	8	9	10	11	12
Corners:	A 3.3 nM	3.3 nM	3.3 nM	BUF					BUF 3.3 nM	3.3 nM	3.3 nM	3.3 nM
Sensitivity/ Linearity:	B BUF	BUF	BUF	BUF					BUF BUF	BUF	BUF	BUF
C	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
D	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
E	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
F	1.0 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
Corners:	G BUF	BUF	BUF	BUF					BUF BUF	BUF	BUF	BUF
H	3.3 nM	3.3 nM	3.3 nM	BUF					BUF 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 µL of buffer on top of the existing SF solutions and buffer. This will dilute the wells as shown in the following map:

Sensitivity/ Linearity:	C	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM					BUF	BUF	BUF
	D	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM					BUF	BUF	BUF
	E	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM					BUF	BUF	BUF
	F	0.75 nM	0.375 nM	0.1875 nM	0.0938 nM	0.0469 nM					BUF	BUF	BUF

Fluorescence Polarization (FP) Test

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

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

Time-Resolved Fluorescence (TRF) Test

- Pipette 200 µL of deionized water into wells A6–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 µL 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:
Top, with 510 nm dichroic mirror	5 pM (2 pg/mL)

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:
EX 485 nm, EM 528 nm	Top/Bottom: 15 pM (5.64 pg/mL)

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 ≥ 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 red:
 - Calculate the Mean Blank (wells A6–H6)
 - Calculate the Signal for each HPR well: Subtract the Mean Blank from its measurement value.
 - Calculate the Signal for each LPR well: Subtract the Mean Blank from its measurement value.
 3. Calculate the G-Factor for each LPR well:

$$\text{G-Factor} = \frac{\text{Parallel LPR Signal} * (1-0.02)}{\text{Perpendicular LPR Signal} * (1+0.02)}$$
 4. Calculate the Mean G-Factor.
 5. Calculate the Polarization value in mP for each HPR well (“PHPR”):

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

6. Calculate the Mean PHPR, in mP.

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

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

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

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

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

Time-Resolved Fluorescence (TRF) Test

1. Calculate the Mean and Standard Deviation of the wells containing the deionized water (wells A6–H6).
2. Calculate the Mean and Standard Deviation of the wells containing the europium solution (wells A8–B8).

3. 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:
Top, with 400 nm dichroic mirror	250 fM

Troubleshooting

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 wheels?
- 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 a 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, clean the internal components according to the instructions in the **Preventive Maintenance** chapter, and rerun the tests.
- When testing the Fluorescence Polarization module 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, or using the Greiner SensoPlate (see **Required Materials**). 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.
- When performing the Fluorescence Intensity tests, if a test fails because one or more wells overranged, reduce the Sensitivity value in the Gen5 protocol by 1–5 counts and reread the plate.
- The Read steps in the protocols use the Gen5 Automatic Sensitivity Adjustment feature to determine optimum sensitivity values for the plate. If an AutoSensitivity 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 Sensitivity value is low, the 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, clean the internal components according to the instructions in the **Preventive Maintenance** chapter, and rerun the tests.
- The PMT 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 134).

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

FI_T.prt

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

Plate Type: Costar 96-well black opaque (#3915)

Read Step:	Read Wells:	Corners: Full Plate; Sensitivity/Linearity: C1–F12 "Corners"/"Sensitivity/Linearity"
	Name:	"Corners"/"Sensitivity/Linearity"
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	40
	Wavelengths:	1
	Light Source:	Tungsten
	Excitation:	485/20 nm
	Emission:	528/20 nm
	Optics Position:	Top 510 nm
	Sensitivity:	75 (for both Corners and Sensitivity/Linearity)
	Top Probe Vertical Offset:	5.00 mm

FP.prt

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

Plate Type: Costar 96-well black opaque (#3915)

Read Step:	Read Wells:	A5–H9
	Name:	"Corners"/"Sensitivity/Linearity"
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	60
	Wavelengths:	1
	Synchronized Mode:	Plate Mode with Timing Control
	Light Source:	Tungsten
	Polarization:	Enabled
	Excitation:	485/20 nm
	Emission:	528/20 nm
	Optics Position:	Top 510 nm
	Sensitivity:	100
	Top Probe Vertical Offset:	5.00 mm

TRF.prt

- ❖ This procedure contains one Read step with Time-Resolved enabled.

Plate Type: Costar 96-well white (#3912)**Shake Step:** 30 seconds at Medium intensity**Delay Step:** 3 minutes

Read Step:	Read Wells:	A5–H9
	Name:	"Corners"/"Sensitivity/Linearity"
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	20
	Wavelengths:	1
	Delay Before Collecting Data:	300 µsec
	Data Collection Time:	1000 µsec
	Light Source:	Xenon Flash
	Time-Resolved:	Enabled
	Excitation:	360/40 nm
	Emission:	620/40 nm
	Optics Position:	Top 400 nm
	Sensitivity:	125
	Top Probe Vertical Offset:	5.00 mm

FI_H4_T_SF.prt and FI_H4_B_SF.prt

Plate Type:	Greiner SensoPlate	
Shake Step:	Medium for 15 seconds	
Delay Step:	For 5 seconds	
Read Step:	Read Wells:	A1–A3
	Name:	"Corners 1"
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	100
	Wavelengths:	1
	Excitation:	485 / 13.5 nm
	Emission:	528 / 13.5 nm
	Optics Position:	Top (or Bottom)
	Sensitivity:	Auto, Scale to High Wells, A1, 50000
	Top Probe Vertical Offset:	8.00 mm
Read Step:	Read Wells:	A10–A12
	Name:	"Corners 2"
	Sensitivity:	Use first filter set sensitivity from FIRST Read Step
	All other parameters same as for first read step	
Read Step:	Read Wells:	H1–H3
	Name:	"Corners 3"
	Sensitivity:	Use first filter set sensitivity from FIRST Read Step
	All other parameters same as for first read step	
Read Step:	Read Wells:	H10–H12
	Name:	"Corners 4"
	Sensitivity:	Use first filter set sensitivity from FIRST Read Step
	All other parameters same as for first read step	

Read Step:	Read Wells:	C1–F12
Name:	"Sensitivity"	
Detection Method:	Fluorescence	
Read Type:	Endpoint	
Read Speed:	Normal	
Delay After Plate Movement:	350 msec	
Measurements Per Data Point:	100	
Wavelengths:	1	
Excitation:	485 / 13.5 nm	
Emission:	528 / 13.5 nm	
Optics Position:	Top (or Bottom)	
Sensitivity:	Auto, Scale to High Wells, C1, 50000	
Top Probe Vertical Offset:	8.00 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.
- 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 microplate, such as Corning Costar #3915, or equivalent. A Greiner SensoPlate (Mfr. #655892) may also be used. If your reader is equipped with the filter- **and** monochromator-based fluorescence systems, the same plate is used to test both systems.
 - Excitation filter wheel with a 360/40 nm filter installed; if you are testing the monochromator-based system, ensure that the reader is initialized with this Excitation filter wheel installed
 - Emission filter 460/40 nm installed
 - 50% mirror installed
 - 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 described on page 142:
 - FI_MUB.prt tests the filter-based fluorescence system
 - FI_H4_T_MUB.prt tests the monochromator-based fluorescence system

Test Solutions

	<p>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.</p> <p>Wrap the vial containing the MUB stock solution in foil to prevent exposure to light.</p> <p>Discard any open, unused solutions after seven days.</p>
---	---

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 142.
2. If you have not already done so, prepare the test solutions. See page 139.
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 solid black plate.
 - Create an experiment based on the **FI_MUB.prt** protocol. Read the plate and then save the experiment.
4. If your reader is equipped with the monochromator-based fluorescence system, perform the Sensitivity/Linearity tests for that 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 **FI_H4_T_MUB_M.prt** protocol. Read the plate and then save the experiment.
5. Calculate and evaluate the results as described under **Results Analysis**, starting on page 141.

Pipette Map

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

- Pipette 200 µL of buffer into columns 10–12.
- Pipette 150 µL of buffer into columns 2–5 (**not column 1**). Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into column 1. Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into column 2. Do not discard the tips.
- Aspirate 150 µL from column 2 and dispense it into column 3. Mix the wells using the pipette. Do not discard the tips.

- Aspirate 150 µL from column 3 and dispense it into column 4.
Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 4 and dispense it into column 5.
Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL 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												

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 µL of buffer on top of the existing MUB solutions and buffer. This will dilute the wells as shown below.

	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

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:
Top, with 50% mirror	0.16 ng/mL

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:
EX 360 nm, EM 460 nm	0.16 ng/mL

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:

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 ≥ 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 134).

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

FI_MUB.prt

Plate Type: Costar 96 black opaque

Shake Step: Medium for 15 seconds

Delay Step: For 5 seconds

Read Step:	Read Wells:	C1–F12
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	40
	Wavelengths:	1
	Excitation:	360 / 40 nm
	Emission:	460 / 40 nm
	Optics Position:	Top 50% mirror
	Sensitivity:	90
	Top Probe Vertical Offset:	5.00 mm

FI_H4_T_MUB.prt

Plate Type: Costar 96 black opaque

Shake Step: Medium for 15 seconds

Delay Step: For 5 seconds

Read Step:	Read Wells:	C1–F12
	Detection Method:	Fluorescence
	Read Type:	Endpoint
	Read Speed:	Normal
	Delay After Plate Movement:	350 msec
	Measurements Per Data Point:	100
	Wavelengths:	1
	Excitation:	360 / 13.5 nm
	Emission:	460 / 13.5 nm
	Optics Position:	Top
	Sensitivity:	Auto, Scale to High Wells, C1, 50000
	Top Probe Vertical Offset:	8.00 mm

Luminescence Test

This section applies to models with the Luminescence module only.

For Synergy H4 models with luminescence capability, BioTek provides two methods for verifying the performance of the luminescence module. 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 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.

Harta Plate Test

Materials

- Harta Luminometer Reference Microplate and Adapter, PN 8030015
- Gen5 protocol (see page 147)

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 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												
F	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer
G	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer
H												

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 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-Mean of the buffer cells)/(3 * Standard deviation of buffer cells)
 4. Calculate the detection limit:
A2 NIST measurement in attomoles/signal-to-noise ratio
 - If the reader is equipped with the low-noise PMT, the detection limit must be **< 50 amol** to pass.
 - If the reader is equipped with the red-shifted PMT, the detection limit must be **< 500 amol** to pass.
- ❖ To determine which PMT is installed, check the label on the side 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)
- Glowell Adapter Plate, available from BioTek, PN 7160006
- Gen5 protocol (see page 147)

Procedure

1. If you have not already done so, insert the Glowell ("window" side up) into well D8 of the Adapter Plate.
 - ❖ Verify that there is a hole in one of the filter positions of the EM filter wheel.
2. If you have not already done so, create the Gen5 protocol as described on page 147.
3. Create an experiment based on the LumTest.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									Buffer	Buffer	Buffer	
B									Buffer	Buffer	Buffer	
C									Buffer	Buffer	Buffer	
D								GloWell	Buffer	Buffer	Buffer	
E									Buffer	Buffer	Buffer	
F									Buffer	Buffer	Buffer	
G									Buffer	Buffer	Buffer	
H									Buffer	Buffer	Buffer	

Results Analysis

- ❖ A manual ATP correlation process determined that 0.021pW R radiant Flux is equivalent to approximately 1800 attomoles of ATP.

1. Locate these items on the Glowell's Calibration Certificate: Calibration Date, R radiant Flux (pW), Measurement Uncertainty of the R radiant Flux.
2. Calculate the number of days between the Calibration Date and the date the test was performed.
3. Correct the Glowell's R radiant Flux value for deterioration over time:

$$\text{R radiant Flux} * e^{(-0.0001536 * \text{number of days since calibration})}$$
4. Convert the Corrected R radiant Flux value to attomoles (see Note above):

$$(\text{Corrected R radiant Flux} / 0.021) * 1800$$
5. Calculate an error factor for the Corrected R radiant Flux (amol):

$$(\text{Corrected R radiant Flux in amol} * \text{Measurement Uncertainty}) / 100$$
6. Calculate the min/max criteria for the Corrected R radiant Flux (amol):
 MIN: Corrected R radiant Flux in amol - Error Factor
 MAX: Corrected R 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:

$$\text{Corrected R radiant Flux in amol} / \text{Signal-to-Noise Ratio}$$

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 < **50 amol** to pass.
- If the reader is equipped with the red-shifted PMT, the detection limit must be < **500 amol** to pass.

Gen5 Protocol Reading Parameters

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

LumTest.prt (Glowell)

Plate Type:	Costar 96 black opaque
Delay Step:	For 3 minutes
Read Step :	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
	Emission: Hole
	Optics Position: Top
	Sensitivity: 150
	Top Probe Vertical Offset: 1.00 mm

LumTest.prt (Harta Plate)

Plate Type:	Costar 96 black opaque
Delay Step:	For 3 minutes
Read Step 1:	Read Wells:
	A2
	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
	Emission: Hole
	Optics Position: Top
	Sensitivity: 150
	Top Probe Vertical Offset: 5.00 mm
Read Step 2:	Read Wells:
	F1-G12
	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
Emission:	Hole
Optics Position:	Top
Sensitivity:	150
Top Probe Vertical Offset:	4.00 mm
Read Step 3: Read Wells:	A7–A8
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
Emission:	Hole
Optics Position:	Top
Sensitivity:	50
Top Probe Vertical Offset:	5.00 mm

Troubleshooting

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

- Ensure that the reading is performed through a hole in the EM filter wheel, not through a glass filter.
- Verify that the filter wheel settings in Gen5 match the physical wheel.
- 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 µL of deionized water into all wells of Columns 9, 10, and 11 (the background wells).

Dispense Module Tests

This section applies to models with the Dispense module only.

BioTek Instruments, Inc., 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 that you perform 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 µL, 5.0% for 20 µL, and 20.0% for 5 µL. It is assumed that one gram is equal to one milliliter.

The test uses a single green dye test solution and one 96-well microplate (per injector) to test the three different volumes. The balance is tared with the empty plate, and then the 80 µL dispense is performed for columns 1–4. The fluid is weighed and the balance is tared again (with the plate on the balance). This process is repeated for the 20 µL and 5 µL dispenses. It is assumed that the solutions used are at room temperature. A precision balance (three-place) is used to weigh the plate.

- The **Precision Test** is a measure of the variation among volumes dispensed to multiple wells. For each volume dispensed (80 µL, 20 µL, and 5 µL) to four columns, the %CV (coefficient of variation) of 32 absorbance readings is calculated. Pass/Fail criteria depends on the per-well volume dispensed: 2.0% for 80 µL, 7.0% for 20 µL, and 10.0% for 5 µL. The plate is read in an absorbance reader at 405/750 nm for columns 1–4 and at 630/750 nm for columns 5–12.

The two tests are performed simultaneously and use the same plate.



Each dispense module is calibrated to perform with a specific Synergy H4 reader. Make sure the dispense module and reader have the same serial number.

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 Synergy H4 may be used if it is equipped with the Absorbance module and has passed the Absorbance Plate Test or Absorbance Liquid Test 2, and Absorbance Liquid Test 1.

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200 µL hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250-mL beaker
- New 96-well, clear, flat-bottom microplates
- BioTek's Green Test Dye Solution (PN 7773003) undiluted, **or** one of the alternate test solutions listed in the next section
- 100-mL graduated cylinder and 10-mL pipettes (if not using BioTek's Green Test Dye Solution)
- Gen5 software installed on the host PC
- Gen5 protocols as defined by the procedure on page 154 or 160

Test Solution Formulas

- ❖ 80 µL of test solution with 150 µL of deionized water should read between 1.300 and 1.700 OD at 405/750 nm. The solutions should be at room temperature.

If you do not have BioTek's Green Test Dye Solution (PN 7773003), prepare a dye solution using one of the following methods:

Using BioTek's Blue and Yellow Concentrate Dye Solutions:

Ingredient	Quantity
Concentrate Blue Dye Solution (PN 7773001, 125 mL)	4.0 mL
QC (Yellow) Solution (PN 7120782, 125 mL)	5.0 mL
Deionized water	90.0 mL

Using FD&C Blue and Yellow Dye Powder:

Ingredient	Quantity per Liter
FD&C Blue No. 1	0.200 grams
FD&C Yellow No. 5	0.092 grams

Tween 20	1.0 mL
Sodium Azide N ₃ Na	0.100 gram
Deionized water	Make to 1 liter

Procedure for Models with the Absorbance Module

1. If you have not already done so, create Gen5 protocols **Disp 1 Test.prt** and **Disp 2 Test.prt**. Instructions begin on page 154.
 2. Prime both dispensers with 4000 μ L of deionized or distilled water.
 3. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000 μ 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 μ L of the solution. When finished, remove the priming plate from the carrier.
 5. In Gen5, create an experiment based on the **Disp 1 Test** protocol.
 6. Place a new 96-well microplate on the balance and tare the balance.
 7. Place the plate on the microplate carrier.
- ❖ 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 will prompt 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:
 - Dispense 80 μ L/well to columns 1–4.
 - Remove the plate and weigh it. Record the weight and tare the balance.
 - Place the plate on the carrier and dispense 20 μ L/well to columns 5–8.
 - Remove the plate and weigh it. Record the weight and tare the balance.
 - Place the plate on the carrier and dispense 5 μ L/well to columns 9–12.
 - Remove the plate and weigh it. Record the weight.
 - Manually pipette 200 μ L of deionized or distilled water into all 12 columns, on top of the green test dye solution.
 - Place the plate on the carrier for a 15-second shake, the “80 μ L” read at 405/750 nm, and the “20 and 5 μ L” read at 630/750 nm.
 10. When processing is complete, select **File > Save As**. Enter an identifying file name and click **Save**.

11. Remove the plate from the carrier and set it aside.
12. Repeat steps 5–11 using the Disp 2 Test protocol.
13. See page 153 for instructions on analyzing the results.
14. When all tests are complete, prime both dispensers with at least 5000 µL of deionized water to flush out the green dye solution.

Procedure for Models without the Absorbance Module

- ❖ If you will not be using a BioTek absorbance reader for this procedure, prepare your reader to perform two reads with the following characteristics:

	80 µL Read	20 & 5 µL Read
Primary Wavelength:	405 nm	630 nm
Reference Wavelength:	750 nm	750 nm
Plate Columns:	1–4	5–12

1. If you have not already done so, create the necessary Gen5 protocols as described on page 154.
2. Prime both dispensers with 4000 µL of deionized or distilled water.
3. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000 µL. 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 µL of the solution. When finished, remove the priming plate from the carrier.
5. In Gen5, create an experiment based on the Disp 1 Test protocol.
6. Place a new 96-well microplate on the balance and tare the balance.
7. Place the plate on the microplate carrier.

- ❖ 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 will prompt 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:
 - Dispense 80 µL/well to columns 1–4.
 - Remove the plate and weigh it. Record the weight and tare the balance.
 - Place the plate on the carrier and dispense 20 µL/well to columns 5–8.

- Remove the plate and weigh it. Record the weight and tare the balance.
 - Place the plate on the carrier and dispense 5 µL/well to columns 9–12.
 - Remove the plate and weigh it. Record the weight.
 - Manually pipette 200 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.
 - Carefully set the plate aside.
10. Close the experiment without saving it.
-
- ❖ If you are **not** using a BioTek reader for taking the absorbance measurements, read the plate using the wavelengths shown in the table on the previous page, and then perform the Results Analysis as described on page 153.
11. Configure Gen5 to communicate with the reader.
12. Create an experiment based on the Disp Test Other Reader protocol.
13. Select **Plate > Read** and click **Read**. Place the plate on the carrier and click **OK** at the Load Plate dialog. The absorbance reader will:
- Shake the plate for 15 seconds.
 - Perform the “80 µL” read at 405/750 nm.
 - Perform the “20 and 5 µL” read at 630/750 nm.
14. When processing is complete, select **File > Save As**. Enter an identifying file name and click **Save**.
15. Repeat steps 5–14 using the Disp 2 Test protocol for the dispense portion.
16. See page 153 for instructions on analyzing the results.
-
- ❖ When all tests are complete, prime both dispensers with at least 5000 µL of deionized water, to flush out the green dye solution.

Results Analysis

-
- ❖ For your convenience, worksheets are included at the end of this chapter for recording the dispense weights, Delta OD values, calculations, and pass/fail.

The pass/fail criteria for each set of 32 wells with the same dispense volume is based on the calculated coefficient of variation (% CV) and Accuracy % Error.

For each volume dispensed (80 µL, 20 µL, 5 µL), for each dispenser (1, 2):

- Calculate the Standard Deviation of the 32 wells
- Calculate the Mean of the 32 wells
- Calculate the %CV: (Standard Deviation / Mean) × 100

- Calculate the Accuracy % Error:

$$\frac{(\text{Actual Weight} - \text{Expected Weight})}{\text{Expected Weight}} * 100$$

❖ Expected Weights for 32 wells: 80 µL (2.560 g), 20 µL (0.640 g), 5 µL (0.160 g). It is assumed that one gram is equal to one milliliter.

Dispense Volume	To pass, % CV must be	To pass, Accuracy % Error must be:
80 µL	≤ 2.0%	≤ 2.0%
20 µL	≤ 7.0%	≤ 5.0%
5 µL	≤ 10.0%	≤ 20.0%

Failures

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

If the test(s) fail again:

- The injectors may require cleaning (see the Preventive Maintenance chapter).
- Each dispense module is factory-calibrated for the Synergy H4 it ships with. Verify that the serial number on the dispense module matches the serial number on the reader. Even if the serial numbers match, it is still possible that the calibration values have been inadvertently changed. Contact BioTek's Technical Assistance Center.

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

Gen5 Test Protocols for Models with the Absorbance Module

This section contains instructions for creating two Gen5 protocols specifically for performing the Synergy H4 Dispense Precision and Accuracy test.

1. Select **System > Reader Configuration**, and add/configure the **Synergy H4** (if it is not already there).
2. Select **File > New Protocol**. A “menu tree” will appear.
 - To edit a protocol category, double-click its “branch” in the tree.
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 μ L dye to columns 1–4
- Dispense 20 μ L dye to columns 5–8
- Dispense 5 μ 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, as demonstrated below.

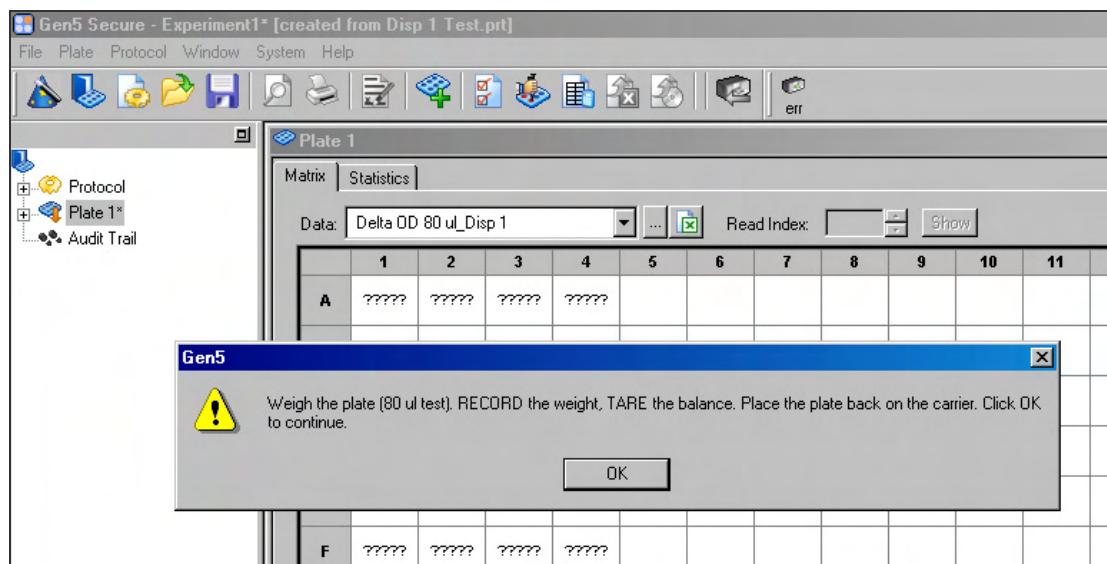


Figure 4: Sample comment associated with a Plate Out/In step

Gen5 Procedure Steps		
#	Step Type	Details
1	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
2	Plate Out,In	Suggested comment: <i>Weigh the plate (80 µL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.</i>
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: <i>Weigh the plate (20 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click OK to continue.</i>
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: <i>Weigh the plate (5 µL test). RECORD the weight. PIPETTE 200 µL/well of DI water into all 12 columns. Place the plate back on the carrier. Click OK to perform the Read steps.</i>
7	Shake	Medium intensity for 15 seconds
8	Read	Step label: "80 ul Read_Dis 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_Dis 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**.
- 3 Click the **Browse (...)** button associated with the **ID** field. Assign the first three controls as Disp_80, Disp_20, and Disp_5. Click **OK**.
- 4 Set ID to **Disp_80** and highlight wells **A1** to **H4**.
- 5 Set ID to **Disp_20** and highlight wells **A5** to **H8**.
- 6 Set ID to **Disp_5** and highlight wells **A9** to **H12**.

The Plate Layout should resemble the following:

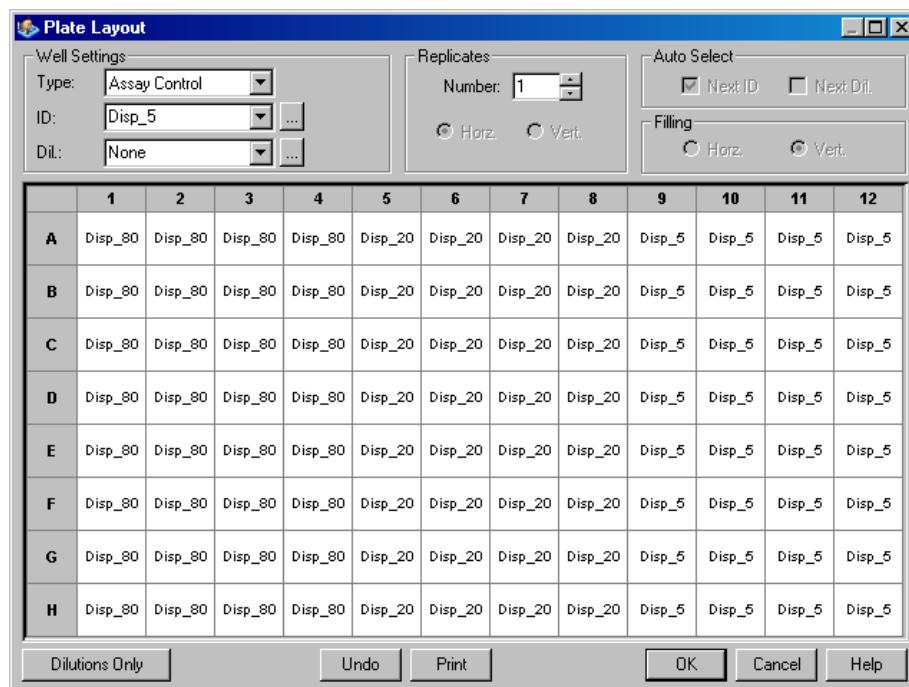


Figure 5: Sample plate layout.

- 7 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 **Transformation**.
- 2 Click **Select Multiple Data Sets** and then select **DS2**.
- 3 Set the Data In for DS1 to the **80 µL** Read step at **405 nm**.
- 4 Set the Data In for DS2 to the **80 µL** Read step at **750 nm**.

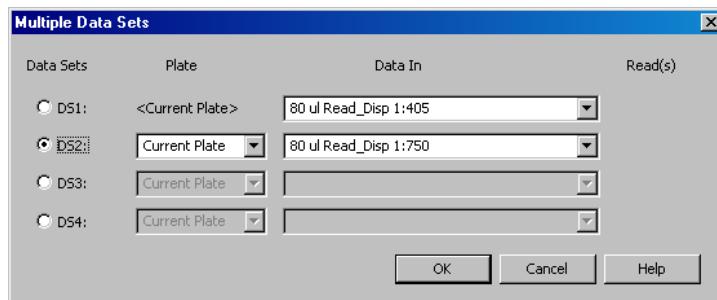


Figure 6: Creating a data reduction step.

- 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 µl_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.

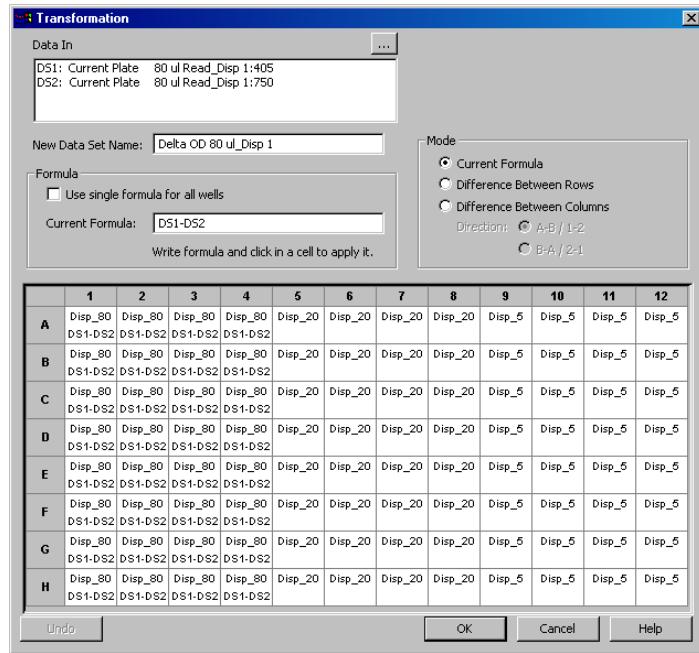


Figure 7: Defining a Delta OD transformation.

- 9 Click **OK** to add the transformation to the Data Reduction list.
- 10 Create another Transformation similar to the above, with these characteristics:
 - DS1 set to the **20 and 5** μL Read step at **630 nm**
 - DS2 set to the **20 and 5** μL Read step at **750 nm**
 - New Data Set Name resembling Delta OD 20 and 5 μL _Disp 1
 - Remember to clear **Use Single Formula**
 - Formula DS1-DS2 applied to wells **A5 to H12**
- 11 When you are finished, the Data Reduction Steps list shows two Delta OD transformations:

Data Reduction	
Data Reduction Steps	
Add Step	
Transformation	Description Data Out
	Transformation Delta OD 80 ul_Disp 1
	Transformation Delta OD 20 and 5 ul_Disp 1

Figure 8: Sample Data Reduction Steps.

- 12 Click **OK** to close the Data Reduction dialog.

Gen5 Test Protocols for Models without the Absorbance Module

This section contains instructions for creating Gen5 protocols specifically for performing the Synergy H4 Dispense Precision and Accuracy test.

The test procedure on page 152 dispenses three different 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 be using a BioTek absorbance reader that is supported by Gen5, you will create one additional protocol to perform the Read steps.

The Dispense protocols (one per dispenser) follow the sequence below. After each Dispense step, the plate is ejected to allow the operator to weigh it and then tare the balance.

- Dispense 80 µL dye to columns 1–4
- Dispense 20 µL dye to columns 5–8
- Dispense 5 µL dye to columns 9–12

The optional Read protocol follows this sequence:

- Shake the plate for 15 seconds
- Read columns 1–4 at 405/750 nm, calculate the Delta OD
- Read columns 5–12 at 630/750 nm, calculate the Delta OD

To create the Dispense protocols (required):

1. Select **System > Reader Configuration**, and add/configure the Synergy H4 (if it is not already there).
2. Select **File > New Protocol** and then **Protocol > Procedure**.
3. Define the procedure as 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.

Gen5 Procedure Steps		
#	Step Type	Details
1	Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
2	Plate Out,In	Suggested comment: <i>Weigh the plate (80 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click OK to continue.</i>

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: <i>Weigh the plate (20 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click OK to continue.</i>
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: <i>Weigh the plate (5 µL test). RECORD the weight. Set the plate aside and click OK.</i>
The last step is a brief Read step—necessary because Gen5 requires a Read step within any Dispense procedure. When this test is run, the measurement value is not used.		
7	Read	Wells: A1 Detection Method: <select any valid method> Read Type: Endpoint Read Speed: Normal Wavelength: <select any valid wavelength(s)>

4. When finished, click **OK** to close the Procedure dialog.
5. Select **File > Save As** and save the file as **Disp 1 Test.prt**.
6. Repeat steps 2–5 to create **Disp 2 Test.prt** for Dispenser #2.

To create the Read protocol (optional):

1. Start by selecting **System > Reader Configuration**, and add/configure the BioTek absorbance reader (if it is not already there).
2. Select **File > New Protocol** and then **Protocol > Procedure**.

3. Define the procedure as described below.

Gen5 Procedure Steps		
#	Step Type	Details
1	Shake	Medium intensity for 15 seconds
2	Read	Step label: "80 µL Read" Wells: A1..H4 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 405 and 750 nm
3	Read	Step label: "20 and 5 µL Read" Wells: A5..H12 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 630 and 750 nm

4. When finished, click **OK** to close the Procedure dialog.
5. (Optional) Customize the Plate Layout. The 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.
- In the protocol, open the Plate Layout dialog.
 - Set the Type set to **Assay Control**.
 - Click the **Browse (...)** button associated with the **ID** field. Assign the first three controls as Disp_80, Disp_20, and Disp_5. Click **OK**.
 - Set ID to **Disp_80** and highlight wells **A1** to **H4**.
 - Set ID to **Disp_20** and highlight wells **A5** to **H8**.
 - Set ID to **Disp_5** and highlight wells **A9** to **H12**.
 - 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.

6. 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.

❖ For guidance, refer to the sample screen shots under **Add Data Reduction Steps** on page 158. The data set names will be slightly different.

- In the protocol, open the Data Reduction dialog and click **Transformation**.
 - Click **Select Multiple Data Sets** and then select **DS2**.
 - Set the Data In for DS1 to the **80 µL** Read step at **405 nm**.
 - Set the Data In for DS2 to the **80 µL** Read step at **750 nm**.
 - Click **OK** to return to the Transformation dialog.
 - In the New Data Set Name field, type an identifying name such as **Delta OD 80 ul**.
 - Clear **Use single formula for all wells**.
 - In the Current Formula field, type **DS1-DS2** and then highlight wells **A1** to **H4** to assign the formula.
 - Click **OK** to add the transformation to the Data Reduction list.
 - Create another Transformation similar to the above, with these characteristics:
 - DS1 set to the **20 and 5 µL** Read step at **630 nm**
 - DS2 set to the **20 and 5 µL** Read step at **750 nm**
 - New Data Set Name resembling Delta OD 20 and 5 µL
 - Formula DS1-DS2 applied to wells **A5** to **H12**
 - Click **OK** to close the Data Reduction dialog.
7. When finished, select **File > Save As** and save the file as **Disp Test Other Reader.prt**.

Synergy H4 Dispense Accuracy & Precision Tests - Dispenser #1

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
A				
B				
C				
D				
E				
F				
G				
H				

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

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

Standard Deviation:
 Mean: _____

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

Reader Model: _____
 Reader S/N: _____
 Reading Date: _____
 Comments: _____

20 µL weight: _____ g
 Expected weight: 0.6400 g

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

Standard Deviation:
 Mean: _____

%CV: _____ %
 Must be < = 10.0% P F

Reviewed/
 Approved By: _____
 Signature: _____

5 µL weight: _____ g
 Expected weight: 0.1600 g

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

Standard Deviation:
 Mean: _____

%CV: _____ %
 Must be < = 10.0% P F

Synergy H4 Dispense Accuracy & Precision Tests - Dispenser #2

80 µL Dispense Delta ODs @405/750 nm				20 µL Dispense Delta ODs @630/750 nm				5 µL Dispense Delta ODs @630/750 nm			
1	2	3	4	5	6	7	8	9	10	11	12
A											A
B											B
C											C
D											D
E											E
F											F
G											G
H											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: %

%CV: %

%CV: %

Must be <= 2.0%

P F

Must be <= 7.0%

P F

Must be <= 10.0%

P F

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

Tested By:

Reviewed/
Approved By:

Signature:

Signature:

Appendix A

Specifications

This appendix contains BioTek's published specifications for the Synergy H4.

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General Specifications

Microplates	
<p>The Synergy H4 accommodates standard 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with 128 x 86 mm geometry, the Take3 Multi-Volume Plate, and Terasaki microplates.</p>	
Maximum Plate Height:	
<ul style="list-style-type: none"> ▶ Absorbance mode: plates up to 0.8" (20.30 mm) high ▶ Fluorescence/Luminescence modes: plates up to 1.2" (30.48 mm) high ▶ PCR tube trays up to 1.25" (31.75 mm) high (may require an adapter) ▶ The system auto-calibration for the top probe is 1.30" (33.1 mm) 	
Hardware & Environmental	
Light Source	
Absorbance, Fluorescence (FI), monochromator-based:	Xenon flash light source, 40W maximum average power, lamp life 1 billion flashes (not user-changeable)
Fluorescence (FI/FP), filter-based:	(Primary) Tungsten quartz halogen, 20W power, lamp life 1000 hours (user-changeable) (Optional) Xenon flash light source, 60W maximum average power, lamp life 1 billion flashes (not user-changeable)
TRF:	Xenon flash light source, 60W maximum average power, lamp life 1 billion flashes (not user-changeable)
Dimensions	21.53" D* x 17" W x 15" H 54.7 cm D* x 43.5 cm W x 38.1 cm H *Depth measurement includes the rubber feet on the back of the reader
Weight	With all modules installed, without power supply or dispense module attached, < 80 lbs. (36.3 kg)
Environment	Operational temperature 18° to 40°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	250W maximum
Incubation	Temperature control range from 4° over ambient to 65°C. Temperature variation ± 0.50°C across the plate @ 37°C (250 µL per well with the plate sealed).
Plate Shaking	Low, Medium, High and Variable shaking speeds. Shake time is programmable by the user.

Dispense/Read Specifications

Dispense/Read	
<i>Specifications apply to models with the dual-reagent dispense module</i>	
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
Volume Range	5–1000 µL with a 5–20 µL tip prime
Accuracy	Dispensing deionized water with 0.1% Tween 20 at room temperature: +/- 1µL or 2.0%, whichever is greater
Precision	Dispensing a 200-µL solution of deionized water, 0.1% Tween 20, and dye at room temperature: ≤ 2.0% for volumes of 50–200 µL ≤ 4.0% for volumes of 25–49 µL ≤ 7.0% for volumes of 10–24 µL ≤ 10.0% for volumes of 5–9 µL

Absorbance Specifications

Accuracy, Linearity, Repeatability	
<i>All qualifications were conducted using 96-/384-well, flat-bottom microplates. For the performance described here, the Gain on the Optics Test should be below 10.0.</i>	
Measurement Range: 0.000 to 4.000 OD	Resolution: 0.0001 OD
Accuracy	
96-well plate, normal read speed	
0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 0	
2–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 0	
384-well plate, normal read speed	
0–2 OD: +/-2% +/-0.010 OD, delay after plate movement = 0	
2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 0	
96-well and 384-well plate, sweep read speed	
0–1 OD: +/-1% +/-0.010 OD	
Linearity (by liquid dilution)	
96-well plate, normal read speed	
0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 0	
2–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 0	
384-well plate, normal read speed	
0–2 OD: +/-2% +/-0.010 OD, delay after plate movement = 0	
2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 0	
96-well and 384-well plate, sweep read speed	
0–1 OD: +/-1% +/-0.010 OD	
Repeatability (measured by one standard deviation: 8 measurements/data points)	
96-well and 384-well plate, normal read speed	
0–2 OD: +/-1% +/-0.005 OD, delay after plate movement = 0	
2–2.5 OD: +/-3% +/-0.005 OD, delay after plate movement = 0	
96-well and 384-well plate, sweep read speed	
0–1 OD: +/-2% +/-0.010 OD	

Optics	
λ range	230 to 999 nm
λ accuracy	\pm 2 nm
λ repeatability	\pm 0.2 nm
λ band pass	2 nm (230–285 nm) to 4 nm (> 285 nm)
Detector	Photodiodes (2). Measurements are reference channel-corrected for light source fluctuation.

Fluorescence Specifications (Mono-Based)

The Synergy H4 reads fluorescence intensity 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.

The following requirements apply to 96-well plate reads. All tests are conducted in a Greiner clear-bottom Sensoplate (BioTek p/n 98226). All tests use 100 flashes or fewer per data point and 350 msec delay before read with 200 μ L in each well.

Monochromator-based Fluorescence	
Excitation range	250–900 nm
Emission range	250–900 nm (for reads); 300–900 nm for emission scans
Bandpass (excitation and emission)	4 slits: approximately 9 nm, 13.5 nm, 17 nm, and 20 nm

Sodium Fluorescein in phosphate buffered saline (PBS)
DL <= 15 pM top read
DL <= 15 pM bottom read
Excitation 485nm, 13.5nm bandwidth
Emission 528nm, 13.5nm bandwidth

Methylumbellifерone (MUB) in carbonate-bicarbonate buffer (CBB)
DL <= 0.16 ng/mL (0.91 nM) top read
Excitation 360nm, 13.5nm bandwidth
Emission 460nm, 13.5nm bandwidth

Propidium iodide (PI) in PBS
DL <= 62.5 ng/mL bottom read
Excitation 485nm, 13.5nm bandwidth
Emission 645nm, 13.5nm bandwidth

Fluorescence Specifications (Filter-Based)

Optical Probes	
Top position	3-mm diameter fixed, with motor-driven moveable apertures to reduce the diameter to support different plate formats

Sensitivity	
<i>The following specifications apply to 96-well read formats using the Tungsten bulb</i>	
3-mm fixed optical probe with movable apertures, Top reading, 96-well aperture	<p>DL ≤ 2 pg/mL (5pM) solution of Sodium Fluorescein in PBS 40 reads per location averaged, 5-mm probe offset, 350-ms delay before read 200 µL per well signal-to-noise ratio greater than 2 Excitation 485/20, Emission 528/20, Dichroic 510 nm Corning Costar 96-well solid black plate</p> <p>DL ≤ 0.16 ng/mL (0.91 nM) solution of Methylumbelliflone in CBB 40 reads per location averaged, 5-mm probe offset, 350-ms delay before read 150 µL per well signal-to-noise ratio greater than 2 Excitation 360/40, Emission 460/40, 50% mirror Corning Costar black strips</p>

Time-Resolved Fluorescence	
96-/384-well plates	250 fM Europium (plate and well modes), 20 reads per location, 5-mm probe offset, 350-ms delay before read
Integration Interval	20 to 16000 µs
Delay	0 to 16000 µs
Granularity	1-µs steps

Fluorescence Polarization	
96-/384-well plates	5 mP at 1 nM Sodium Fluorescein, 60 reads per location, 5-mm probe offset, 350-ms delay before read

Luminescence Specifications

DL <= 50 amol/well flash ATP in a 96-well plate (low-noise #49984 PMT)

DL <= 500 amol/well flash ATP in a 96-well plate (red-shifted #49721 PMT)

Appendix B

Error Codes

This appendix lists and describes Synergy H4 error codes that may appear in Gen5.

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Overview

When a problem occurs during operation with the Synergy H4, an **error code** will appear 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

- ❖ For errors that are displayed during operation of the Synergy H4 with the BioStack Microplate Stacker, refer to the *BioStack 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
0601	Tungsten Lamp reference voltage out of range A test of the tungsten lamp is performed when the instrument is first turned on and then periodically during background functions. This error may indicate that the lamp is weak or defective. Refer to the As Needed Maintenance chapter for instructions to replace the lamp. If the error still appears after replacing the lamp, contact BioTek TAC.
230x	Expected plug/hole/filter not found in EX Filter Wheel $x = 0:$ plug, $1:$ open hole, $4:$ bandpass filter This error indicates that the locations of the plugs/holes/filters in the EX filter wheel do not match the Wavelengths Table in Gen5. Check the physical contents of the filter wheels and update Gen5 if necessary.
2B0A	Priming plate not detected
2B0x	Dispenser syringe 1 or 2 (respectively) did not home $x=1-3$ Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. (Refer to the Installation chapter for instructions.) Restart the reader.
2B04	Dispenser syringe 1 or 2 (respectively) failed position verify Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. Restart the reader. (Refer to the Installation chapter for instructions.)
37x0/47x0 38x0/48x0 39xy/49xy	Noise Test Errors Offset Test Errors Dark Range Errors $x=0, 1; y=0-6$ This series of System Test errors may indicate too much light inside the chamber. Make sure the reader's shroud is properly fastened, and 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 back of the reader, re-install the plastic plugs that shipped with the instrument (or cover the holes with black tape). Restart the reader.
4Fxy	Filter is defined in the wrong location $x=0, 1; y=0-6$ Ensure that Gen5 Fluor/Lum wavelengths table matches the actual filter installed in the filter wheels.

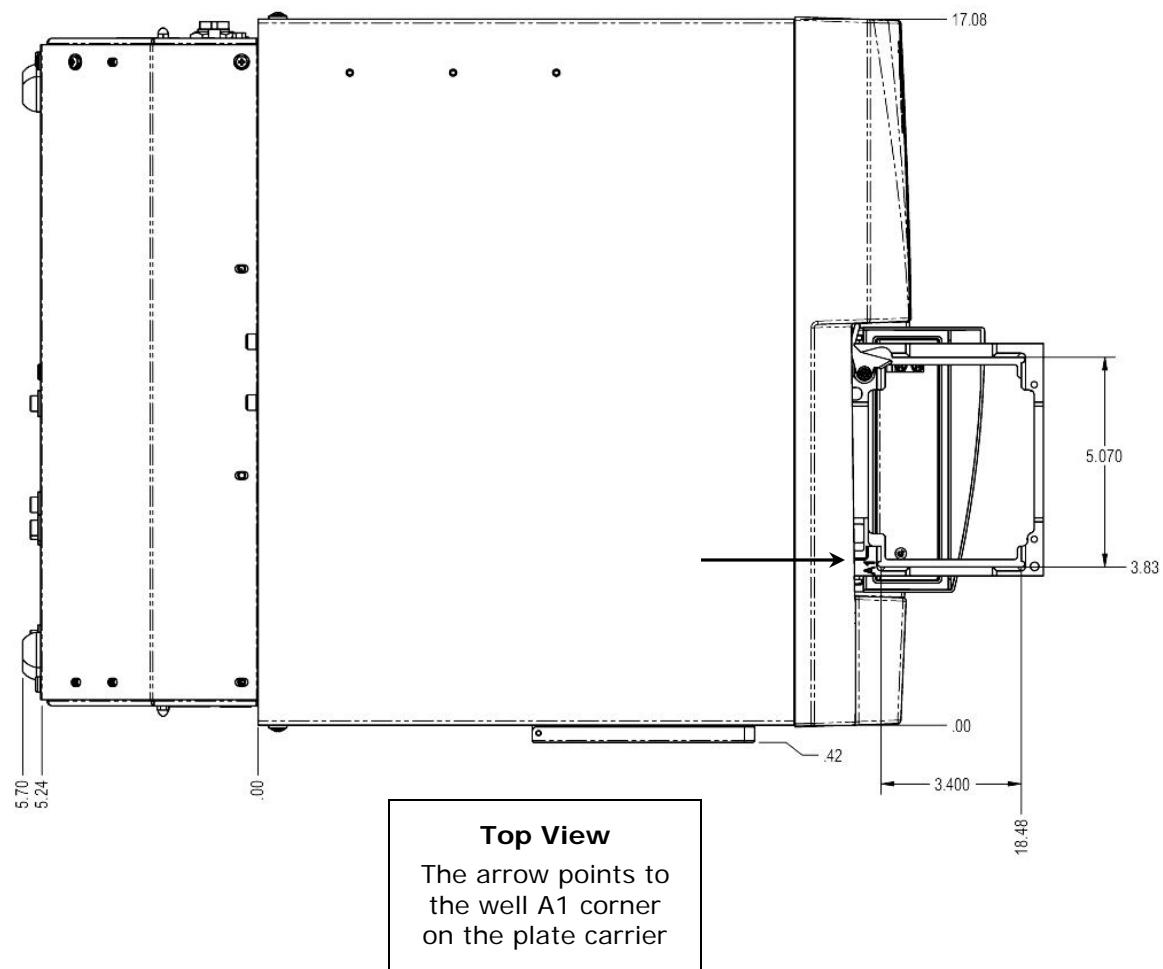
Code	Description and Possible Remedy
4xxx	<p>PMT overload well error at <well #xxx></p> <p>This error typically means that the fluid in a well has oversaturated the PMT (i.e., the well is too bright). Try lowering the sensitivity value in the read step.</p> <p>To identify the well:</p> <p>Wells are counted 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"> 1. Convert the ASCII hex string to a decimal equivalent. Ex: "057" indicates 57 hex, yielding a well code of 87 decimal. 2. Row = (well code) / (columns in plate), rounded up to a whole number. Ex: 87/12 = 7.25, indicating row 8 (or H). 3. Column = (well code) - ((row-1) * (columns in plate)). Ex: 87 - ((8 - 1) * 12) = column 3. <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, NOT the actual well where the error occurred.</p>
4Exy	<p>Detector saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).</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>For models with the dispense module, the internal chamber may require cleaning (see Preventive Maintenance).</p> <p>If a 4E18 error is detected during monochromator-based fluorescence, the luminescence probe may be picking up stray light. Try installing a plug in the Emission filter wheel. Restart the reader.</p>
2D46	<p>Fluorescence wavelength not found in table</p> <p>This error indicates that the wavelength specified in the procedure is not detected in the instrument's filter table. In Gen5, verify the Fluorescence filter table has the wavelengths loaded into the reader. Compare the contents of the table with the Excitation and Emission filters installed (see the Gen5 Help system for more information). Restart the reader.</p>
500x 510x	<p>EX/EM filter wheel (respectively) did not home</p> <p>x=4: excitation wheel, 5: emission wheel</p> <p>Generally, this error indicates the Excitation or Emission filter wheel 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>
540x	<p>EX/EM filter wheel (respectively) failed positional verify</p> <p>x=4: excitation filter, 5: emission filter</p> <p>Generally, this error indicates the Excitation or Emission filter wheel 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>

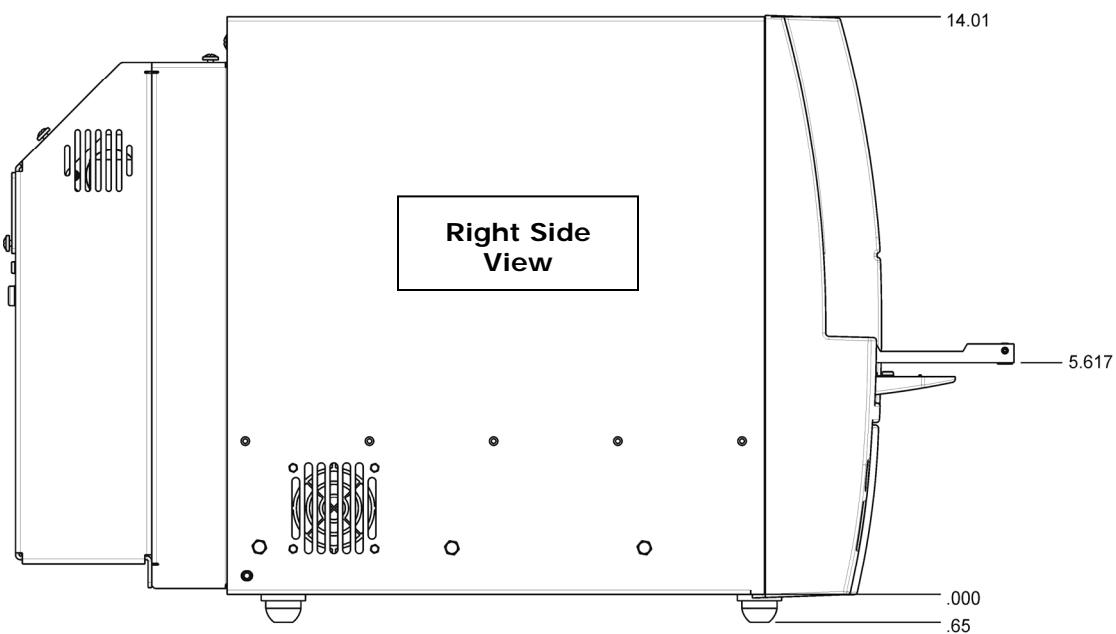
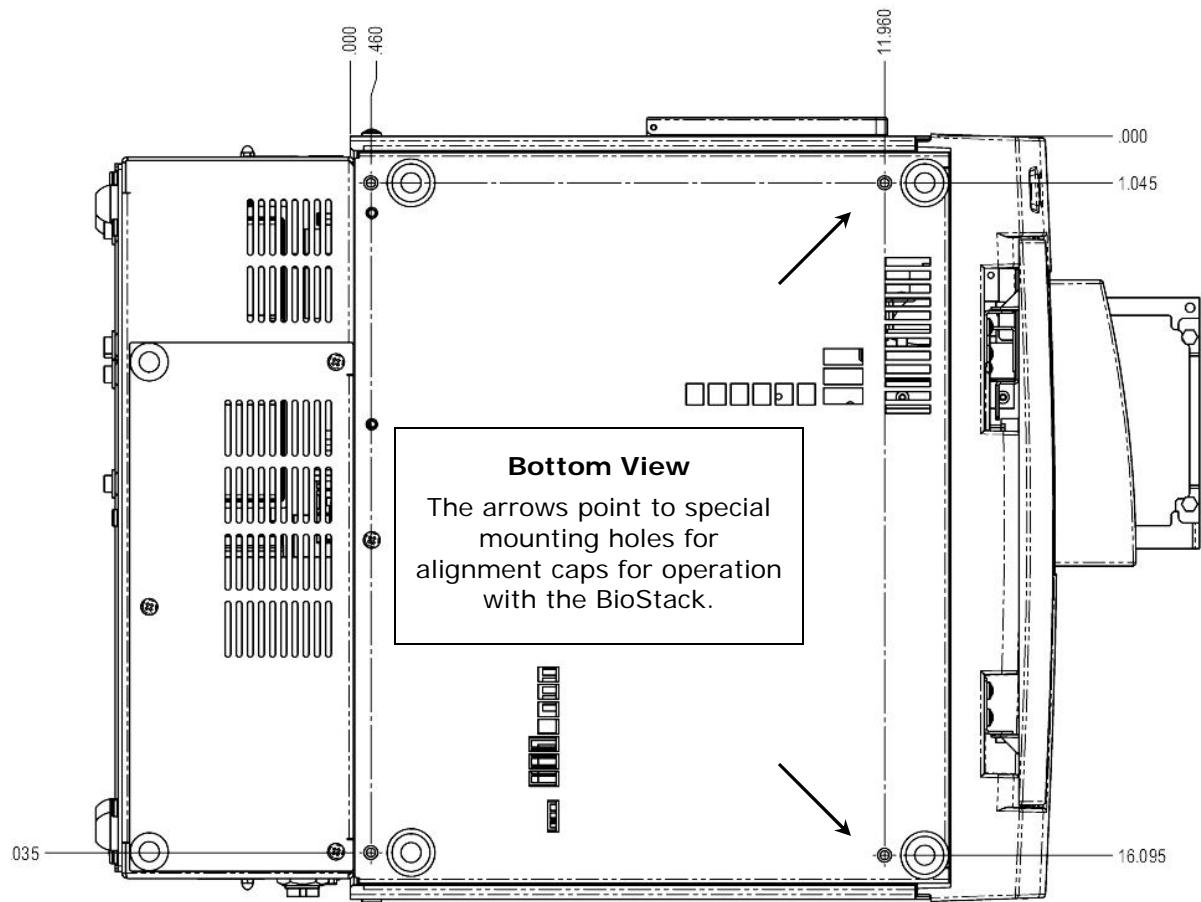
Code	Description and Possible Remedy
55xy	<p><Motor> not homed successfully xy=axis This error indicates that an axis failed a previous verify function and now needs to be homed. Check for any obstructions that may prevent the carrier, syringes, or filter wheel from moving normally. Restart the reader.</p>
5A0x	<p>Plate could not be moved inside $x=0, 1$ Make sure the Plate Type defined in the Gen5 Protocol matches the plate you are using. This error can also occur if the plate type is correct but the lid was left on the plate. If you wish to read the plate with a lid on it, create a new plate type in Gen5 with the correct Plate Height.</p>
5B00	<p>Required carrier in when expected to be outside The carrier is inside the read chamber and the probe needs to move down for the requested operation. Press the Carrier Eject button.</p>

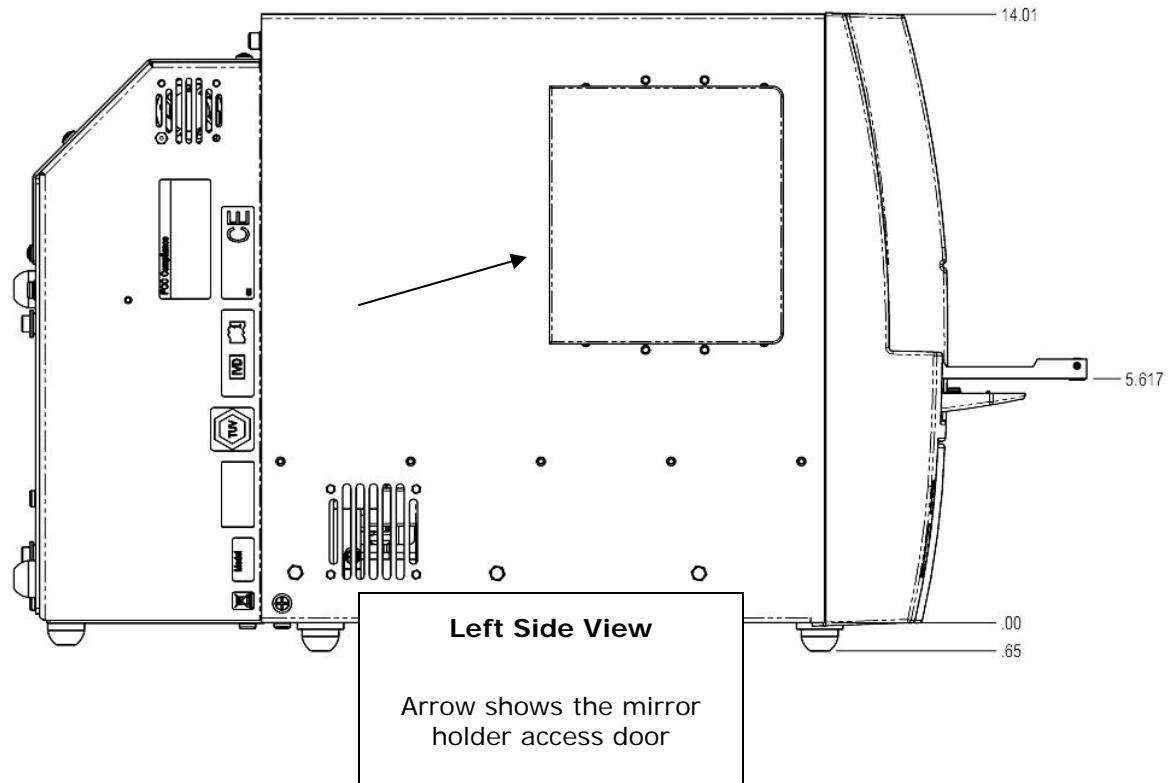
Appendix C

Instrument Dimensions for Robotic Interface

This section shows the location of the microplate carrier in reference to the exterior surfaces of the Synergy H4 and the mounting holes on the bottom. Use the illustrations to facilitate system setup with a robotic instrument, such as the BioStack Microplate Stacker. Dimensions are in inches.







BioStack users: Special alignment hardware is included in the BioStack's alignment kit for correct positioning with the Synergy H4. Refer to the **Installation** chapter in the *BioStack Operator's Manual* for instructions.

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