Multi-Mode Microplate Reader

Synergy™ 2

Operator's Manual

BioTek®

Synergy 2

Multi-Mode Reader

Operator's Manual

BioTek® Instruments, Inc.
Part Number 7131000
Revision K
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Notices

BioTek® Instruments, Inc.

Highland Park, P.O. Box 998

Winooski, Vermont 05404-0998 USA

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Contents

Contact Information

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Global Service and Support

BioTek product service and repair is available worldwide at one of BioTek's International Service Centers and in the field at your location. To arrange for service or repair, contact the office nearest you; visit www.biotek.com for up-to-date contact information. For customer service, sales, and technical assistance, refer to the information below.

Customer Service and Sales

Internet: www.biotek.com

Phone: 888-451-5171 (toll-free in the U.S.) or 802-655-4740 (outside the U.S.)

Fax: 802-655-7941

Email: customercare@biotek.com

Service/Technical Assistance Center (TAC)

Phone: 800-242-4685 (toll free in the U.S.) or 802-655-4740 (outside the U.S.)

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European Coordination Center/Authorized European Representative

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Revision History

Rev	Date	Changes
A	8/2006	First issue
B	12/2007	General: Changed all instances of "multi-detection" to "multi-mode". Changed all instances of "filter cartridge" to "filter wheel". Preface: Added Glowell™ and FluoSpheres® to Trademarks. Removed Figures list. Ch. 1, Introduction: Updated Package Contents and Accessories lists. Added note stating that part numbers are subject to change. Ch. 2, Installation: Revised unpacking/repackaging instructions. Added note stating that packaging materials are subject to change. Ch. 3, Getting Started: Simplified Gen5 Software usage instructions. Ch. 4, Filters and Mirrors: Mirrors section: Added information on Visible and UV-polarizing filters. Corrected high end of EM Range for 510 nm mirror (changed 780 nm to 640 nm). Changed mirror label location from "lower left corner" to "face up and readable" and added an illustration. Updated list of dichroic mirrors available from BioTek. Ch. 5, Instrument Qualification: Added materials and procedure for a Luminescence Test. Fluorescence Liquid Tests section: Added option to use Greiner SensoPlate to FI tests for the top optics. Corrected instances of "505 nm" mirror to "510 nm" and "410 nm" to "400 nm". Under "Results Analysis" for the FP Test, corrected the wells used for the Mean Blank calculations—changed from A1-H6 to A6-H6. Added option to use Sodium Borate instead of PBS. Reconfigured the SF test solutions and dilutions for efficiency and consistency with other BioTek products. Added troubleshooting tip to reduce the Gain/Sensitivity value in the Gen5 protocol if well(s) are overranging during the Fluorescence Intensity tests. To minimize the opportunity for overranging during these tests, reduced the recommended Gain/Sensitivity values for the SF Fluorescence Intensity Corners and Sensitivity top probe tests to 75. Updated the materials list and solution preparation steps for the TRF test to support the use of FluoSpheres® from Invitrogen. Removed screen shots of sample BioTek data sheets. Under "Gen5 Protocol Reading Parameters" added the Gain/Sensitivity value as an example of a protocol parameter that may need to be adjusted for some readers. Added FI tests using Methylumbelliferone. Ch. 6, Preventive Maintenance: Removed task to clean supply bottles. Ch. 7, As Needed Maintenance: Changed the replacement tungsten lamp part number to 7080500. Appendix B, Error Codes: Revised to focus on only the most common, most easily-fixed error codes that may appear in Gen5.

Rev	Date	Changes
C	1/2010	Throughout: Added information for purchasing and using the Harta Luminometer Reference Microplate. Added support for the BioTek Take3 Multi-Volume Plate. Preface: Updated the Intended Use Statement; the Synergy 2 may be used for In Vitro Diagnostic, research and development, or other non-clinical purposes. Added information for registering products online through the BioTek Customer Resource Center. Updated the list of Hazards and Precautions. Updated CE Mark information and Safety Symbols. Ch 1, Introduction: Updated the list of Optional Accessories and added a reference to the BioTek online Accessories search tool. Removed the BioTek shipping address; customers will be notified of the address when they contact TAC/Service for a Return Materials Authorization number. Ch 2, Installation: Combined reader unpacking/inspection and shipping panel removal instructions. Moved dispense module figures from step 2 to "Repackaging and Shipping Instructions." Corrected USB Driver Software CD instructions under "Establish Communication". Ch 3, Getting Started: Added "Modular Design," "External Components," and "Internal Components." Clarified/updated content in remaining sections. Ch 4, Filters and Mirrors: Clarified instructions throughout. Added "Filters Available From BioTek." Moved mirror cleaning instructions to Chapter 7. Ch 5, Instrument Qualification: Corrected unit of measure typos. Added reference to new BioTek test kits for fluorescence liquid testing. Added Luminescence Test using the new Harta plate. Corrected Accuracy % Error calculation for Dispense Module tests. Ch 6, Preventive Maintenance: Added instructions for inspecting/ cleaning mirrors. Appendix A, Specifications: Added support for the Take3 plate. Appendix B, Error Codes: Updated error code descriptions.
D	3/2010	*Preface*: Updated Directive 98/79/EC: In Vitro Diagnostics. *Ch 2, Installation*: Corrected typo in the step heading numbers. *Ch 5, Instrument Qualification*: For the luminescence test using the Harta Luminometer Reference Microplate, moved the background (buffer) wells from rows C and D to rows F and G. For the background read step, changed the Top Probe Vertical Offset to 4.00 mm. *Ch 6, Preventive Maintenance*: Under "Run a Dispense Protocol (Optional)," added instruction to set the Plate Type in the Gen5 protocol to match the plate being used for the test.
E	4/2010	*Ch 5, Instrument Qualification*: For the luminescence test using the Harta Luminometer Reference Microplate, corrected errors in the Gen5 protocol reading parameter table on page 123 (read step 3).
F	7/2011	Updated Gen5 instructions for new Gen5 version 2.x. Added information for new side-access door. Added Take3 Trio to "Optional Accessories."

Rev	Date	Changes
G	7/2012	*Preface*: Updated the Intended Use Statement and the heading for the In Vitro Diagnostics directive to refer to the instrument's IVD label (if one exists). Added 'Service' and 'Accessories' hazard warnings. Added a warning to use two people to lift/carry the instrument. Added 'Spare Parts' precaution. Updated 'Power Supply' precaution. Added EN 61010-2-010 to Directive 2006/95/EC.
H	2/2014	*Preface*: Updated contact information to include global info. *Chapter 1, Introduction*: Updated part number of power supply and updated incubation range in Product Description. *Chapter 2, Installation*: Updated power supply section to reflect current power supply. *Chapter 3, Getting Started*: Updated photo of Synergy 2, added note to Recommendations about using partial plates during incubation.
I	10/2014	*General*: Updated references to USB drivers to state that they now reside on the Gen5 software media. Removed statements that the serial numbers must match between the reader and dispense module. Changed 'RMA' to 'service authorization number'. *Chapter 2, Installation*: Added a new step 11 to verify/set dispenser calibration values. *Chapter 5, Instrument Qualification*: Under 'Harta Plate Test', updated instructions for checking the test plate's battery and updated the attomole conversion and battery check formulas. Under 'Glowell Test', revised the radiant flux correction formula.
J	4/2015	*Preface*, Contact Information: To reduce the risk of providing outdated contact information for BioTek's offices worldwide, replaced the former detailed information for every location with a simpler instruction to visit www.biotek.com for the most up-to-date information. CE Mark: Updated Directive headings. Notices: Remove trademark information for Glowell. *Chapter 3, Getting Started*: To 'Recommendations for Optimum Performance' added information on the use of acids, corrosives, and solvents. *Chapter 5, Instrument Qualification*, Luminescence Test: Removed the Glowell Test procedure. In the Gen5 [Harta] protocol parameters table, changed the Dynamic Range for the Battery Check read step to Extended.
K	10/2015	*General*: Added information for purchasing and using the BioTek 340 nm Absorbance Test Plate (BTI #7260551). *Preface*, CE Mark section: Updated Directive headings. Created a new Chapter 7, *Instrument Qualification Process* to describe the tests designed to qualify the Synergy 2. Added Gen5 protocol parameters tables for Absorbance Testing. Renamed the former Chapter 7, Instrument Qualification as Chapter 8, *Instrument Qualification Procedures* and moved the description content to the aforementioned new chapter.

Document Conventions

	This icon identifies information that protects the **safety** of the operator and the integrity of data.
Warning!	A **Warning** indicates the potential for bodily harm and tells you how to avoid the problem.
Caution	A **Caution** indicates the potential for damage to the instrument and tells you how to avoid the problem.

Note:	**Bold text** is primarily used for emphasis.
	This icon calls attention to **important** information.

This style calls attention to usage instructions and helpful facts. For example, "Refer to *Figure 2-3* when performing these steps" and "Part numbers are subject to change."

Topics that apply only to specific reader models are presented in this style. For example, "Applies only to models equipped with injectors."

Intended Use Statement

- The Synergy 2 is a single-channel 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.
- If the instrument has an “IVD” label it may be used for clinical and nonclinical purposes, including research and development. If there is no such label the instrument may be used only for research and development or other nonclinical purposes.

Quality Control

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

Warranty and Product Registration

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. Register online through the Customer Resource Center at www.biotek.com or call (888) 451-5171 or (802) 655-4740.

Repackaging and Shipping

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

Warnings

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

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

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

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these vary depending on the instrument type. 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 plug adapter to connect primary power to the external power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.

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

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

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

Warning! The instrument with all available modules weighs up to 57 pounds (25.8 kg). Use two people when lifting and carrying the instrument.

	Warning! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, abort the program and turn the instrument off. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid. **Warning! Unspecified Use.** Failure to operate the equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition. **Warning! Software Quality Control.** The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading or dispensing methods. **Failure to conduct quality control checks could result in erroneous test data.** **Warning! Reader Data Reduction Protocol.** No limits are applied to the raw measurement data. All information exported via computer control must be thoroughly analyzed by the operator.
	**Warning! Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument or removing its top case.
	**Warning! Hot Surface.** The 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.
	**Warning! Potential Biohazards.** Some assays or specimens may pose a biohazard. This hazard is noted by the symbol shown here. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemically resistant rubber gloves and apron.
	**Warning! Pinch Hazard.** Some areas of the dispense module can present pinch hazards when the instrument is operating. The module is marked with the symbol shown here. Keep hands and fingers clear of these areas when the instrument is operating.

Precautions

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

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

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

Caution: Environmental Conditions. Do not expose the instrument to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in the **Specifications** chapter. 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, and operate it within the range of line voltages listed on it. The power supply was tested for use with the BioTek Synergy 2; do not use it with any other product. Using this power supply with another product may create a hazardous condition.

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

Caution: Warranty. Failure to follow preventive maintenance procedures may **void the warranty**.

Caution: Shipping Hardware. All shipping hardware (e.g., shipping panel, carrier shipping screw, etc.) must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

Caution: Electromagnetic Environment. Per EN 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

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

See the Declaration of Conformity for more specific 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: 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 take measures 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 2006/95/EC Low Voltage (Safety)

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

EN 61010-1, "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."

EN 61010-2-081, "Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."

EN 61010-2-010, "Particular requirements for laboratory equipment for the heating of materials."

Directive 2012/19/EU: Waste Electrical and Electronic Equipment

Disposal Notice: Dispose of the instrument according to the Directive, "on waste electrical and electronic equipment (WEEE)" or local ordinances.

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

Product registration with competent authorities.

Traceability to the U.S. National Institute of Standards and Technology (NIST).

EN 61010-2-101, "Particular requirements for in vitro diagnostic (IVD) medical equipment."

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

RADIO AND TELEVISION INTERFERENCE

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

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

Canadian Department of Communications Class A

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

Le present appareil numerique n'émet pas de bruits radioélectriques depassant les limites applicables aux appareils numérique de la Class A prescrites dans le Réglement sur le brouillage radioélectrique édicté par le ministère des Communications du Canada.

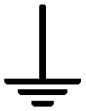
User Safety

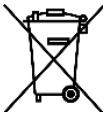
This device has been type-tested by an independent laboratory and found to meet the requirements of the following:

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

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 sejeción 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
	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
	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
	Consult instructions for use Consulter la notice d’emploi Gebrauchsanweisung beachten Consultar las instrucciones de uso Consultare le istruzioni per uso
	Laser radiation: Do not stare into beam Rayonnement laser: Ne pas regarder dans le faisceau Laserstrahlung: Nicht in den strahl blicken Radiación de láser: No mire fijamente al rayo Radiazione di laser: Non stare nel fascio

Chapter 1

Introduction

This chapter introduces the Synergy 2 Multi-Mode Reader, describes its key features, lists its package contents, and provides contact information for technical assistance.

Product Description

The Synergy 2 is a single-channel microplate reader for research and development and in vitro diagnostic use. Depending on the model, detection modes include Fluorescence Intensity (FI), Fluorescence Polarization (FP), Time-Resolved Fluorescence (TRF), Luminescence, and UV-visible Absorbance.

Synergy 2 uses a unique combination of monochromator, filters, and dichroic mirrors. Its three broad-spectrum light sources have been chosen for optimal illumination and excitation in all applications. The reader is computer-controlled using BioTek's Gen5 PC software for all operations including data reduction and analysis. The Synergy 2 is robot accessible and compatible with BioTek's BioStack Microplate Stacker. Gen5 supports OLE automation to facilitate integration into an automated system.

Two light sources are available for Fluorescence determinations: a tungsten halogen lamp (part of the FI and FP modules) or a xenon flash (part of the TRF module) along with interference filters and dichroic mirrors for wavelength specificity, and a photomultiplier tube (PMT) detector. The Synergy 2 has both top and bottom probes for fluorescence measurements. Bottom probe readings do not use the mirrors. Models with Fluorescence Polarization (FP) capability are equipped with polarizing filters.

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 by switching to a xenon flash lamp and a monochromator for wavelength selection. The xenon lamp allows for both UV and visible light absorbance measurements. The monochromator provides wavelength selection from 200 to 999 nm in 1 nm increments. Area and spectral scanning, and Pathlength Correction are available read methods.

The Synergy 2 has a 4-Zone™ temperature control from 4°C (39°F) over ambient to 65°C (149°F). Internal plate shaking is supported to ensure that reagents are thoroughly mixed prior to reading.

The Synergy 2 supports the reading of 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with standard 128 x 86 mm geometry, as well as the BioTek Take3 Micro-Volume Plate.

The Luminescence system does not support the reading of 1536-well plates.

ⓘ 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-, and 96-well microplates. An external dispense module pumps fluid from the supply bottles to the two injectors located inside the instrument. Both injectors are positioned directly above the bottom probe, and fluid is injected into one well at a time.

Package Contents & Accessories

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

Item	BTI Part #
Synergy 2 Operator's Manual (on USB flash drive)	7131000
Power supply	01317
Power cord set (specific to installation environment):	
Europe (Schuko)	75010
USA/International	75011
United Kingdom	75012
Australia/New Zealand	75013
USB cable	75108
RS-232 serial cable	75034
Wrench	48576
Filter "plugs" (2) (also referred to as "dummy filters" or "blanks")	7082073
Spare filter retaining clips (2)	7082075
Storage bag and fastener strips	--
Models with injectors ("D" models), an external dispense module with the following accessories:	
Outlet tubes (2, plus 2 spare) from dispense module to reader	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	7092133
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 storage bag	2872304

Optional Accessories

Availability and part numbers are subject to change. Please contact BioTek Customer Care with any questions, or visit our website and use the Accessories search tool.

Item	BTI Part #
Absorbance Test Plate (400-800 nm)	7260522
Absorbance Test Plate (340 nm)*	7260551
Luminometer Reference Microplate (includes adapter BTI #8042028 for Synergy 2)	8030015
Take3 Micro-Volume Plate	TAKE3
PCR Tube Adapter Plates	6002072, 6002076
Terasaki Adapter Plate	7330531
UV-Range (300 nm and above) excitation polarizer	7132041
3-foot (1-meter) external dispense module tubing	7112186
Empty filter wheel	7080541
Filter retainer clip	7082075
Filter wheel plug (dummy, or blank, filter)	7082073
Empty mirror holder	7130564
Empty mirror holder, FP-compatible	7130563
Handle for mirror holder Securing screws (order 2) Optional label	8032006 19189 8031007
Replacement tungsten lamp	7080500
Replacement shipping materials	7130016
BioCell Adapter Plate	7270512
BioCell Quartz Vessel	7272051
Synergy 2 Qualification and Maintenance (IQ/OQ/PQ) package	7130012
Additional filters, filter wheels, mirrors, and mirror holders; contact BioTek Customer Care for availability and part numbers	
The Synergy 2 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.	

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

Materials for Conducting Liquid Tests

Manufacturer part numbers are subject to change.

Item	Part Number
Absorbance Liquid Tests	
BioTek Wetting Agent Solution	BTI #7773002
BioTek QC Check Solution #1 (25 mL)	BTI #7120779
BioTek QC Check Solution #1 (125 mL)	BTI #7120782
Phosphate-Buffered Saline (PBS) tablets, pH 7.2-7.6	Sigma #P4417
β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, reduced form)	BTI #98233 or Sigma #N6785-10VL
Fluorescence Liquid Tests	
Test Kits	
Kit with microplates and test solutions for conducting Corners/Sensitivity/Linearity (FI) tests using Sodium Fluorescein and Methylumbelliferone, and Time-Resolved Fluorescence (TRF) tests using Europium	BTI #7160010 (contains 7160013, 7160012, and 7160011 described below)
Kit for FI tests using Sodium Fluorescein	BTI #7160013
Kit for FI tests using Methylumbelliferone	BTI #7160012
Kit for TRF tests using Europium	BTI #7160011
Kit for Fluorescence Polarization (FP) test	BTI #7160014 or Invitrogen #P3088
Individual Materials	
Sodium Fluorescein Powder, 1-mg vial	BTI #98155
Methylumbelliferone, 10-mg vial	BTI #98156
Carbonate-Bicarbonate Buffer (CBB) capsules	Sigma #3041
Phosphate-Buffered Saline (PBS) tablets, pH 7.2-7.6	Sigma #P4417
Sodium Borate, pH 9.18	Fisher Scientific #159532, or equivalent
Injection System Tests	
BioTek Green Test Dye	BTI #7773003

Product Support & Service

See also *Contact Information* on page x.

Technical Assistance Center (TAC)

If your BioTek product fails 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 repair or other service, 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.

TAC@biotek.com

Phone: (800) 242-4685 or (802) 655-4740

Fax: (802) 654-0638

Please be ready with the following information:

- Your name and company, email address, daytime phone or fax number
- The product name, model, and serial number
- The onboard software part number and basecode version (available through Gen5 by selecting System > Instrument Control > Information)
- Gen5 software version information (Help > About Gen5)
- For troubleshooting assistance or instruments needing repair, the specific steps that led to the problem and any error codes that were reported (see also **Error Codes** starting on page 163)

If you need to send an instrument to BioTek, please contact the TAC for a Service Call Notice (SCN) number and the shipping address. Package the instrument according to the instructions in *Repackaging and Shipping Instructions* starting on page 25.

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.

Applications@biotek.com or (888) 451-5171

Chapter 2

Installation

This chapter includes instructions for unpacking and setting up the Synergy 2 and, if applicable, the external dispense module. Instructions are also included for preparing the reader and dispense module for shipment.

Product Registration

Please register your products with BioTek to ensure that you receive important information and updates about the products you have purchased.

Register online through BioTek's Customer Resource Center (CRC) at www.biotek.com or by contacting BioTek Customer Care.

Important Information

This chapter contains installation and setup tasks for a Synergy 2 model equipped with all of the available modules. Your model may be different; for example, it may not have injection capability. Perform the 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 chapter. You will also need a small wrench; this item is supplied with the instrument.

Remove the shipping hardware before turning on the instrument.

Reinstall the shipping hardware before repackaging the instrument for shipment.

1: Unpack and Inspect the Synergy 2

	The Synergy 2 with all available modules weighs up to 57 pounds (25.8 kg). Use two people when lifting and carrying the instrument. 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 the warranty**. Improper packaging the results in damage to the reader may lead to additional charges. 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.

1. Set the outer shipping box close to the intended work surface. Open the outer shipping box and remove the foam blocks to access the inner shipping box.
2. Open the inner shipping box. Remove the accessories that are stored inside the cardboard shipping insert and then remove the insert.
3. The reader is attached to a shipping panel, which has two handles for lifting. With one person on each side, locate and grasp the handles. 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. See *Figure 2-1* on page 10.
4. Using a 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 in the shipping boxes for reuse if the reader needs to be shipped again.

Figure 2-1: The reader on its back with the shipping panel attached to the bottom (left), and removing the screws (right)

⚠	Reattach the shipping panel before repackaging the reader for shipment.

2: Select an Appropriate Location

Install the reader on a level, stable 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 2, you may wish to seat the instruments in their aligning plates now. Refer to the *BioStack Operator's Manual* for more information.

3: Remove the Shipping Hardware

Remove all shipping hardware before turning on the reader.

1. Locate the two screws that secure the shroud, one on each side of the reader, in the lower rear corners. Remove the screws and slide off the shroud.

Figure 2-2: Locating and removing the two side screws

Figure 2-3: Sliding off the shroud

2. Identify the areas inside the instrument where the shipping hardware is currently installed, and where some items will be stored.

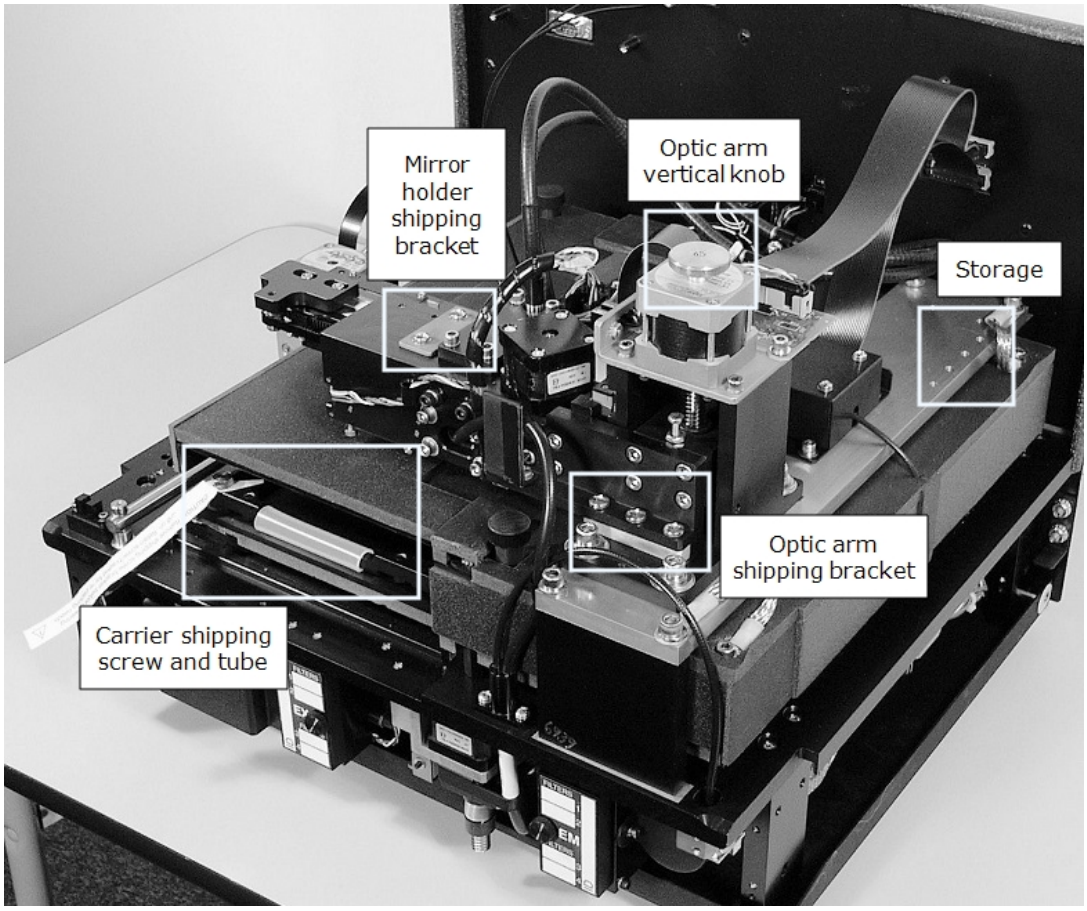


Figure 2-4: Internal compartment, with shipping hardware

3. Use the supplied wrench to remove the carrier shipping screw. Store the screw on the base plate as shown in *Figure 2-7*. Pull the shipping tube off the carrier and place it in the storage pocket. Attach the pocket to the back of the reader.

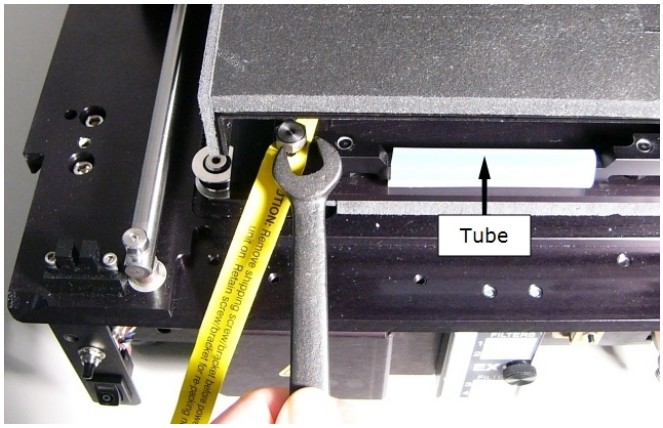


Figure 2-5: Removing the carrier shipping screw and tube

4. 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.

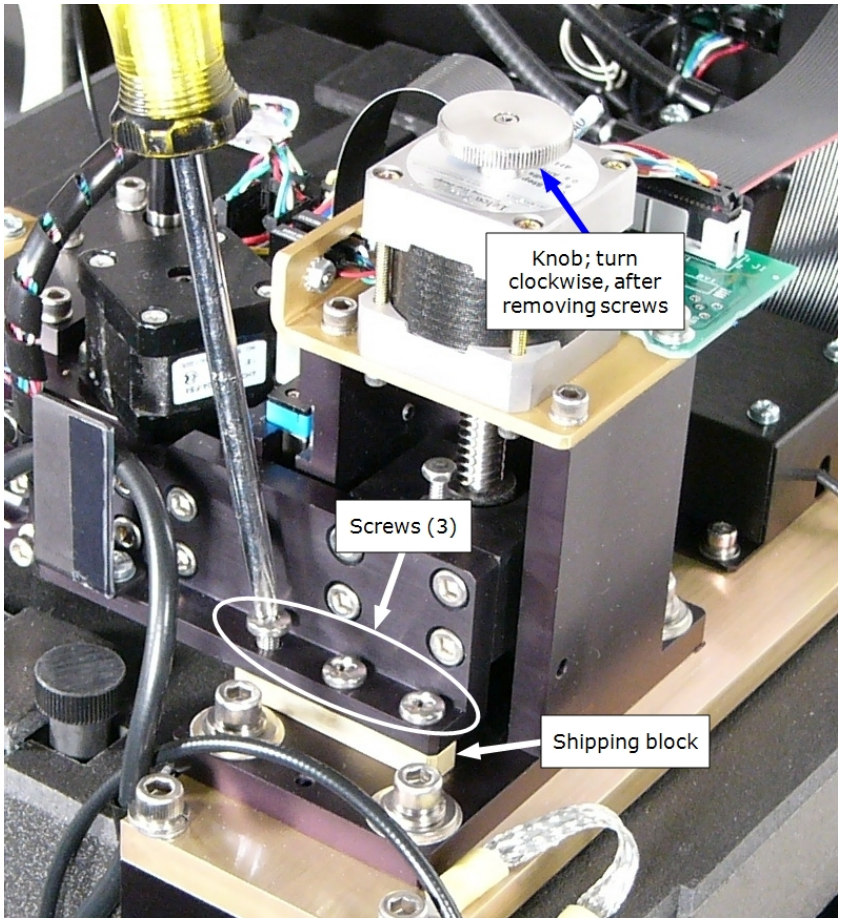


Figure 2-6: Removing the optic arm shipping block

5. Store the optic arm shipping block and three screws/washers on the base plate.

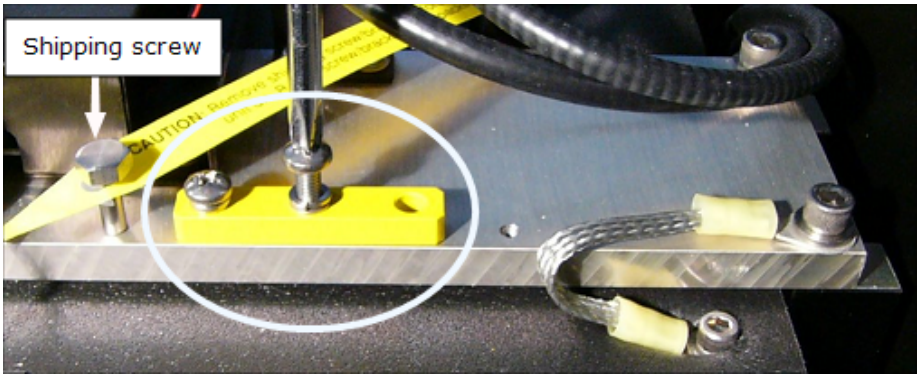


Figure 2-7: Carrier shipping screw and optic arm shipping block stored on the base plate

6. Remove the two screws that secure the mirror holder shipping bracket.

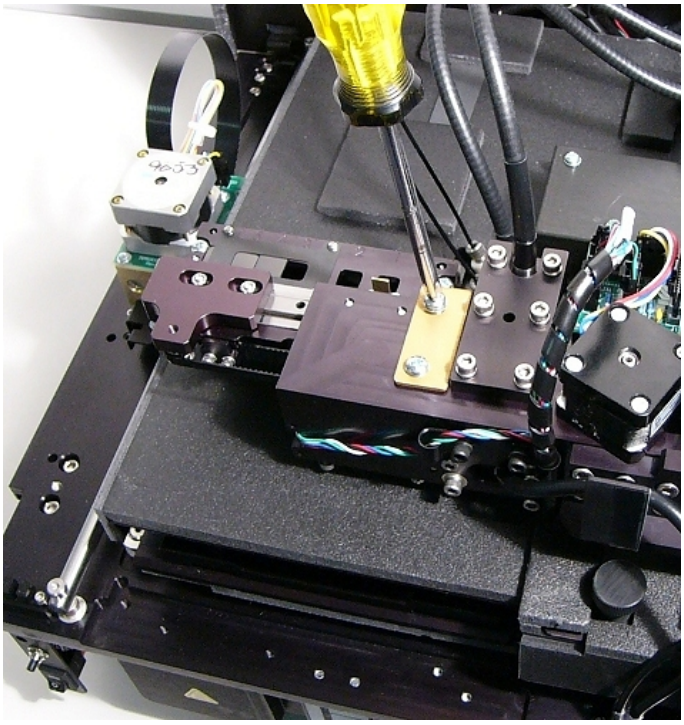


Figure 2-8: Removing the mirror holder shipping bracket

7. Turn the bracket over and replace the screws.

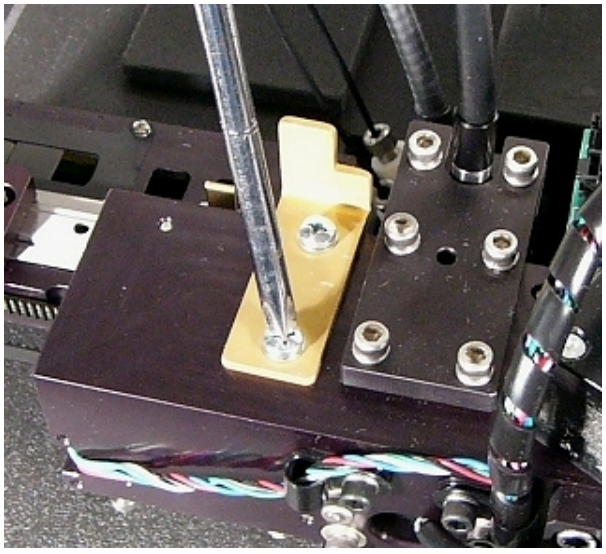


Figure 2-9: Mirror holder shipping bracket, in its storage position

8. If you plan to access the mirror holder through the reader's side door, remove two additional shipping screws.

Figure 2-10: Mirror holder shipping screws

ⓘ	Replace these screws before shipping the reader. We suggest taping them to the back of the instrument to store them.

9. Slide the shroud back onto the reader and replace the two side screws.

4: Install the Power Supply

⚠	**Power Rating.** The instrument must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards. **Electrical Grounding.** Never use a plug adapter to connect primary power to the instrument. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to an appropriate receptacle with a functional ground.

1. Plug the power supply's cord into the power inlet on the rear of the reader.
2. Connect the power cord to the power supply.
3. Plug the power cord into an appropriate power receptacle.

5: Unpack and Inspect the Dispense Module

Applies only to models equipped with injectors

	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.

Refer to Figure 2-15 and Figure 2-16 starting on page 28.

1. Open the outer shipping box. Remove the foam cap, inner shipping box, and accessories box.
2. Using no sharp tools, open the box containing the dispense module. Remove the two reagent bottle holders and the cardboard shipping insert. Lift out the module and place it on a level surface.
3. Open the accessories box and remove its contents. Refer to *Package Contents & Accessories* on page 3 for the expected items.
4. Place all packaging materials into the shipping box for reuse if the dispense module needs to be shipped again.

6: Install the Dispense Module

Applies only to models equipped with injectors

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

ⓘ Do not place the dispense module *next to* the reader.

Figure 2-11: 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. 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 shrouds in the plastic tool storage bag. Use the supplied fastener strips to attach the bag to the rear panel of the dispense module.
5. Remove the two inlet tubes from their canisters.
6. Identify the two syringe valves on the dispense module (see below). Each is labeled with a left-pointing arrow.

ⓘ When installing the 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 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.

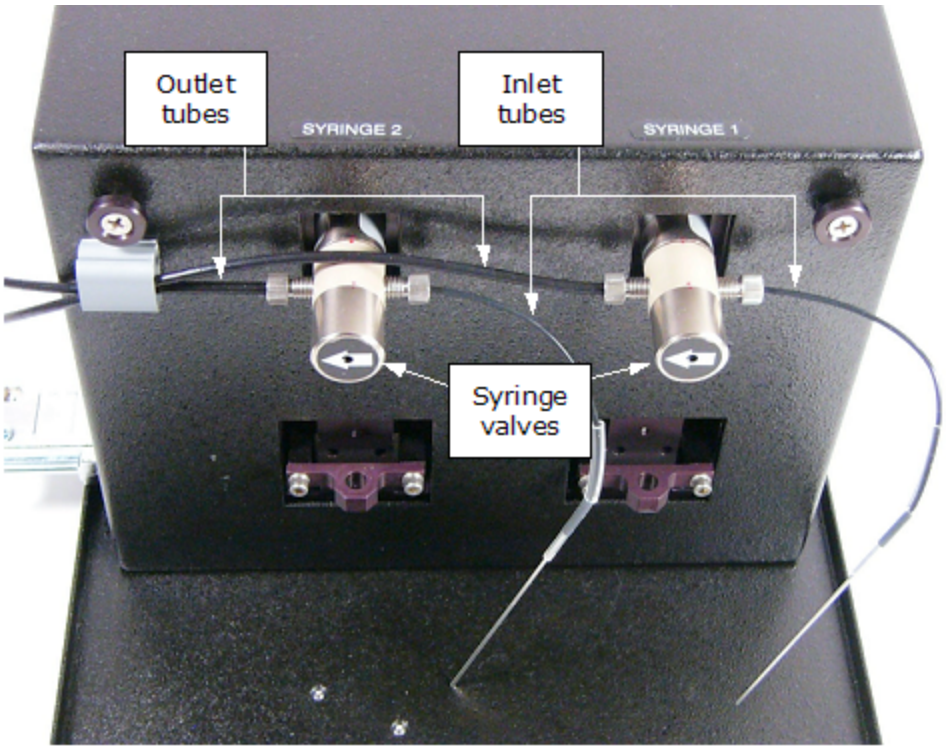


Figure 2-12: Dispense module components

It is critical that the outlet tubes are correctly connected between the syringe valves and the ports on the instrument's rear panel. **Otherwise, injected fluid may miss the intended well.**

12. Remove the two syringes from their boxes. They are identical and interchangeable. Each 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 the syringes:
    - Hold the syringe vertically with the threaded end at the top.
    - Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
    - Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.

- Pass a thumbscrew up through this hole and thread it into the bottom of the syringe. Hold the syringe to prevent it from rotating while tightening the thumbscrew. Finger-tighten only.

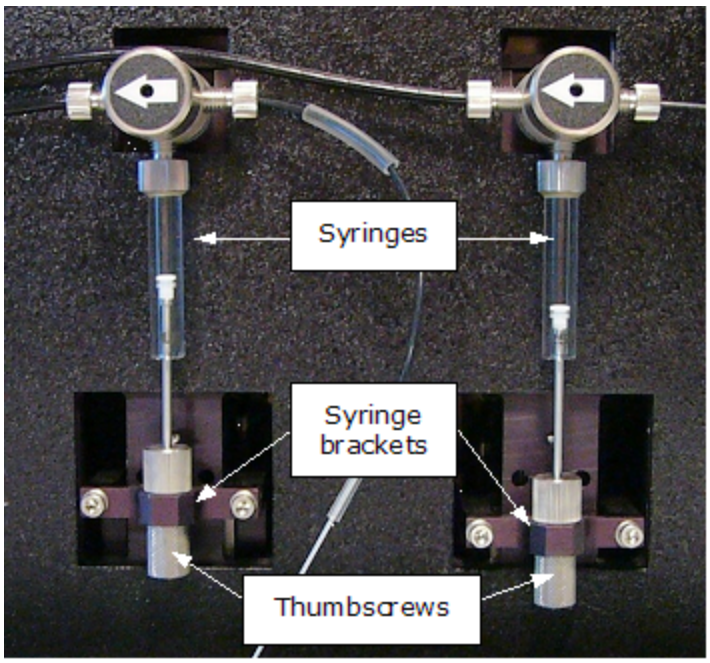


Figure 2-13: Dispense module, close-up view of syringes

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 rear 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 2 is equipped with two communication ports, "USB" and "RS232" (serial), located on the back of the reader. Connect one end of the supplied communication cable to the appropriate port on the reader, and the other end to an appropriate port on the host computer.

8: Install Gen5 Software

	The Synergy 2 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 the *Gen5 Getting Started Guide* to install the software.

9: Turn on the Reader

1. If Gen5 is open, close it now.
2. The power switch is located on the lower-left corner of the front panel; turn on the Synergy 2. The reader performs a System Test. When the test is completed, the reader extends the microplate carrier.

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

10: Establish Communication

If using the USB cable, refer to the instructions that shipped with the USB drivers on the Gen5 software media to install the necessary drivers.

1. Start Gen5 and log in if prompted.
2. From the main screen select **System > Instrument Configuration**.
3. Click **Add Reader** and select **Synergy 2**. Click **OK**.
4. Set the **Com Port** to the computer's COM port to which the reader is connected. (If using the USB cable, the information can be found via the Windows Control Panel, under Ports in the Hardware/Device Manager area of System Properties.)

5. Click **Test Comm**. Gen5 attempts to communicate with the reader. If the communication attempt is successful, return to Gen5's main screen.

   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?
   - Try a different Com port.
   - Did you install the USB driver software?
   - If you remain unable to get Gen5 and the reader to communicate with each other, contact BioTek's Technical Assistance Center.

11: Verify/Set Dispenser Calibration Values

Applies only to models equipped with injectors

Confirm that the reader is configured with calibration values for the dispense module.

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

1. If you have not already done so, turn on the instrument and establish communication with Gen5.
2. In Gen5, go to **System > Instrument Configuration**, select the **Synergy 2**, and click **View/Modify**.
3. Click **Setup** and select the **Dispenser 1** tab.
4. Click **Get Volumes**.
5. Compare the Calibration Volumes in the dialog with the Syringe #1 values on the rear panel of the dispense module.

   If the values match, skip to step 6.

   If there is a mismatch:

   - Press CTRL+SHIFT+M to enter maintenance mode for the Dispenser 1 window.
   - Enter the syringe calibration values from the corresponding label on the rear of the dispense module.
   - Click **Send Volumes** and then **Get Volumes** to verify that the entered values were sent to the reader.
6. Select the **Dispenser 2** tab and repeat steps 4–5 for Dispenser 2.

12: Run a System Test

Running a System Test will confirm that the reader is set up and operating properly, or will provide an error code if a problem is detected.

If applicable, adjust Gen5's Absorbance Wavelengths table to values that will confirm operation of the reader at its limits. We recommend 200 and 999 nm (the lower and upper limits of the monochromator), and four wavelengths in between that best represent your assays and/or the lowest and highest values typically used in your lab.

1. Turn on the incubator:
   - In Gen5, select **System > Instrument Control > Synergy 2**.
   - Click the **Pre-Heating** tab. Enter a Requested temperature of at least 37°C and then click **On**.

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

2. Select **System > Diagnostics > Run System Test**. Select your reader if prompted and click **OK**.
3. When the test is complete, a dialog requesting additional information appears. Enter the information and click **OK**.

   If a message appears stating that the reader has a *pending* system test report, view the report and then repeat steps 2 and 3.

4. The results report appears and should contain the text "SYSTEM TEST PASS".
   - If required, print the report and store it with your installation records. Note: Gen5 stores results in its database; you can print a report at any time.

   If an error code is returned, refer to **Error Codes** starting on page 163. 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.

Models with injectors: Keep Gen5 open and proceed to the next section.

All other models: The installation and setup process is complete. Close Gen5 and proceed to *Operational/Performance Qualification* on page 24.

13: Test the Injection System

Applies only to models equipped with injectors

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

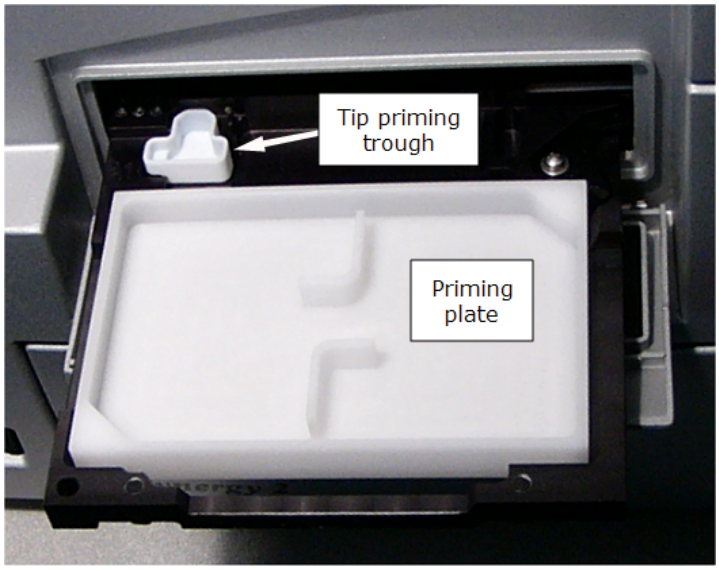


Figure 2-14: Priming trough and plate installed on the carrier

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 > Instrument Control > Synergy 2** and click the **Prime** tab.
6. 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 and pumping it through the tubing and 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.
7. When finished, set the Volume to 2000 µL and click **Purge** to clear the fluid lines.
8. Set the Dispenser to 2 and repeat steps 6 and 7.
9. Remove and empty the priming plate.

Operational/Performance Qualification

Your Synergy 2 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 **Instrument Qualification Procedures** starting on page 123 to learn about BioTek's recommended OQ/PQ procedures for the Synergy 2.

A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Synergy 2 is available for purchase (BTI #7130566). Contact your local BioTek dealer for more information.

Repackaging and Shipping Instructions

If the equipment 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 page 88 for decontamination instructions for the reader and dispense module.

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 Synergy 2 with all available modules weighs up to 57 pounds (25.8 kg). Use two people when lifting and carrying the instrument.

The instrument's packaging design is subject to change. If the instructions in this section do not apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center.

Replace the shipping hardware before repackaging the reader. Please contact BioTek and order part number 7130016 if you need a carrier shipping tube, carrier shipping screw/o-ring, optic arm shipping block/screws, or mirror holder shipping bracket/screws.

When preparing to ship the Synergy 2 and/or the dispense module to BioTek, 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 a Service Call Notice (SCN) number and the shipping address before returning equipment for service.
2. Decontaminate the reader and, if attached, the dispense module, according to the instructions provided under *Decontamination* starting on page 88.
3. If you will also be shipping the dispense module, perform the steps described on page 26.

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

4. If applicable, remove the tip priming trough from the microplate carrier.
5. Retract the microplate carrier. Turn off and unplug the reader.

6. Remove the shroud and replace the shipping hardware (see the photos in *Remove the Shipping Hardware* starting on page 11). Replace the shroud when finished.
7. Carefully tip the reader onto its back. Attach the shipping panel to the bottom of the reader using the four mounting screws and washers (see the photos in *Unpack and Inspect the Synergy 2* starting on page 9).
8. Locate the original outer shipping box. Place four foam blocks in the four bottom corners of the box. Place the inner shipping box inside the outer box.
9. Using two people, grasp the handles on the shipping panel and carefully lower the reader into the inner shipping box.
10. Slide the cardboard insert straight down into place around the reader. Place the accessories inside the pockets in the insert.
11. Close and seal the inner box with tape.
12. Place four foam corner blocks around the inner shipping box. Close and seal the outer box with tape.
13. Write the SCN number on the outside of the box. Ship the box to BioTek.

Prepare the Dispense Module for Shipment

Refer to the illustrations on the next two pages when performing these steps.

1. If you have not already done so, contact BioTek's Technical Assistance Center for a Service Call Notice (SCN) number and the shipping address before returning equipment for service.
2. Decontaminate the module according to the instructions starting on page 88. Be sure to purge the dispense module of all fluid when finished.
3. With the reader on, start Gen5 and select **System > Instrument Control > Synergy 2**.
4. Perform this step twice, once per dispenser: Click the **Prime** tab (or **Dispenser** tab, if using Gen5 v2.05 or lower) and set the number (1 or 2). Click **Maintenance**. The syringe bracket lowers. Remove the thumbscrew from underneath the bracket. Carefully unscrew the top of the syringe from the syringe valve. Lift out the syringe and store it in its original box.
5. Fully detach the dispense module from the reader. Replace the two nylon screws into the Syringe 1 and 2 tubing ports on the rear of the reader.
6. Remove the two inlet tubes from the syringe valves and store them in their plastic canisters.
7. Remove the two outlet tubes from the syringe valves. Attach the clear plastic fitting shrouds to the fittings of the outlet tubes. Place the tubes in a plastic bag.
8. 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.

9. Locate the original accessories shipping box and foam end caps. Place the bottom foam end cap into the box.
10. Place the syringes, the inlet tubes, and the outlet tubes inside the cutouts of the bottom foam end cap in the accessories box. Place the dispense module shroud on top of the accessories.
11. 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.
12. 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.
13. Insert the accessories box alongside the dispense module box.
14. Insert the top foam end cap. Close and seal the outer box with tape.
15. Write the SCN number on the outside of the box. Ship the box to BioTek.

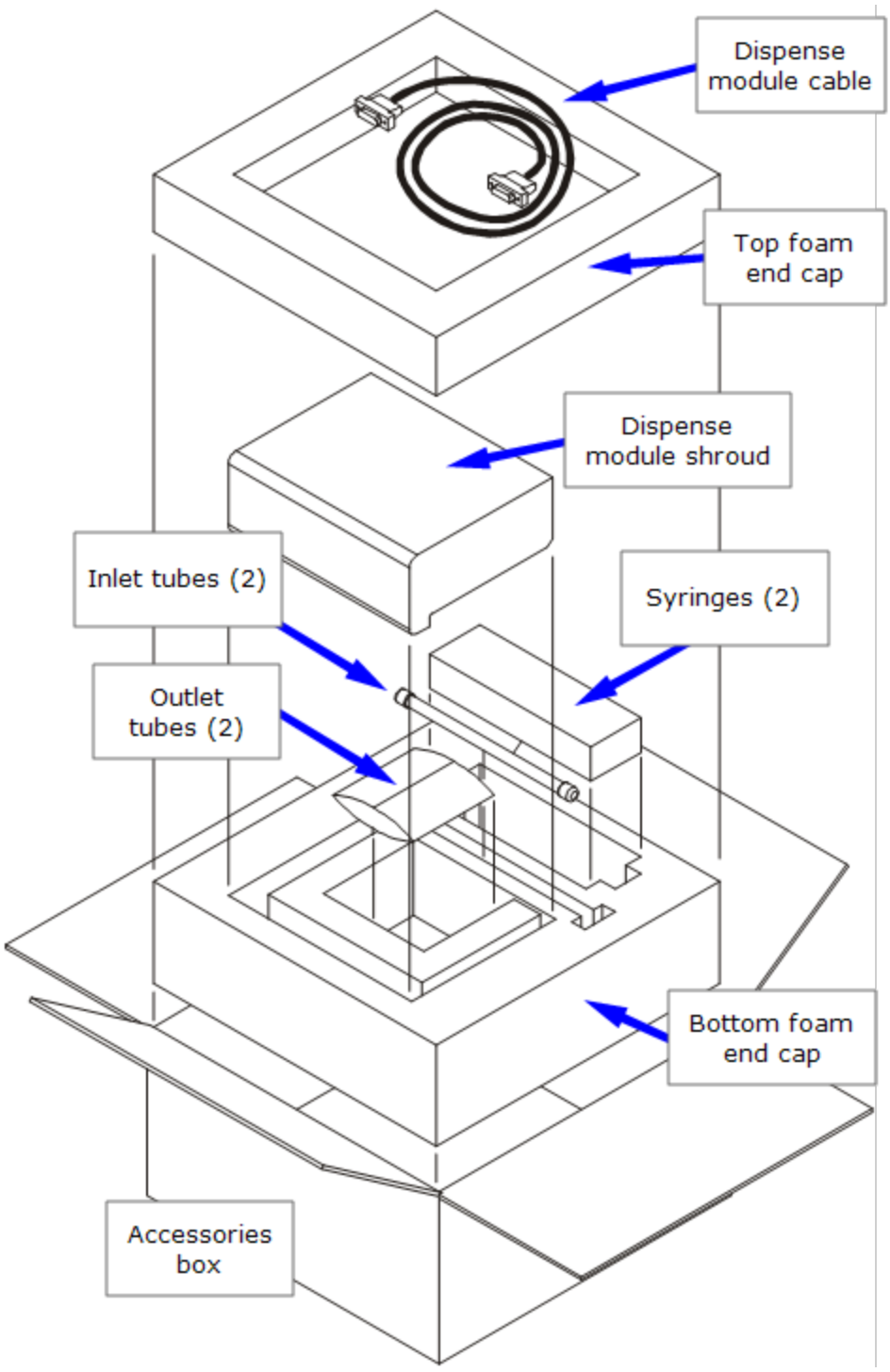


Figure 2-15: Packing the dispense module accessories

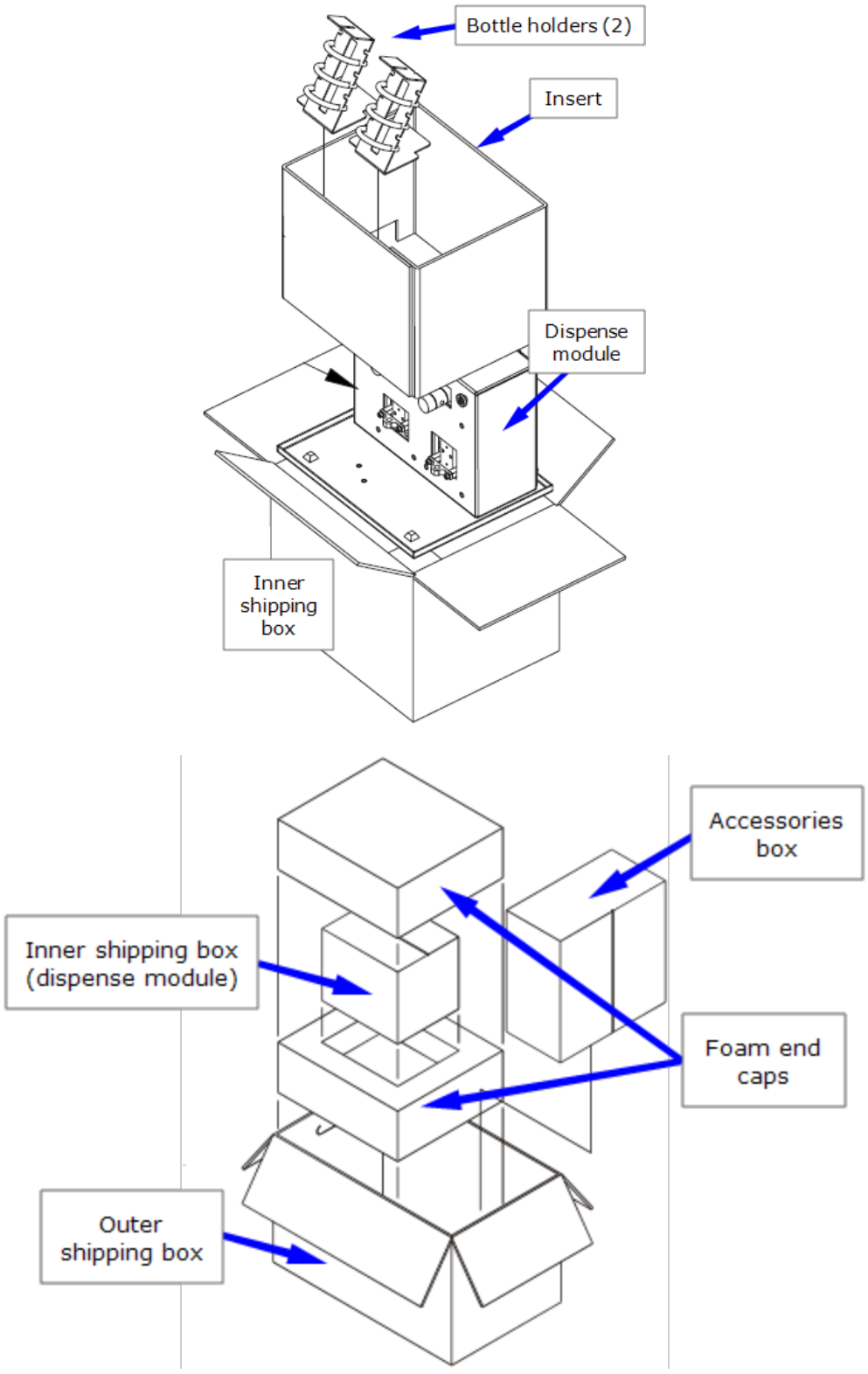


Figure 2-16: The inner (top) and outer (bottom) shipping boxes

Chapter 3

Getting Started

This chapter describes some of the Synergy 2's external and internal components, and provides an introduction to using Gen5 software to control the instrument and, if equipped, dispense module.

Modular Design

The Synergy 2 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 injectors, Gen5 will not provide the option to add a Dispense step to your assay protocol.

The table below briefly describes the available modules. The module letters form the part number for each Synergy 2 model; for example, a reader with all modules installed is an SLFPTAD.

Module	Description
S	Synergy 2 base model. Includes incubation control, shaking, and Gen5 software.
L	Luminescence
F	Fluorescence Intensity, top and bottom. Includes the Tungsten-Halogen light source.
P	Fluorescence Polarization. Requires the "F" module.
T	Time-Resolved Fluorescence. Requires "F" module. Includes the high energy DPR Xenon Flash Lamp.
A	UV-Visible Absorbance (monochromator-based). Includes the SQ Xenon flash lamp.
D	Dual-reagent dispenser

External Components

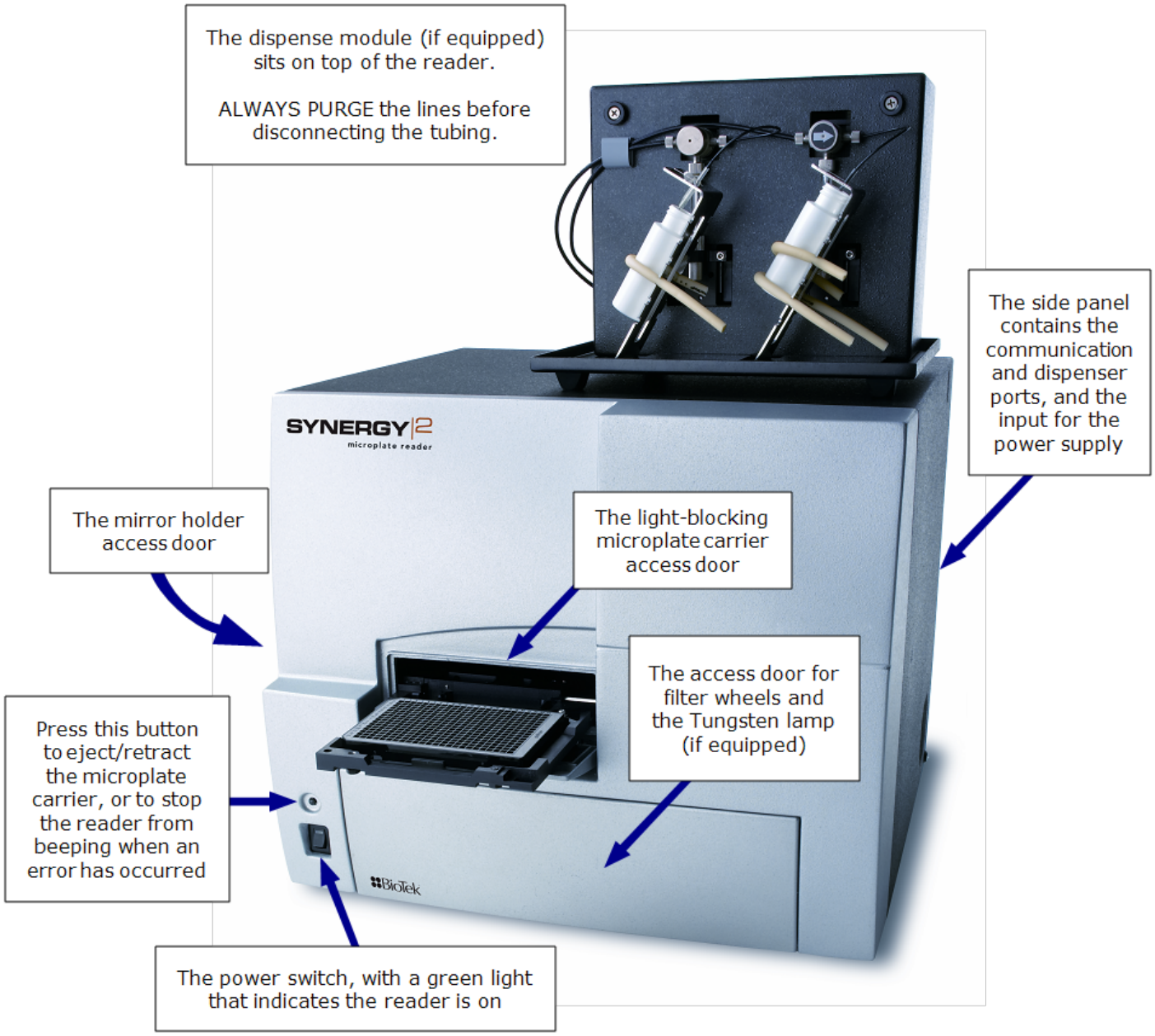


Figure 3-1: Synergy 2, front view

Internal Components

This section introduces some internal components that may require replacement and/or cleaning over time. Each topic provides references to other sections of this manual for more detailed information and instructions. As discussed on page 33, not all of these components exist in all Synergy 2 models.

Component	Comments	Page
Tungsten Lamp	Used with the Fluorescence Intensity (F) and Fluorescence Polarization (P) modules. May require replacement over time.	35
Excitation and Emission filters	The filters and filter wheels can be changed to accommodate your assays, and filters should be cleaned periodically. Used with the Fluorescence Intensity (F), Fluorescence Polarization (P), Time-Resolved Fluorescence (T), and Luminescence (L) modules.	36
Mirrors	The mirrors and mirror holder can be changed to accommodate your fluorescence assays. Mirrors may require cleaning over time. Applies to the filter-based Fluorescence Intensity (F), Time-Resolved Fluorescence (T), and Fluorescence Polarization (P) modules. The mirror holder is accessible via a door on the left side of the reader.  **Warning!** Do not put your hand in the side door during operation; doing so presents a potential pinch hazard.	37
Injection System	The syringes may require replacement over time. The tubing and internal reading chamber may require cleaning over time. Applies to models with the dual reagent dispense module.	38

Tungsten Lamp

Synergy 2 models with the Fluorescence Intensity and/or Fluorescence Polarization modules are equipped with a Tungsten lamp. The lamp is accessed through 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.

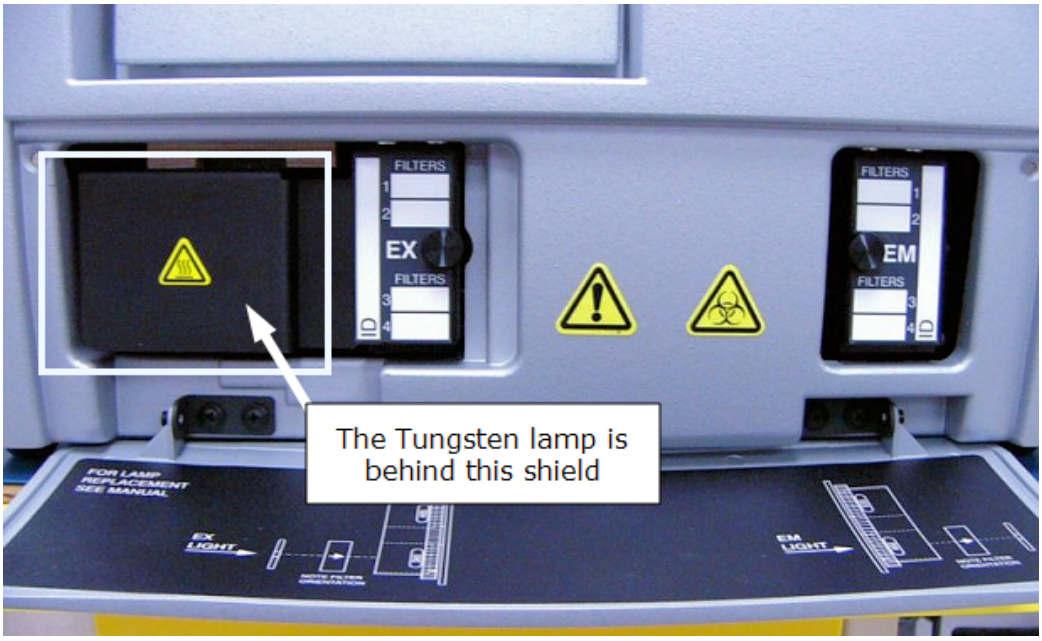


Figure 3-2: Locating the replaceable Tungsten lamp

The Tungsten lamp 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. Turn to page 93 for replacement instructions.

⚠	Keep the front door closed during operation. The intense broad spectrum light of the xenon lamp can cause eye damage. The light shield shown above mitigates the risk.

Excitation/Emission Filters

Synergy 2 models with the Fluorescence Intensity module are equipped with Excitation and Emission filter wheels. (Models with the Luminescence module without Fluorescence Intensity have an Emission filter wheel only.) The filter wheels are labeled as "EX" or "EM" and are accessed through 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.

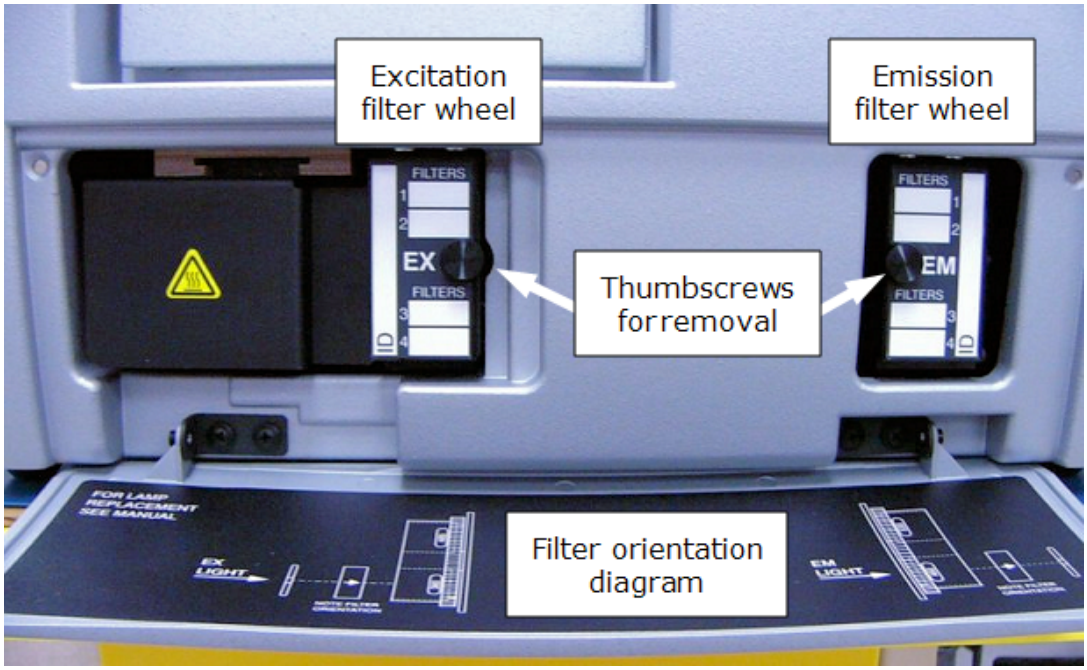


Figure 3-3: Locating the excitation and emission filter wheels

Each filter wheel contains four equal-sized positions. Each position can contain a glass filter or a light-blocking plug, or can be left empty. Filter wheel contents can be changed, as well as entire filter wheels. See **Filters and Mirrors** starting on page 47 for information on working with the filter wheels, and for a list of filters available from BioTek.

⚠	The Synergy 2 is shipped with a set of excitation and emission filters installed, and the reader's onboard software is preconfigured with the filter values and their locations. **If you change the contents of a filter wheel, you must update Gen5's filter table and then download the information to the reader.** The Synergy 2 does not automatically detect which filters are installed. See page 41 for information on updating Gen5's filter table.

EX/EM Configuration for Luminescence

For best results when taking luminescence measurements, the Excitation filter wheel (if equipped) should have no empty locations.

If your tests require that the light emitted from the samples remain unfiltered, the Emission filter wheel should have an empty location in it. When selecting a filter set for a read in Gen5, selecting "Hole" indicates the empty location.

Mirrors

When taking fluorescence (FI, FP, or TRF) measurements from the top, the Synergy 2 uses mirrors to direct the excitation and emission light paths. The mirrors are stored in a mirror holder, which is a rectangular box located inside the reader. The holder stores up to three mirrors. There are two possible mirror types:

- A 50% mirror is a glass slide with silver dots (see #1 below). It works with any wavelength in the range of 200 to 850 nm.
- A dichroic mirror is wavelength specific; it requires the excitation and emission filters to fall within specific ranges. Dichroic mirrors provide better sensitivity than 50% mirrors, but they are dye-specific.

The default mirror configuration is shown below (any changes should be reflected in your sales order). A 50% mirror is easily identified by its "dots," and each dichroic mirror is etched or labeled with its wavelength value.

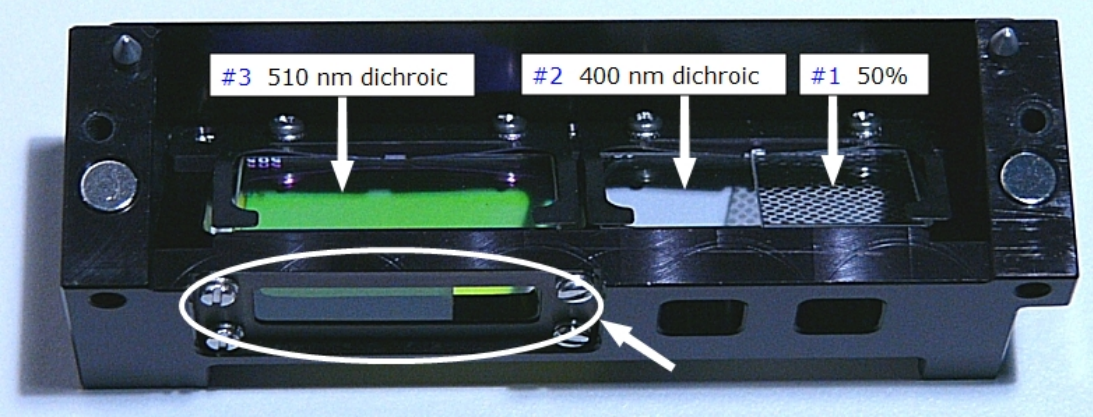


Figure 3-4: Identifying the mirrors in the mirror holder (default configuration shown). Models with the Fluorescence Polarization module also have EX polarizing filters in the mirror holder, as shown inside the oval above.

The entire mirror holder and the individual mirrors are user-changeable. Because the mirrors can be easily smudged or even damaged, however, BioTek strongly recommends changing the entire mirror holder. Contact BioTek for more information on purchasing additional mirrors and holders.

To learn more about how mirrors are used, and how to change or clean them, see **Filters and Mirrors** starting on page 47.

	Do not open the side door to access the mirror holder during instrument operation. **Doing so may result in invalid data.**
	The Synergy 2 is shipped with a mirror holder installed, and the reader's onboard software is preconfigured with the mirror values and their locations. **If you change the contents of a mirror holder, you must update Gen5's mirror table and then download the information to the reader.** The Synergy 2 does not automatically detect which mirrors are installed. See page 41 for information on updating Gen5's mirror table.

Injection System

External Dispense Module

If a syringe is leaking, it may need to be replaced. See **As-Needed Maintenance** starting on page 87 for instructions.

The dispense module pumps fluid from the supply bottles to injector heads located inside the instrument. Fluid is injected into one well at a time.

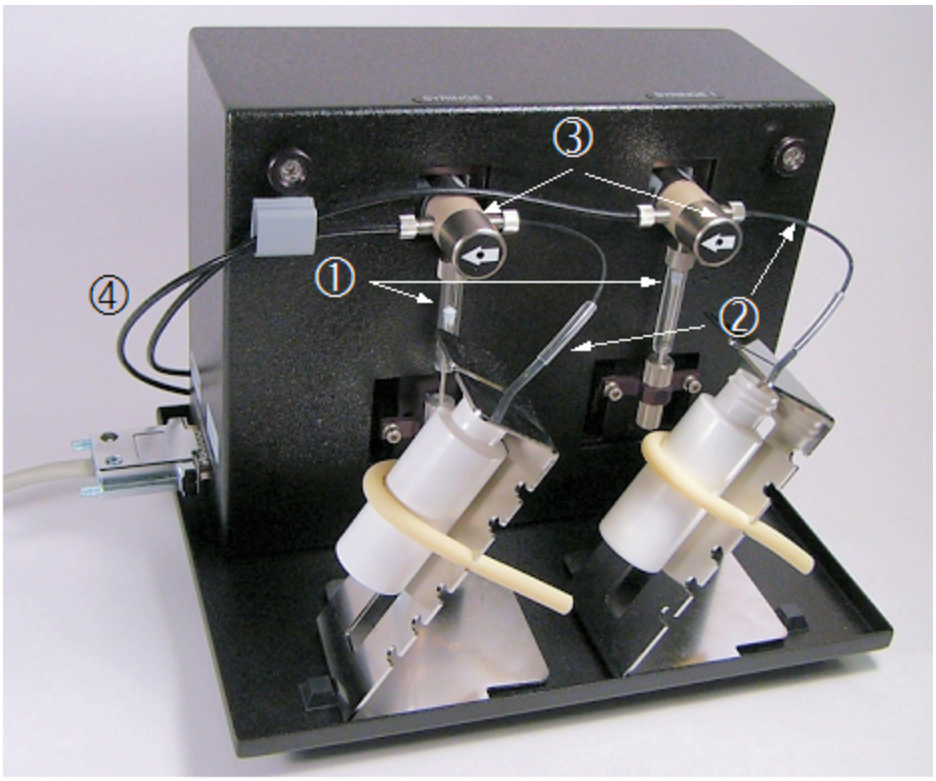


Figure 3-5: Dispense module components

1	Two 250-µL syringes draw fluid from the supply bottles.
2	Inlet tubes transport fluid from the supply bottles to the syringes. These tubes are short pieces of opaque PTFE (Teflon) tubing connected to stainless-steel probes on one end and threaded fittings on the other end.
3	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 reader's rear panel. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

⚠	Avoid continuous contact with harsh chemicals. Rinse the fluid path with deionized water after contact with any strong acid, base, or solvent. For information on the materials used in the injection system, refer to *Injection System—Chemical Compatibility Technical Note* on the USB flash drive supplied with the Synergy 2.

Internal Tubing

Inside the reader, two Teflon tubes transport fluid from the tubing ports on the rear of the instrument to the two injectors. As shown below, both injectors are positioned directly above the bottom fluorescence optical probe.

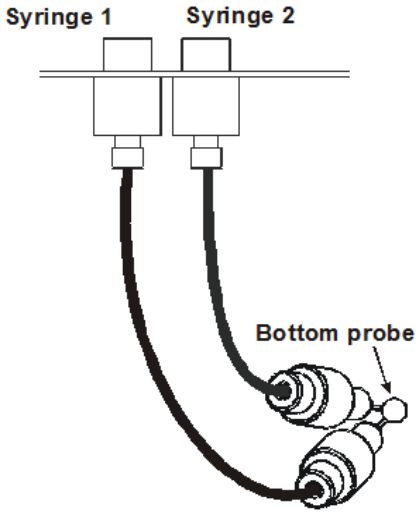


Figure 3-6: Injectors inside the reader

The tubing and injectors should be cleaned at least quarterly. See **Preventive Maintenance** starting on page 65 for more information.

Priming the Injection System

Before running a Dispense assay, prime the system with the reagent or dispensing fluid. Additionally, 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.

Both types of primes require a fluid reservoir to be present on the microplate carrier (see *Figure 2-14* on page 23):

- 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 a small, removable cup located in the left rear 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; tip priming is rarely required for these larger-volume plates.

Gen5 Software

Gen5 supports all Synergy 2 models. Use Gen5 to control the reader, the dispense module (if equipped), and the BioStack (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 Getting Started Guide* and the Help system for more information.

Define Excitation/Emission Filters in Gen5 and on the Reader

For models equipped with filter wheels, the reader's onboard software is configured with the filter values and their locations in the installed filter wheels. When Gen5 communicates with the reader, it "asks" for this information and then stores the values in a Filter Table.

Important! It is critical that the values in the Gen5 Filter Table and in the reader's software exactly match the contents of the installed filter wheels.

If you exchange or modify the filter wheels, update the Gen5 Filter Table and send the information to the reader:

1. In the Gen5 main view, select **System > Instrument Configuration**. Highlight the **Synergy 2** reader, click **View/Modify** and then click **Setup**.
2. In the Fluorescence / Luminescence tab, define/modify information for the Excitation and Emission filter wheels. For each position (1–4) in each filter wheel:
   - Select **Band Pass** to indicate a filter and then enter the Wavelength and Bandwidth (printed on the side of the filter)
   - Select **Plug** to indicate a Plug ("dummy filter")
   - Select **Hole** to indicate an empty position
3. When finished, click **Send Values** to transfer the information to the reader.

Define the Mirrors in Gen5 and on the Reader

For models equipped with a mirror holder, the reader's onboard software is configured with the mirror types, their characteristics, and their position in the installed mirror holder. When Gen5 communicates with the reader, it "asks" for this information and then stores the values in a Mirror Table.

Important! It is critical that the values in the Gen5 Mirror Table and in the reader's software exactly match the contents of the installed mirror holder.

If you exchange or modify the mirror holder, update the Gen5 Mirror Table and send the information to the reader:

1. In the Gen5 main view, select **System > Instrument Configuration**. Highlight the **Synergy 2** reader, click **View/Modify** and then click **Setup**.
2. In the Mirrors tab, define/modify information for the mirror holder. For each mirror position (1, 2, 3; see *Figure 4-3* on page 54), use the drop-down list to select the Type of mirror. For dichroic mirrors, enter the excitation and emission wavelength ranges.
3. When finished, click **Send Values** to transfer the information to the reader.

Protocols and Experiments

In Gen5, a protocol contains instructions for controlling the reader and (optionally) for analyzing 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. In the Gen5 Task Manager, select the Protocols icon and click **Create New**.
2. Open the Procedure dialog (double-click Procedure in the menu tree).
3. Select an appropriate Plate Type.
4. Add steps to the procedure to shake or heat the plate, dispense fluid, read the plate, and more.
5. Click **Validate** to verify that the attached reader supports the defined steps, and then close the Procedure dialog.
6. Optionally, perform any of these steps to analyze and report the results:
   - Open the Plate Layout dialog and assign blanks, samples, controls, and/or standards to the plate.
   - Open the Data Reduction dialog to add data reduction steps. Categories include Transformations, Well Analysis, Curve Analysis, and Qualitative Analysis.
   - Create a report or export template via the Report/Export Builders.
7. Select **File > Save** and give the protocol 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. In the Gen5 Task Manager, select the Experiments icon and click **Create using an existing protocol**.
2. Select the desired protocol and click **OK**.
3. Select a plate in the menu tree and select **Plate > Read Plate #** or click the **Read New** icon.

4. When the read is complete, measurement values appear in Gen5.
5. Select **File > Save** and give the experiment an identifying name.

Dispense Module Control

Applies only to models equipped with injectors

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

Prime

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

1. Place the priming plate on the carrier.
2. Fill the supply bottle with a sufficient volume of the fluid to be used for the prime and the assay. Insert the appropriate inlet tube into the bottle.
3. Select **System > Instrument Control > Synergy 2** and click the **Prime** tab.
4. Select the Dispenser number (1 or 2) associated with the supply bottle.
5. Enter the Volume to be used for the prime. The minimum recommended prime volume is 2000 µL.
6. Select a prime Rate, in µL/second.
7. Click **Prime** to start the process. 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 conserve reagent, Gen5 provides the option to purge fluid from the system back into the supply bottle.

1. Select **System > Instrument Control > Synergy 2** and click the **Prime** tab.
2. Select the Dispenser number (1 or 2) associated with the supply bottle.
3. Enter the desired purge Volume in µL (e.g., 2000).
4. Select a prime Rate in µL/secon.
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.
- Before preparing your microplates, make sure the reader is on and communicating with Gen5. You may want to run a System Test if the reader has not been turned off/on in a few days. Design your Gen5 protocol in advance as well, to ensure that the intended reading parameters are used and to avoid any last-minute corrections.
- Although the Synergy 2 supports standard flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with flat-bottomed wells. See **Specifications** starting on page 155 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, 25 µL in a 384-well plate, and 5 µL in a 1536-well plate (if supported).
- Pipetting solution into 384- [and greater] 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.
- Use of liquids with concentrations of acids, corrosives, or solvents of 3% and greater can begin attacking the materials inside the instrument's chamber. Running multiple plates with concentrations <3% in long kinetics may also have a destructive effect. If the experiment is incubated, deterioration of chamber components will be accelerated. When in doubt about the use of acids, corrosives, or solvents; please contact TAC@biotek.com.

- It is the user's responsibility to understand the volumetric limits of the plate type in use as it applies to the assay being run.

Luminescence Measurements

- For highly sensitive Luminescence assays using white plates, add a Delay step to your Procedure to "dark adapt" the plates in the reading chamber before taking measurements.

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 µL/well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20 µL/well, we recommend a tip prime volume of 20 µL.
- To avoid spillage and possible contamination of the instrument, empty the tip prime trough frequently and do not exceed the total fluid volume of the plate well when dispensing.

Incubation and Partial Plates

When performing a partial plate read that includes an incubation step, the following recommendations can reduce the effects of evaporation of your samples:

- Use microplate lids.
- Fill unused wells with liquid.
- Cluster your sample wells rather than spacing them throughout the plate.
- Place your sample wells in the center of the plate. This placement may lead to less evaporation than if you place the samples in wells on the edge of the plate.

Chapter 4

Filters and Mirrors

The **Getting Started** chapter provided an overview of the filters and mirrors installed in some Synergy 2 models. This chapter provides more detailed information on working with these components.

Excitation/Emission Filters

As described on page 36, Synergy 2 models with the Fluorescence Intensity module are equipped with Excitation and Emission filter wheels. (Models with the Luminescence module without Fluorescence Intensity have only an Emission filter wheel.) 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 are accessed by opening the hinged door on the front of the reader, as shown in *Figure 3-3* on page 36.

Each filter wheel is labeled EX or EM, and can contain up to four filters and/or black plugs (also referred to as "dummy filters"). A filter can be used in either wheel, but it must be oriented properly, as described below. Each filter and plug is held securely in place with a C-clip filter retainer. Each filter has its wavelength and bandpass values printed on its side, with an arrow to indicate the proper direction of light through the filter.

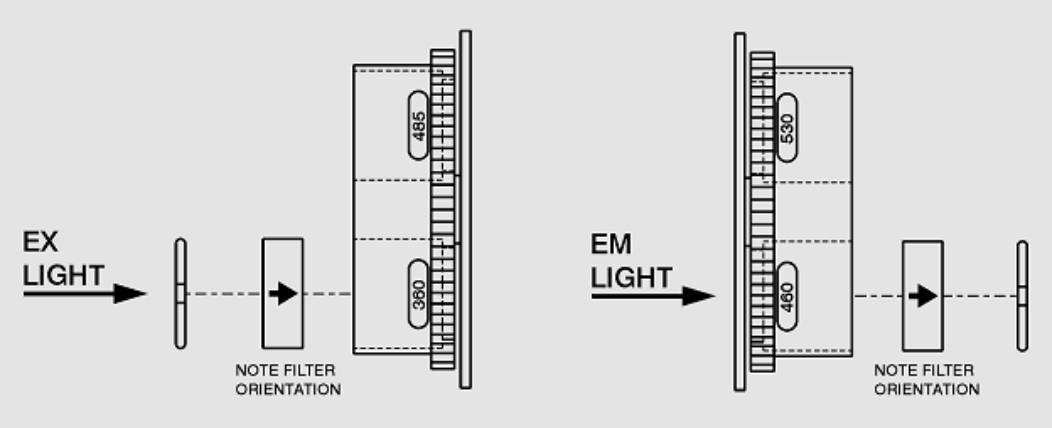


Figure 4-1: Diagram showing the proper orientation of the filters in their wheels

Change the Filter Wheels and Filters

The filter wheels are easily exchanged or modified 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 3-3*, labels on the front of the filter wheels can be marked with the central wavelength and band pass of each filter.

When exchanging/modifying filter wheels:

- 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.
- 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.
- We recommend placing filters in the wheels in ascending wavelength order from position 1 to 4 (no holes in EX2 or EM3), particularly if the reader has generated a 0508 (saturation) error.

Important! It is critical that the filter wheel characteristics defined in Gen5 and on the reader exactly match the contents of the installed filter wheels. If you exchange or modify a filter wheel, update its definition in Gen5 and send the new values to the reader. Instructions are provided on page 41.

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. Note: 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 4-1*).
2. Slide the filter wheel into its chamber.
3. Replace the thumbscrew and close the front door.
4. **Important!** If you modified the filter wheel's contents, use Gen5 to update the reader's internal software with the new configuration; see instructions on page 41.

	When removing or replacing a filter or C-clip retainer, do not use a sharp tool. Use several layers of lens paper and your finger to remove and replace filters and clips. Using a sharp object, 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 above.
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 4-1*): 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 **Chapter 5, Preventive Maintenance**.

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.

PN	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

PN	Wavelength	Main Application
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

For top-reading fluorescence analysis, the Synergy 2 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 gain/sensitivity for fluorescence intensity (FI) and time-resolved fluorescence (TRF) measurements when compared to mapped fiber optics.

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. 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 2 models with the FP module, the reader is equipped with three polarizers:

- Excitation polarizer (visible-range or UV-range)
- Emission polarizer, parallel to excitation polarizer
- Emission polarizer, perpendicular to excitation polarizer

Inside the reader, the mirror holder is labeled with five position numbers which translate to three possible measurement positions. See *Figure 4-2* and *Figure 4-3*.

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 4-4* 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.

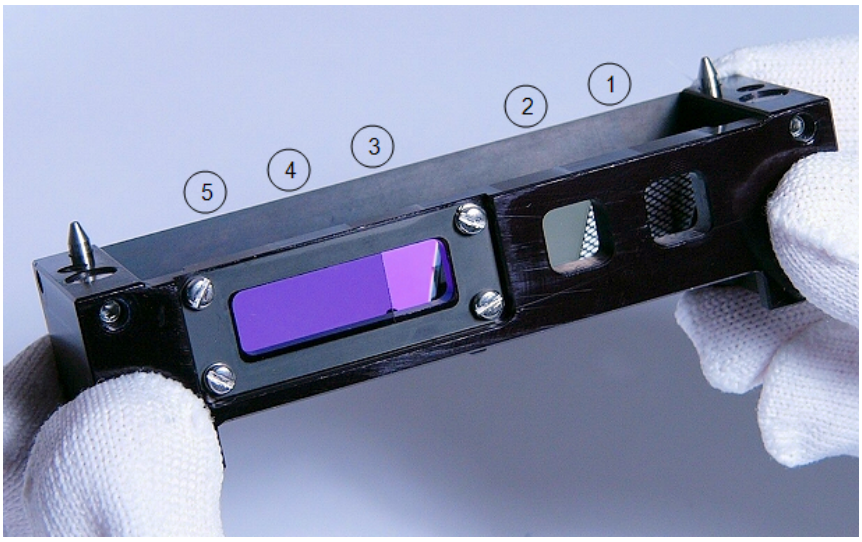


Figure 4-2: Mirror holder disengaged from the reader

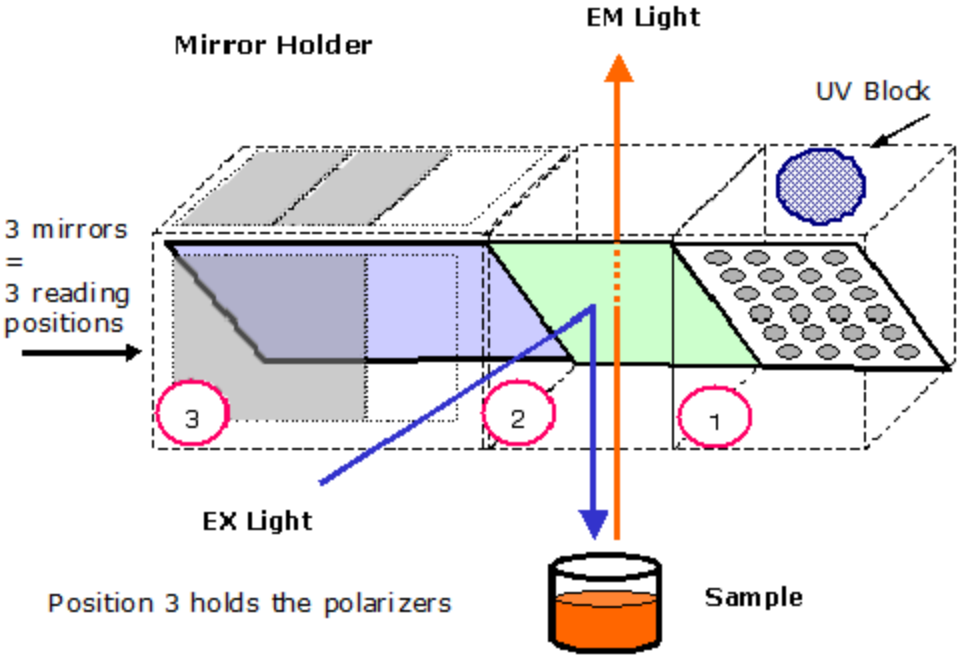


Figure 4-3: Mirror holder diagram; the three reading positions are indicated

ⓘ	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.

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, which use the open positions.

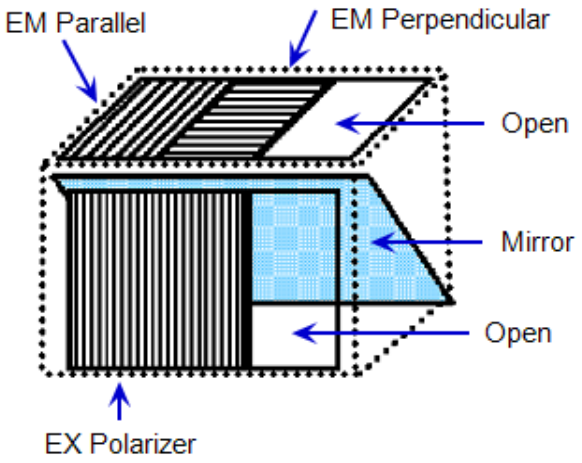


Figure 4-4: Mirror holder, Position 3 diagram

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 64 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			FP
5			EX (visible range) perpendicular EM			

Change the Mirror Holder and Mirrors

⚠	Do not touch the mirrors with your bare fingers, and only touch them along their edges. These optical elements are delicate and must be handled carefully. 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 *Inspect/Clean Mirrors* on page 71.
⚠	**Important!** It is critical that the mirror holder characteristics defined in Gen5 and on the reader exactly match the contents of the installed mirror holder. If you exchange or modify the mirror holder, update its definition in Gen5 and send the new values to the reader. Instructions are provided on page 41.

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

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. BioTek offers additional mirror holders and mirrors as separate accessories.

Removing the mirror holder from the reader is required in either scenario. Before removing the holder, take a moment to identify the reader's components that are described in the following procedures; see *Figure 4-5*.

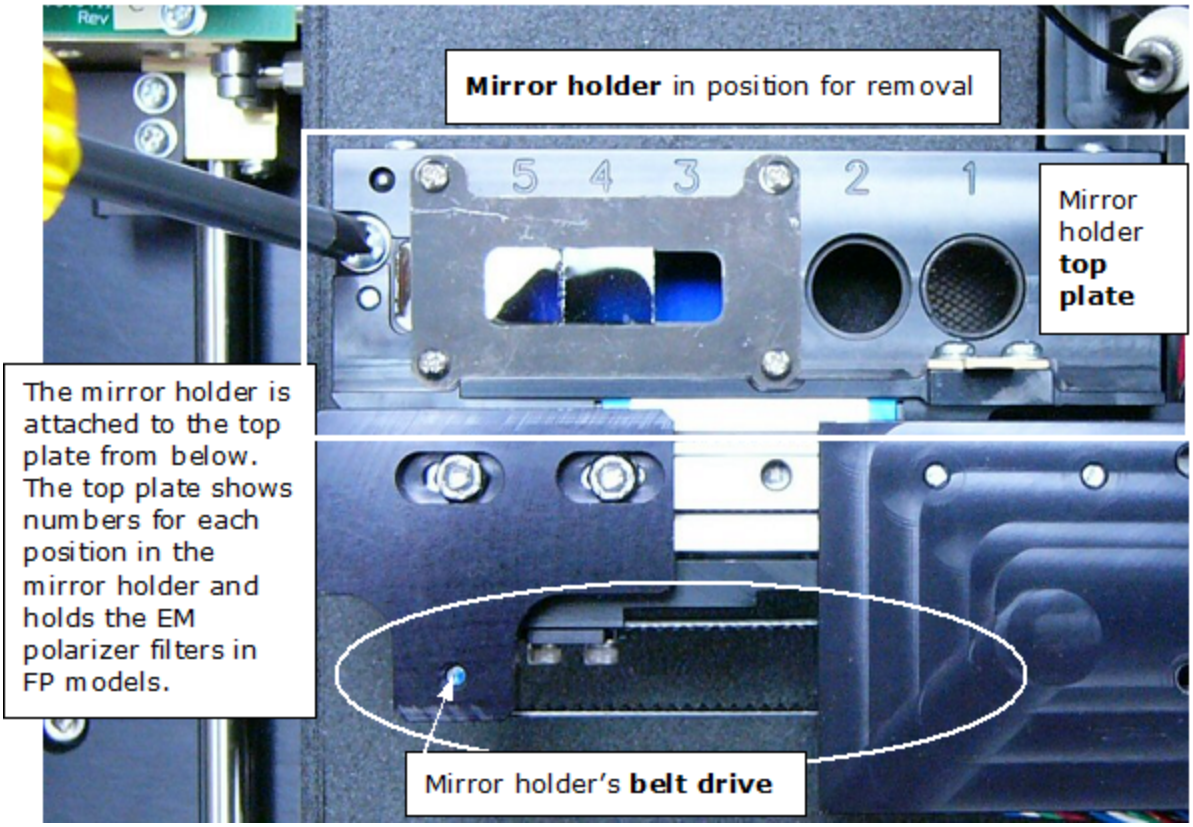


Figure 4-5: Mirror holder positioned for removal

To remove the mirror holder

The mirror holder is held in place by two shipping screws. These screws can be removed and stored for easy replacement of the mirror holder through the side-access door. For more information on using the side-access door, see page 62.

1. Turn off the reader and remove its shroud (see instructions and photos on page 11).
2. The mirror holder will likely be stored in its "home" position; you'll need to move it to an accessible position. Use the holder's belt drive (see *Figure 4-6*) to roll the holder (to the left) away from the optics armature. Use your thumb to turn the belt counterclockwise.

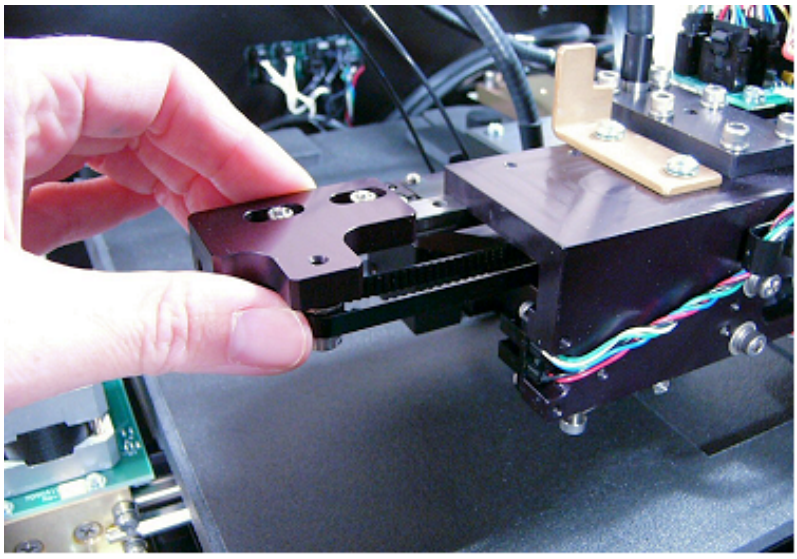


Figure 4-6: Turning the belt drive to position the holder for removal

3. When the mirror holder is fully exposed, carefully remove the two screws and washers on top (see *Figure 4-5*), and set them aside. The holder will continue to be held in place by its magnetized frame.
4. Grasp the handle (if equipped), or the sides of the holder underneath the top plate. Pull the holder down and toward the back of the reader to remove it.

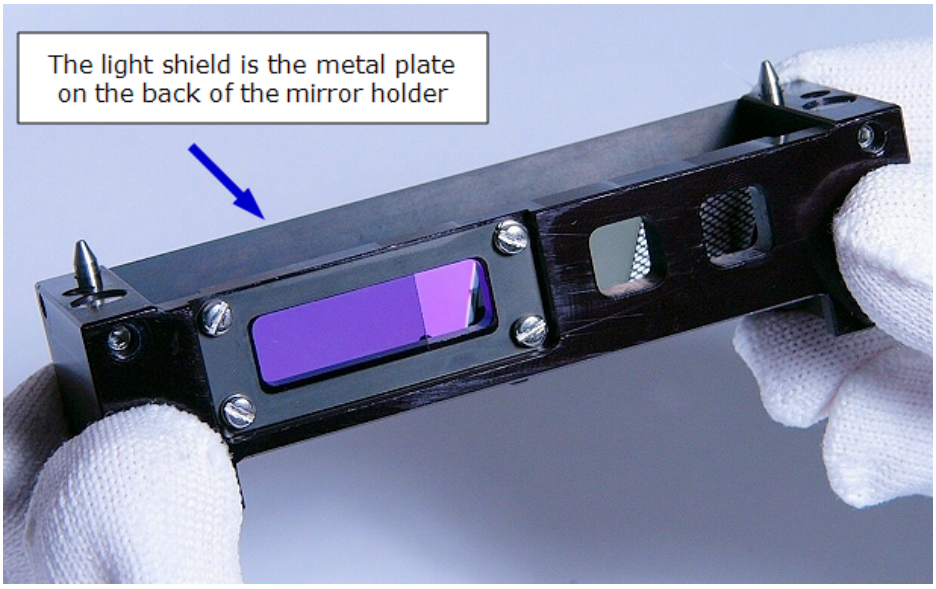


Figure 4-7: Mirror holder removed from reader

ⓘ	If you accidentally touch the mirrors or polarizing filters with your bare fingers, see *Inspect/Clean Mirrors* starting on page 71 for cleaning instructions.

To change a mirror in the mirror holder

Gather these tools:

- Linen or cloth gloves
- Small screwdriver

ⓘ Touch the mirrors as minimally as possible; hold them by their edges only. The mirrors are easily damaged.

1. Remove the mirror holder from the reader.
2. The holder has a light shield to protect the mirrors (*Figure 4-8*). Use a screwdriver to remove the four screws and washers that attach this shield to the holder.
3. Use the screwdriver to remove the bracket that secures the mirrors (*Figure 4-9*). 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.
6. Replace the metal bracket to secure the mirror. The bend in the bracket's arms should point away from the holder.
7. Replace the light shield.

ⓘ The light shield fits the holder in only one way. Check its alignment when reattaching it to the mirror holder.

8. Reinstall the mirror holder (see instructions in the next section).
9. **Important!** Update Gen5 and the reader with the new mirror configuration. Instructions are provided on page 41.

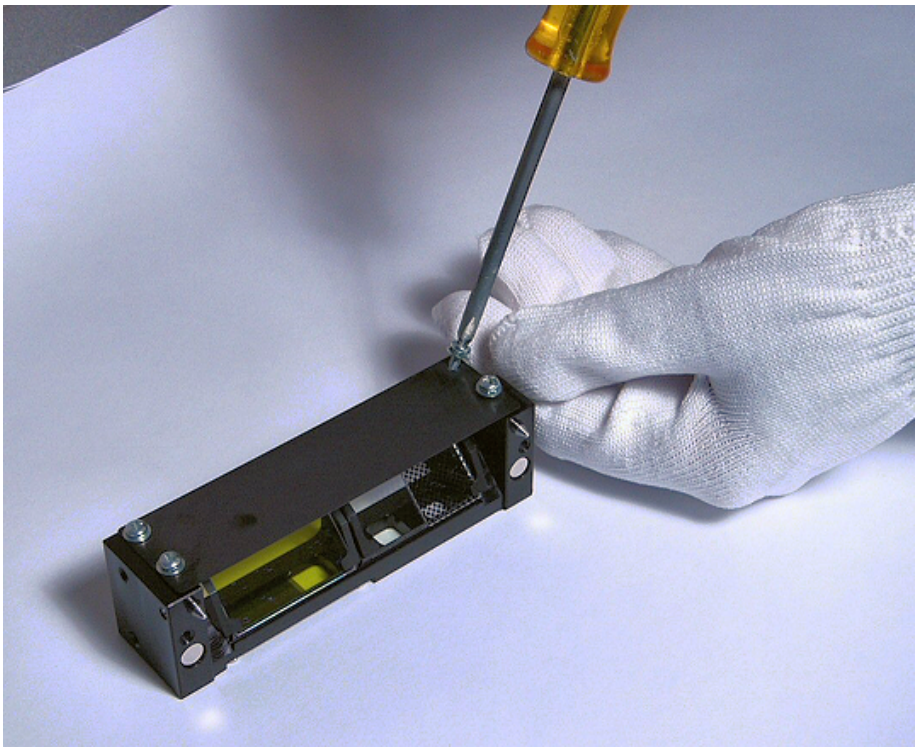


Figure 4-8: Removing the light shield from the mirror holder

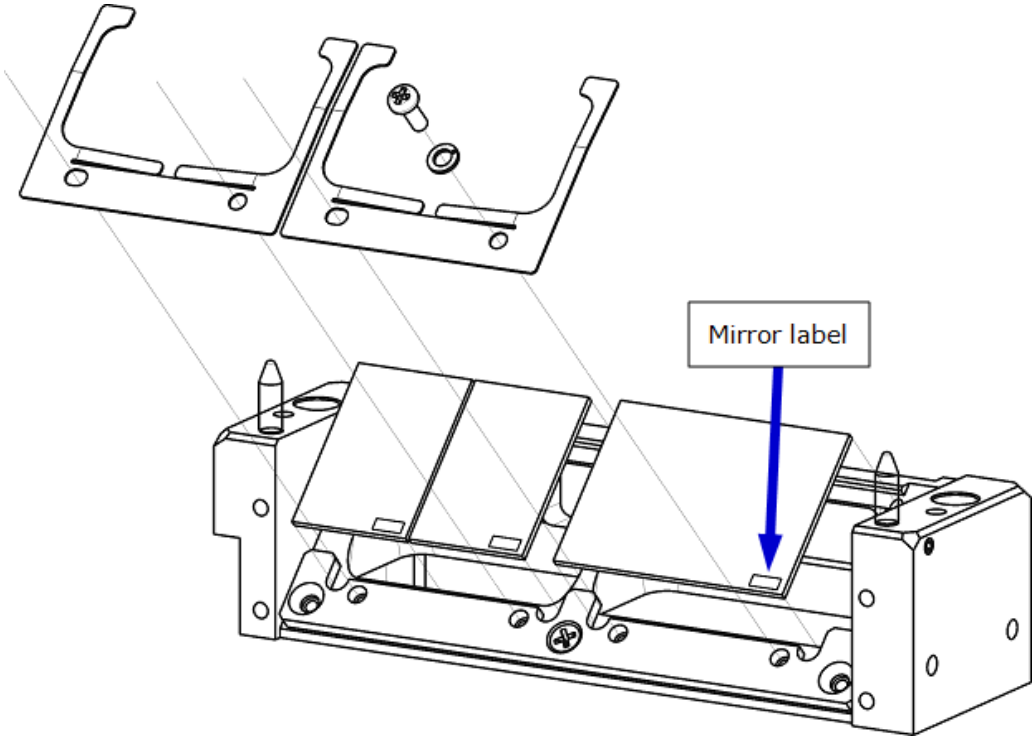


Figure 4-9: Mirror orientation; labels are face-up and readable

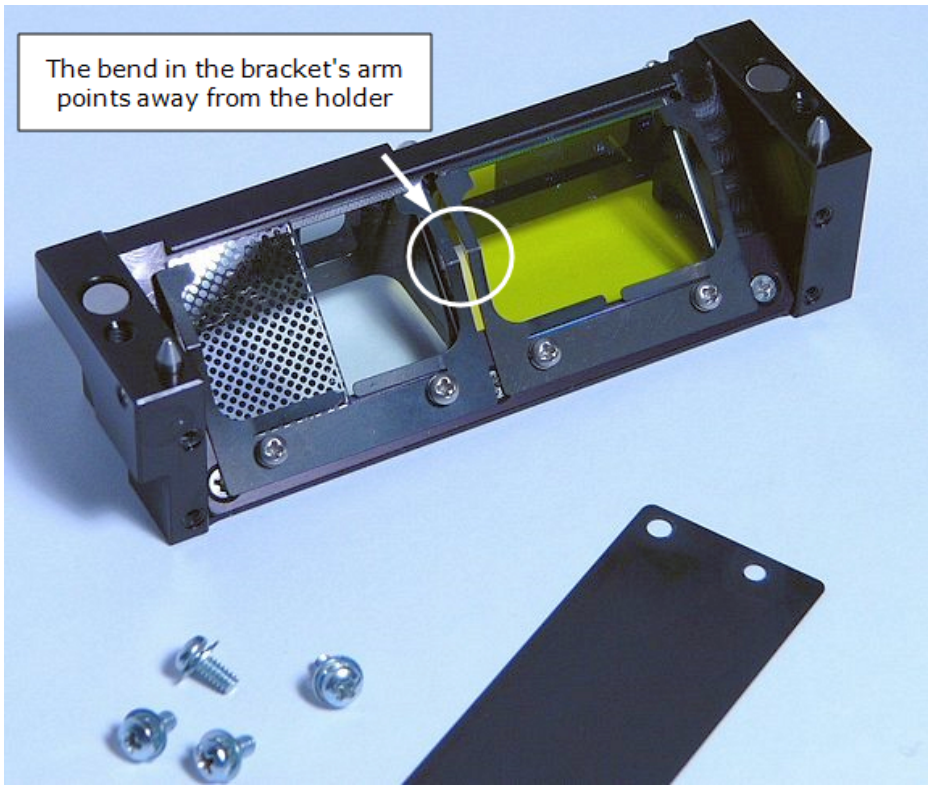


Figure 4-10: Mirror holder with shield removed

To reinstall the mirror holder

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 (see *Figure 4-5*). The holder's alignment pins will help guide it into place and because it is magnetized the holder will stay in place.
3. (Optional) Reinstall the two sets of screws and washers that secure it in the reader.

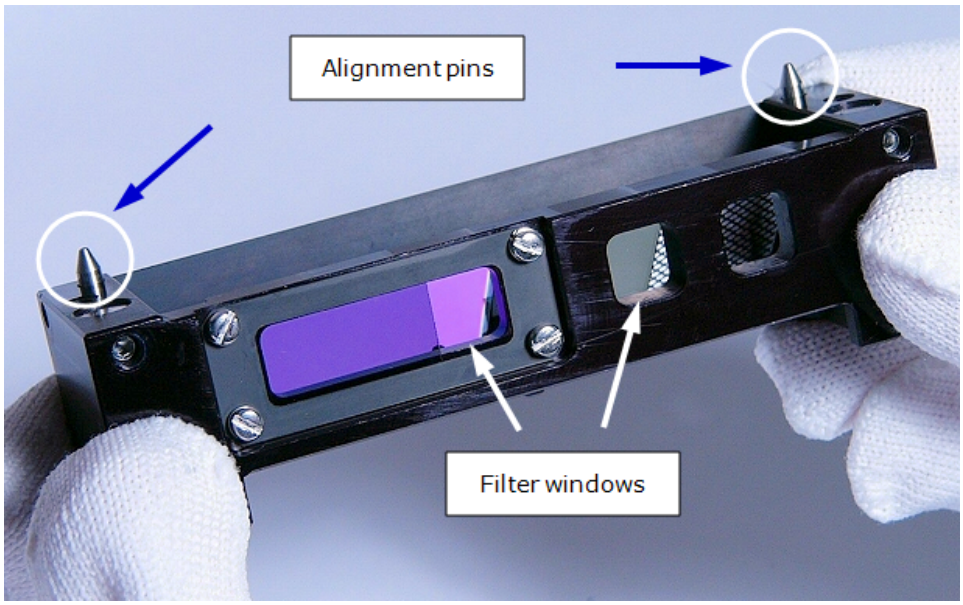


Figure 4-11: Mirror holder positioned for reinstallation

To exchange the mirror holder through the side-access door

The mirror holder shipping screws must be removed and stored before the holder can be removed through the side-access door. See page 57.

1. If the instrument is off, turn it on. Otherwise, perform a System Test.
2. If the carrier is extended outside the instrument, press the carrier eject button to retract the carrier.
3. Open the side-access door and pull the mirror holder (using the handle, if equipped) toward the door. You will detect a rough vibration as you pull against the stepper motor holding torque. This will not damage any components.
4. Pull down on the mirror holder to disengage it from the magnets and then pull it out of the instrument.
5. Re-install the replacement mirror holder in the reverse order. The holder has alignment pins to guide it into position. Once the holder is seated, you do not need to push it back into place.
6. **Important!** Update Gen5 and the reader with the new mirror configuration. See instructions on page 41.
7. Run a System Test. When finished, the new mirror holder is ready for use.

To install the mirror block handle

If your mirror holder is not equipped with a handle, you can order one from BioTek (see *Optional Accessories* on page 4). The handle ensures that you do not damage or get fingerprints on the mirrors and polarizers. Attach the handle as shown below. The optional label can be used to identify the mirror types.

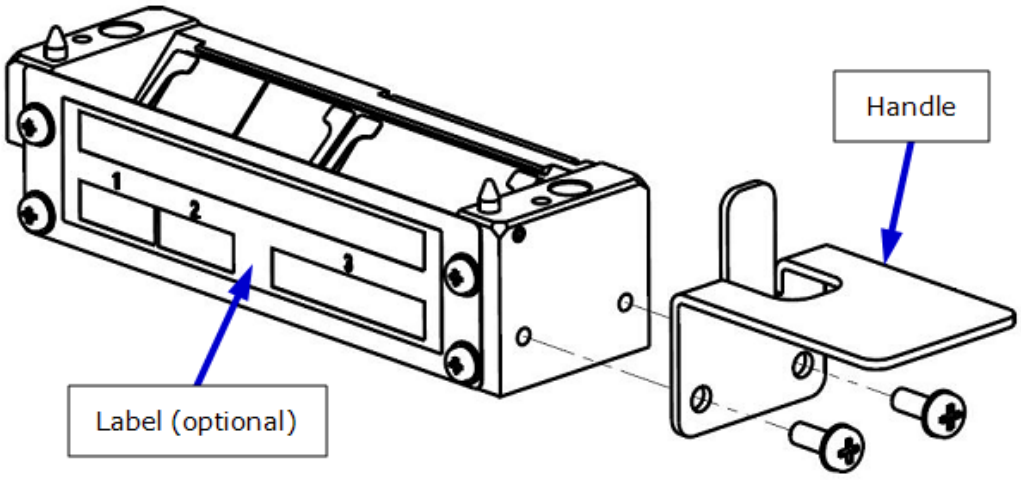


Figure 4-12: Attaching the handle to the mirror block

Clean the Mirrors

Instructions for cleaning the mirrors and polarizers (if equipped) are provided on page 71.

Mirrors Available from BioTek

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	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 2 and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

Overview

A general Preventive Maintenance (PM) regimen for the Synergy 2 includes periodically cleaning all exposed surfaces and inspecting/cleaning the excitation and emission filters. 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 injector heads.

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 and then 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 instructions.

Recommended Maintenance Schedule

The table below contains the recommended Preventive Maintenance tasks for Synergy 2 and the frequency with which each task should be performed.

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

Task	Daily	Quarterly	As Needed
All models:			
Clean exposed surfaces			✓
Inspect/clean excitation and emission filters (if equipped)		✓	
Inspect/clean mirrors (if equipped)			*annually*
Decontaminate the instrument	*before shipment or storage*		
Models with injectors and an external dispense module:			
Flush/purge the fluid path	✓		
(Optional) Run a Dispense protocol			✓
Empty/clean tip prime trough	✓		
Clean priming plate			✓
Clean internal components		✓	✓

Warnings and Precautions

	**Warning! Internal Voltage.** Turn off and unplug the instrument for all maintenance and repair operations.
	**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.
	**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.
	**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.
	**Warning!** The Tungsten lamp assembly is hot when the instrument is powered on. If the instrument is on, turn it off and allow the lamp to cool down before attempting to replace it.
	**Warning!** The instrument with all available modules weighs up to 57 pounds (25.8 kg) depending on the model. Use two people when lifting and carrying the instrument.

	**Important!** Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. **Do not allow water or other cleaning solution to run into the interior of the instrument.** If this happens, contact BioTek's Technical Assistance Center.
	**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.

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 will need:

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

Procedure:

1. **Important!** Turn off and unplug the instrument.
2. Wet a cloth or paper towel with water, or with water and mild detergent, and then **thoroughly wring it out so that liquid does not drip from it**.
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 (not soaked) with water.
6. Use a clean, dry cloth to dry all wet surfaces.

Models with injectors: If the tip priming trough overflows or other spills occur inside the instrument, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. If overflow is significant, you may need to remove the reader's shroud and the incubator housing to better access the interior; see instructions starting on page 75.

Inspect/Clean Excitation and Emission Filters

Applies only to models with fluorescence and/or luminescence capability

BioTek recommends inspecting the filters for dust and other debris every three months. To clean them, you will 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 holds the excitation (EX) filter wheel in place; the right secures the emission (EM) filter wheel. Remove each thumbscrew and pull the filter wheel out of the compartment.

The **Filters and Mirrors** chapter contains illustrations for identifying the filters 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 by the cotton ball.
6. Replace the filter wheels in their respective positions and replace the thumbscrews. Close the hinged door.

Inspect/Clean Mirrors

Applies only to models with fluorescence and/or luminescence capability

BioTek recommends inspecting/cleaning the mirrors and polarizing filters (if equipped) annually, especially if the mirror holder has been handled or changed.

The mirrors (especially the dichroic) and polarizing filters can be easily damaged. Perform the cleaning steps only when necessary and always handle the mirror and filters carefully.

These optical elements are delicate and must be carefully handled. 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.** If performance indicators or obvious defects in the mirrors or filters suggest cleaning them, however, 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

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

Procedure

1. Turn off and unplug the reader. Remove its shroud if you do not normally use the side-access door to remove the mirror holder.
2. Remove the mirror holder (see instructions on page 57).

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 which 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 mirror, moving in a single direction. A minimal amount of pressure can be applied while wiping. However, too much pressure will damage the mirror.
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 Fluid Path

Applies only to models equipped with injectors

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

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

ⓘ If using Gen5 version 2.05 or earlier, the **Prime** and **Purge** options are found under the **Dispenser** tab in the Reader Control dialog.

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

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

To purge the fluid from the system:

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

   After purging the system, you may wish to run a quick Dispense protocol to visually verify the dispense accuracy (see the next section) or the more thorough Dispense Accuracy and Precision Tests (see *Injection System Tests* starting on page 149).

Run a Dispense Protocol (Optional)

Applies only to models equipped with injectors

After flushing/purging the system (described on page 73) and before running an assay that requires dispense, take a moment to visually inspect the dispense accuracy.

	Use a DI H_2O–Tween solution to visually inspect the dispense accuracy following maintenance: e.g., add 1 mL Tween 20 to 1000 mL of deionized water.

1. Create a new protocol in Gen5. Select a Plate Type that matches the plate you are using.
2. 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
3. Add another Dispense step with the same parameters, selecting Dispenser 2.
4. Add a quick Read step with parameters relevant to your reader model (this is necessary because Gen5 requires that a Read step follow the Dispense step).
5. Save the protocol with an identifying name, such as “Dispense Observation.”
6. Fill the supply bottles with the DI H_2O–Tween solution mentioned above.
7. Create and run an experiment based on the Dispense Observation protocol.
8. When the experiment is complete, visually assess the fluid level in the wells. Well volumes should appear evenly distributed across the plate.

   If the well volume appears to be unevenly distributed, clean the internal dispense tubes and injector heads as described in *Clean the Internal Components* starting on page 75 and run the protocol again.

Empty/Clean the Tip Priming Trough

Applies only to models equipped with injectors

The tip priming trough is a removable cup located in the rear pocket of the microplate carrier, used for performing the tip prime. The trough holds about 1.5 mL of liquid and must be periodically emptied and cleaned.

1. Extend the microplate carrier and carefully remove the tip priming trough from its pocket in the left rear of 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.

At the start of an experiment that requires dispensing, Gen5 prompts the user to empty the tip prime trough.

Clean the Priming Plate

Applies only to models equipped 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 models equipped 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 reader's internal dispense tubes and injector heads require routine cleaning at least quarterly and possibly more frequently depending on the type of fluids dispensed.

Cleaning inside the reader is required when 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. Perform a System Test before and after maintenance so that any changes in performance can be noted.

	Wear protective gloves and safety glasses when performing the procedures.

Required Materials

- Protective gloves and safety glasses
- Screwdriver
- Mild detergent or isopropyl alcohol
- Clean, lint-free cotton cloths
- Deionized or distilled water
- Stylus (stored in a cylinder affixed to the back of the dispense module or reader; BioTek PN 2872304)

Procedure

Remove the Reader's Shroud

	Before removing the shroud, purge the system of fluid, and then turn off and disconnect the reader from its power supply, the computer, and the dispense module.

1. Purge the injection system of all 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 lower-rear corner. See *Figure 2-2* on page 11.
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. See *Figure 2-3* on page 11.

To reinstall the shroud, rest its bottom on the table in front of the reader and gently slide it into place. Internal wheels roll along the reader's bottom track to properly reposition the shroud.

Clean the Shroud's Air Filters

If dust has accumulated 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.

Remove the Internal Dispense Tubes and Injector Heads

1. Locate the tubing ports on the reader's back wall. Turn each tube's thumbscrew counterclockwise and gently pull the tube from the port (*Figure 5-2*).
2. Locate the injector heads. Turn each tube's thumbscrew counterclockwise and gently pull the tube from its injector tip (*Figure 5-3*).
3. Turn each injector head counterclockwise and gently pull it out of the socket (*Figure 5-4*).

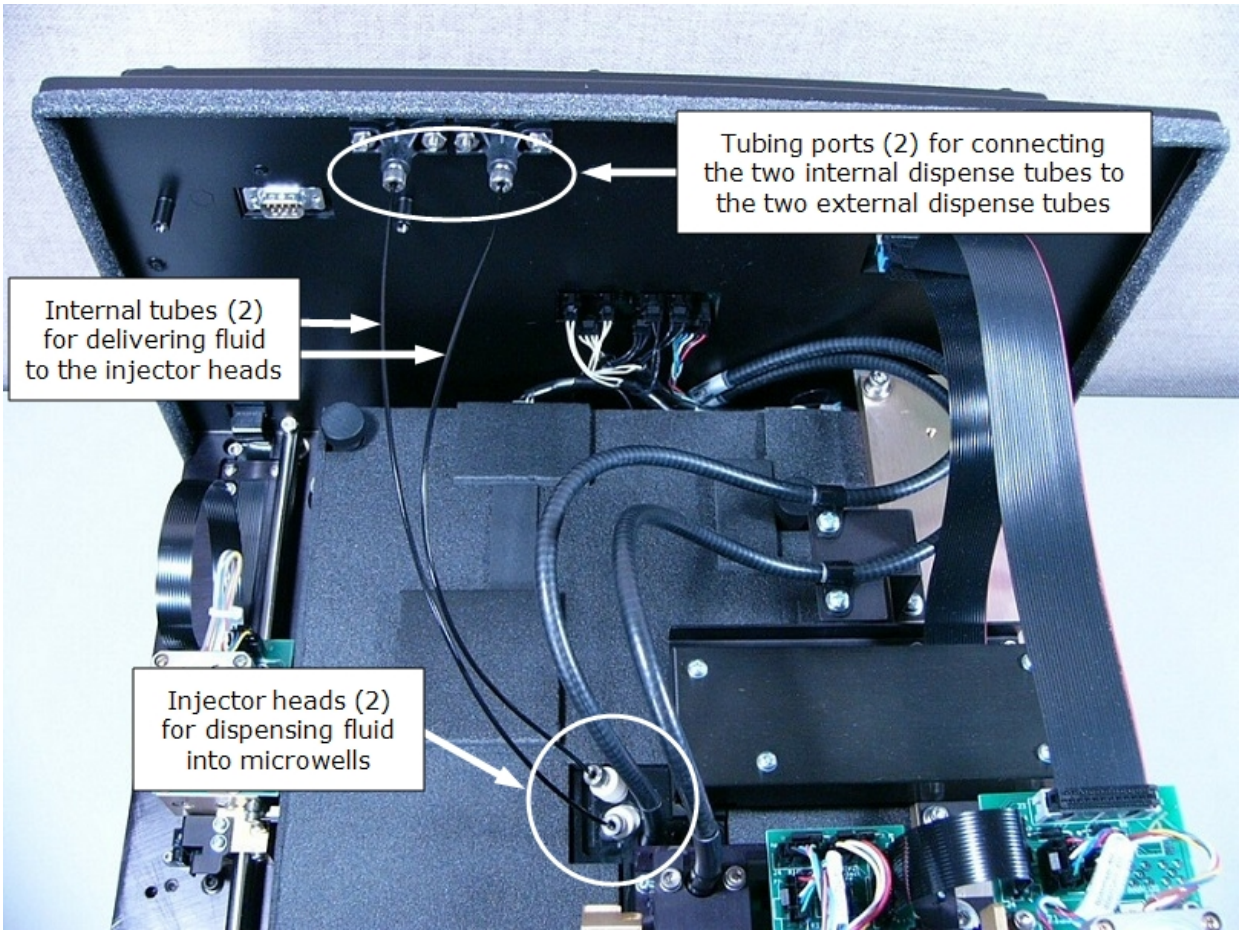


Figure 5-1: Internal components for the injection system

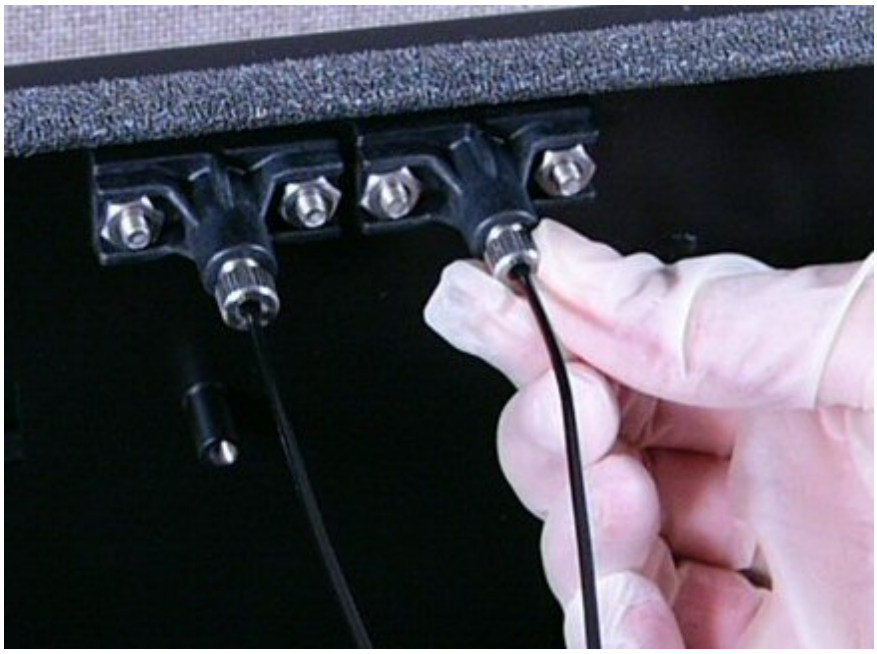


Figure 5-2: Disconnecting the dispense tubes from the back wall

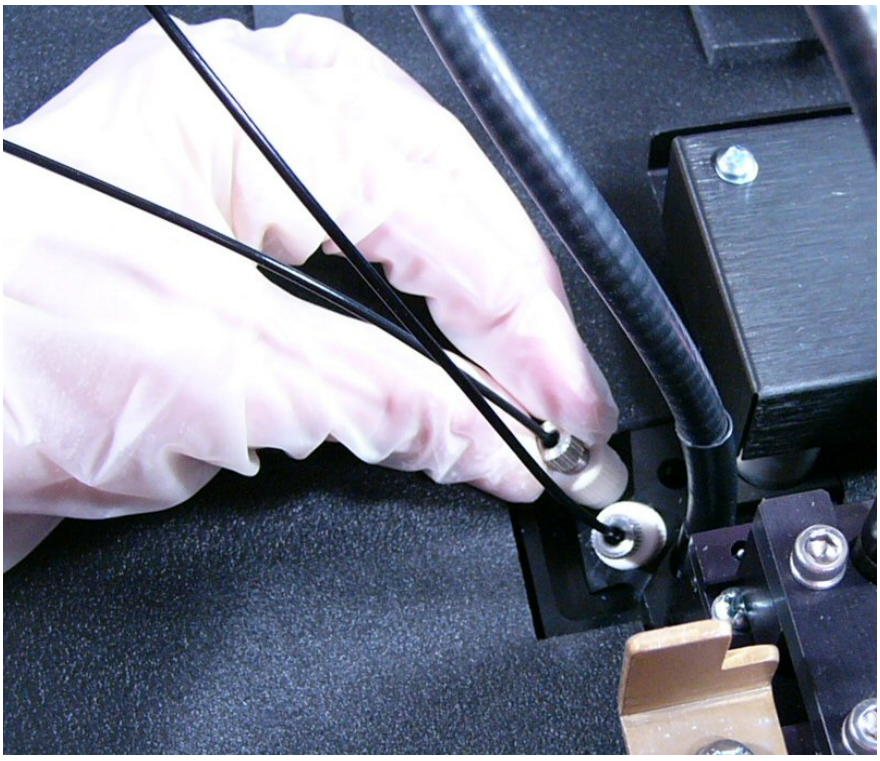


Figure 5-3: Disconnecting the tubes from the injector heads

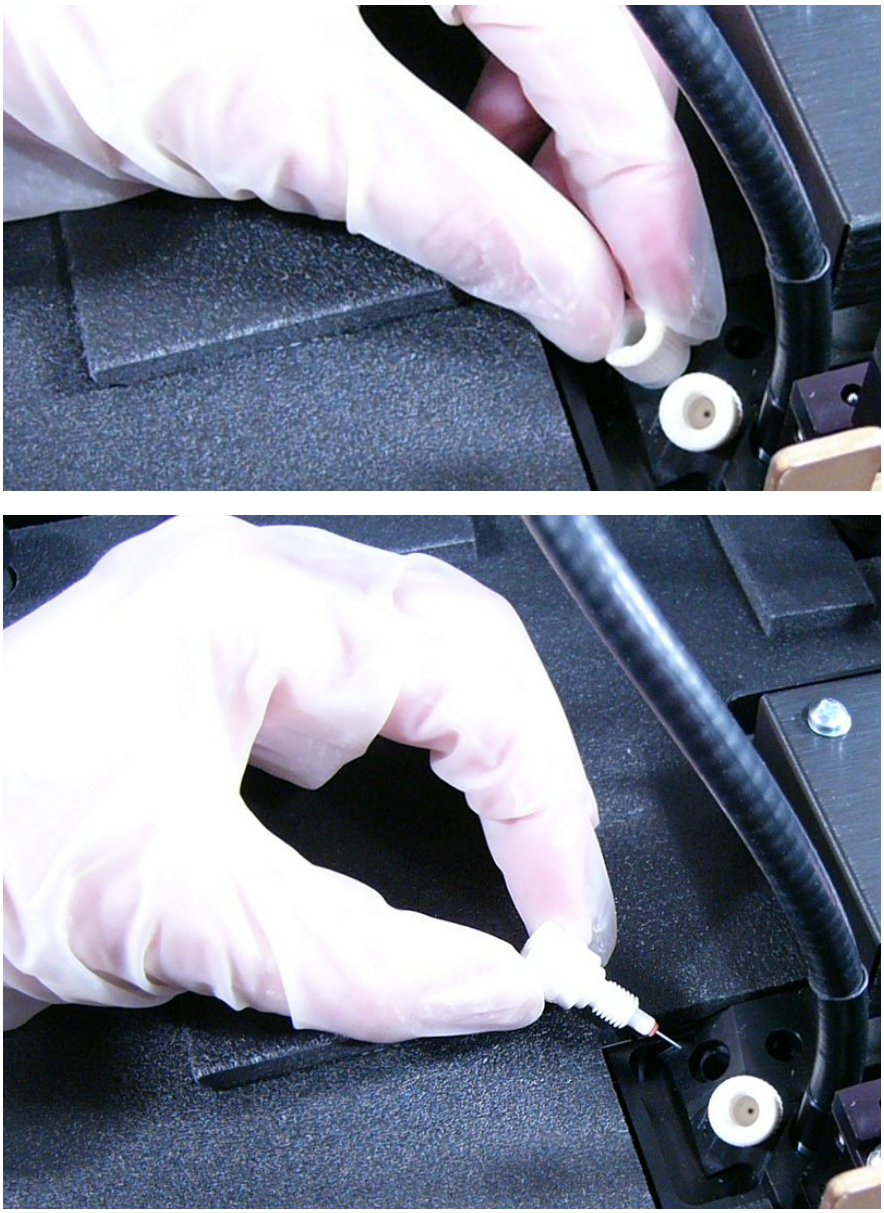


Figure 5-4: Removing the injector heads

Clean the Dispense Tubes and Injector Heads

As discussed on page 66, some reagents can crystallize and clog the tubing and injector heads. Daily flushing and purging can help to prevent this, but more rigorous cleaning may be necessary if reagent has been allowed to dry in the tubing and/or injectors.

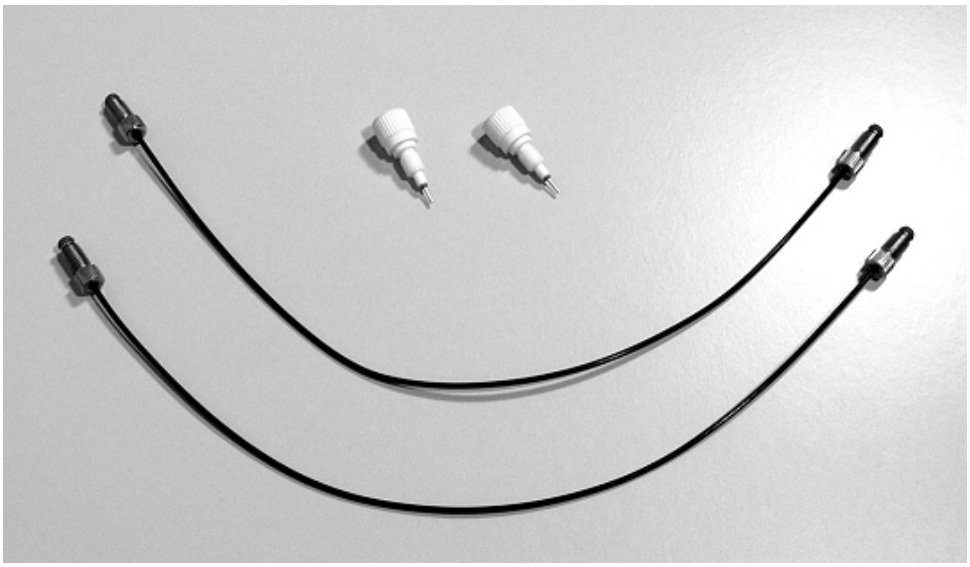


Figure 5-5: Injector heads and internal dispense tubes

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 (BTI #2872304) is stored in a cylinder affixed to the back 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.

⚠	**Caution:** Do not bend the injector tips. A bent tip might not dispense accurately. **Caution:** Do not remove the o-rings (if equipped).

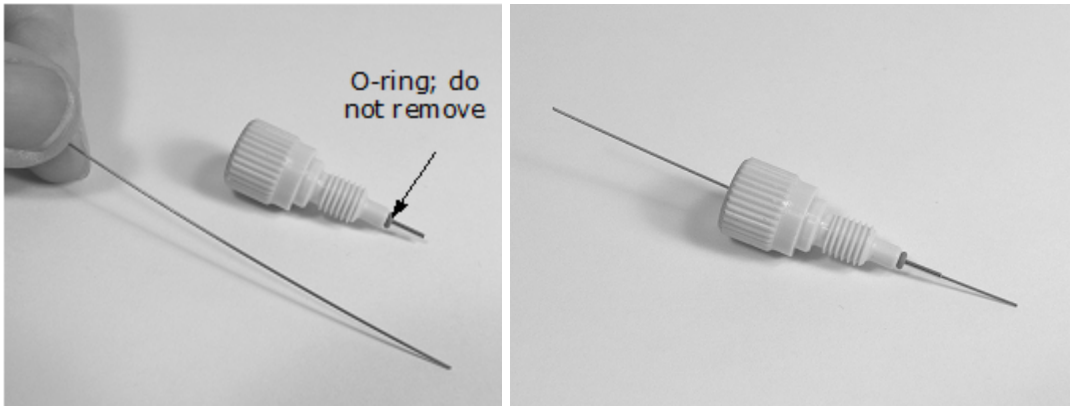


Figure 5-6: Using the stylus to clean an injector head

Clean Inside the Reader

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

BioTek recommends performing this process in conjunction with the previously defined steps for cleaning the dispense tubes and injectors.

For this procedure you will need:

- Deionized or distilled water and mild detergent (optional)
- Two or more lint-free cotton cloths

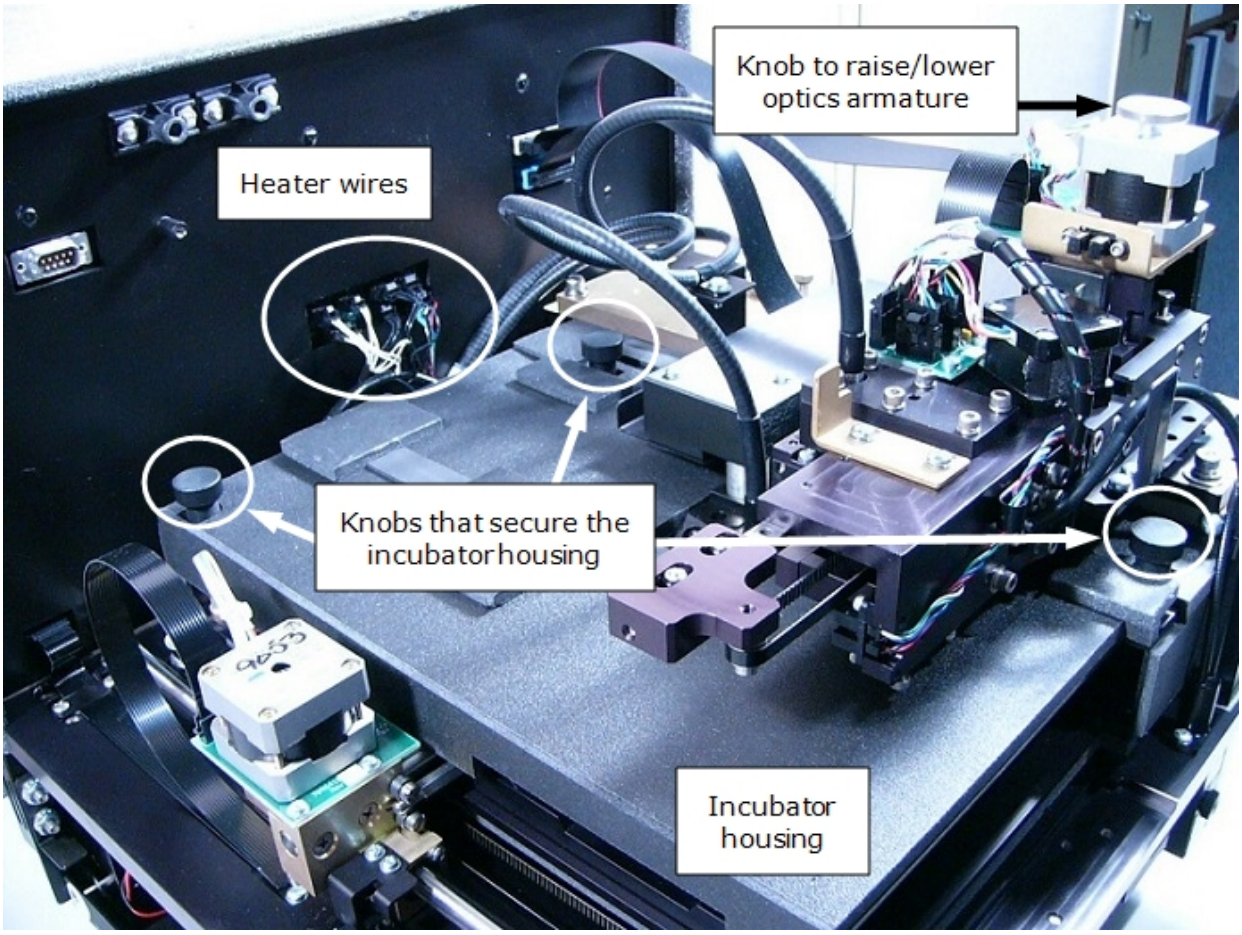


Figure 5-7: Locating the components described in this section

Remove the Incubator Housing

1. If you have not already done so, unplug the reader and remove its shroud.
2. Disconnect two heater wires located on the reader's back wall. The two wires are in the first and third positions in the top row (see below). To disconnect a wire, depress the tab and slide it off the board.

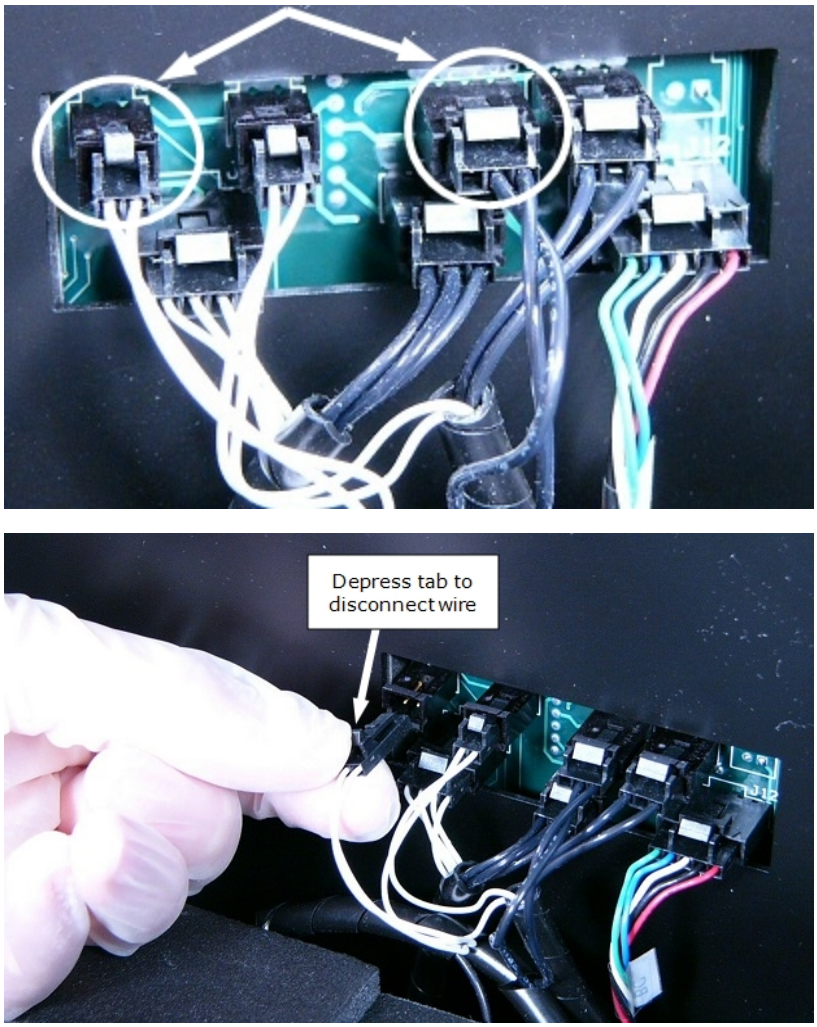


Figure 5-8: Disconnecting heater wires 1 and 3 in the top row

3. Refer to *Figure 5-7* and remove the three knobs that secure the incubator housing.
4. Turn the optics armature knob clockwise to raise the mechanism as high as it will go.

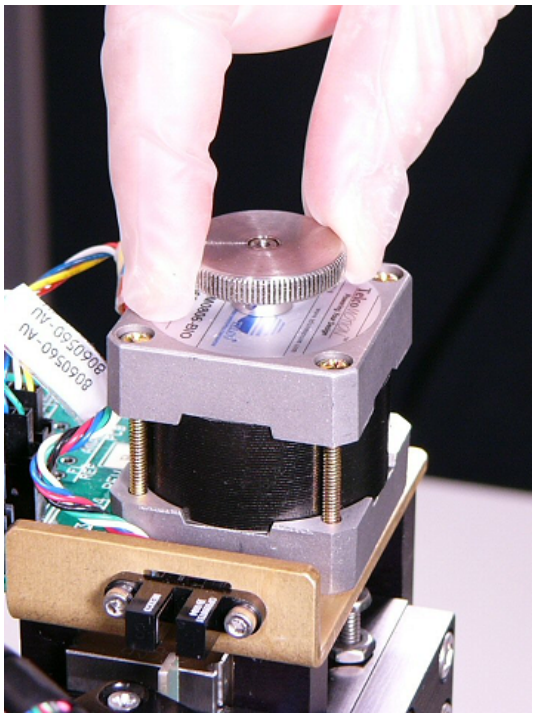


Figure 5-9: Raising the optics armature

5. Lift the left side of the incubator housing and carefully slide it out (see *Figure 5-10*).
6. 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.

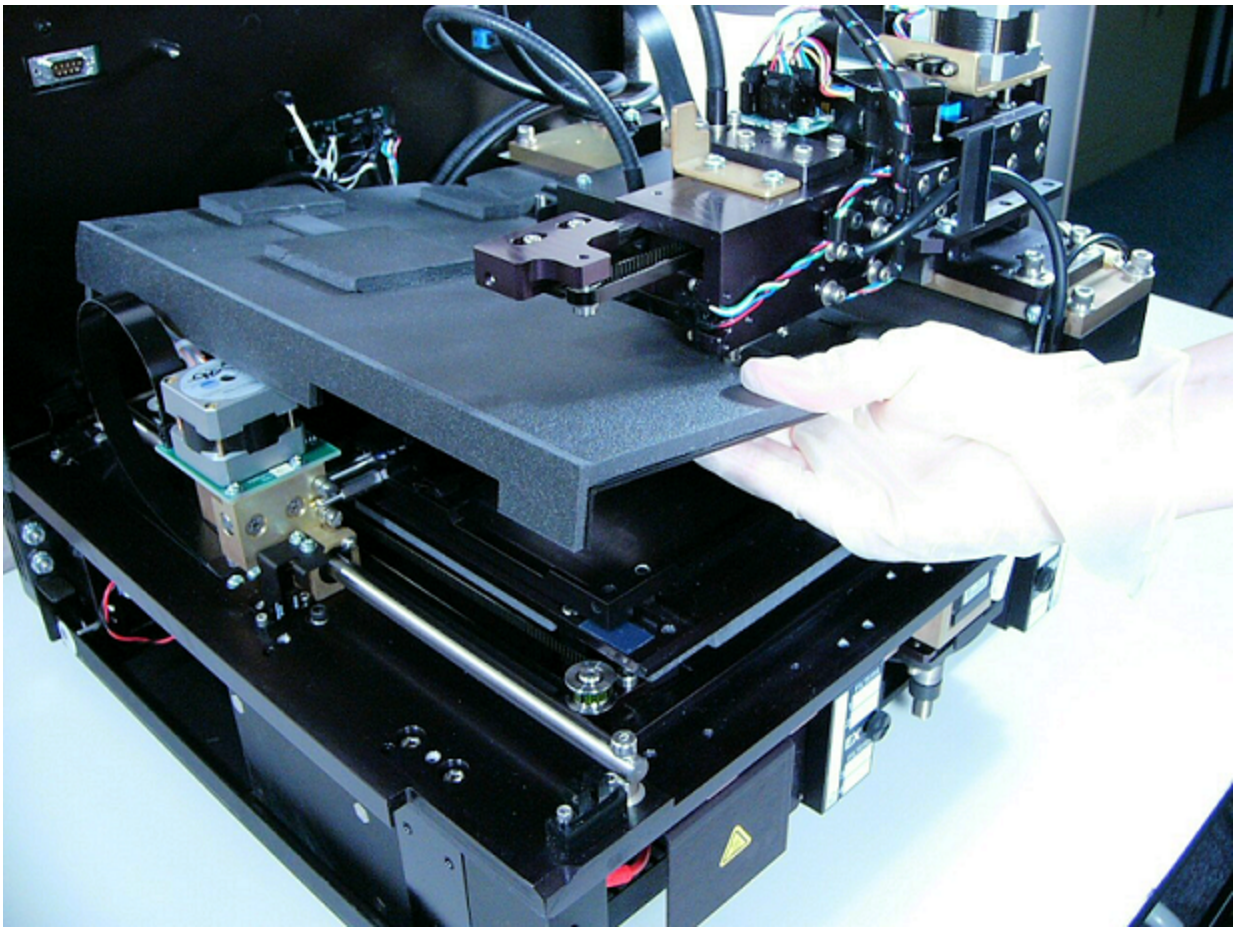


Figure 5-10: Removing the incubator housing

Clean the Reader's Surface

1. After you remove the incubator housing, slide the microplate carrier to the left to engage the support pin and then pull it toward the front of the reader.
2. Moisten (do not soak) a clean cotton cloth with water, or with water and mild detergent, or alcohol. Wipe all sides of the plate carrier. Wipe the instrument's horizontal surface. See *Figure 5-11*.

   If the injector heads were removed from the reader, use the cloth to gently clean the underside of the optical armature around the injector head holders.

   ⓘ Do not apply pressure. Components are 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.

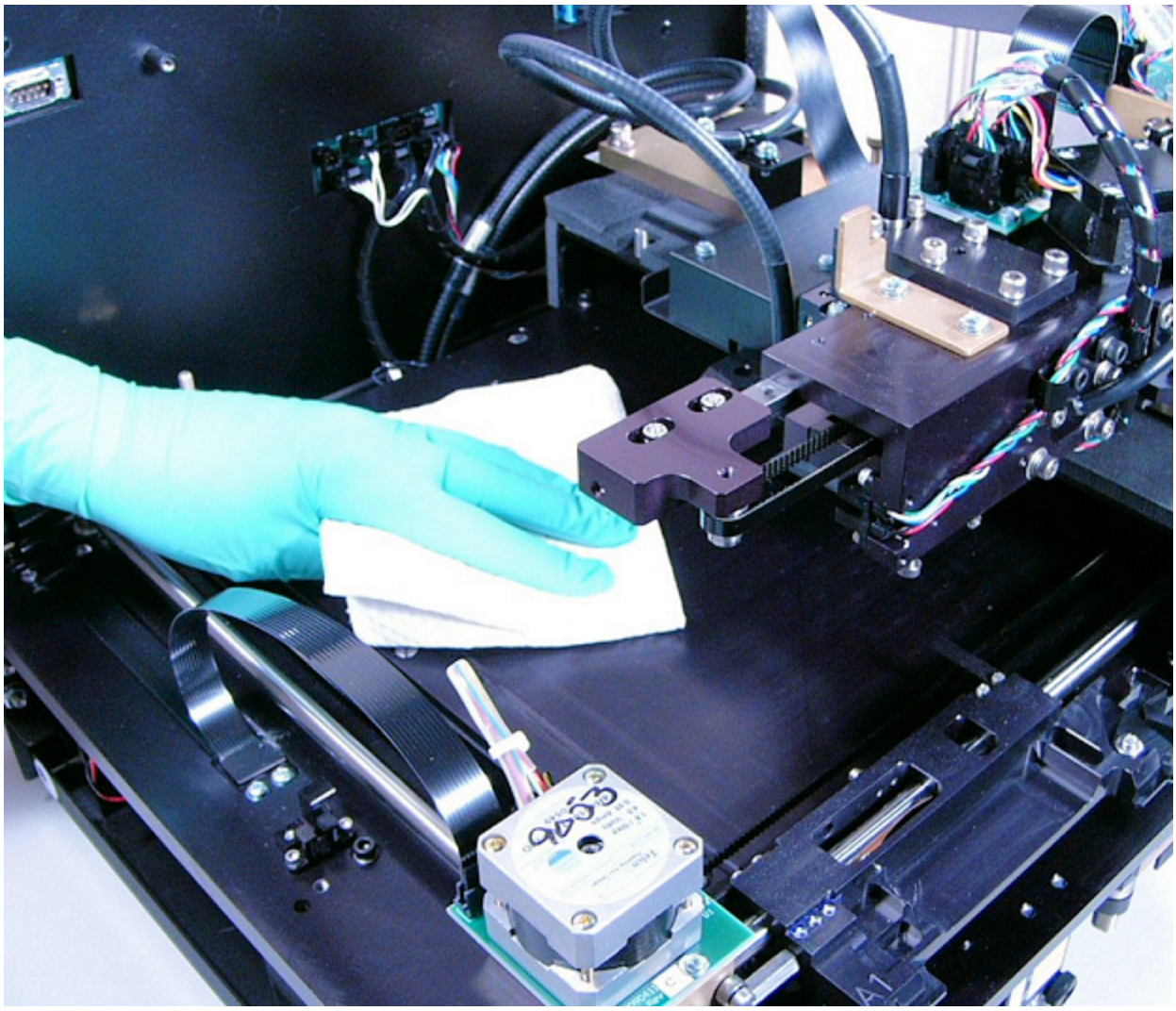


Figure 5-11: Cleaning the inside of the reader

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 screws on either side of the optics armature. Do not slide the forks under the foam. See *Figure 5-12*.
   - Secure the incubator housing with the three knobs (*Figure 5-7*).
   - Reconnect the two heater wires to the rear wall of the reader: the white wire fits into position 1 (*Figure 5-8*) in the top row of wires; the black wire fits into position 3.

Figure 5-12: Positioning the incubator housing fork

3. Insert the two injector heads into their sockets. Ensure that they are properly seated; the knurled plastic should sit flush against the surface, as shown below.

Figure 5-13: Seating the injector head

4. Attach the two internal dispense tubes to the injector heads (*Figure 5-3* on page 78). Do not overtighten the thumbscrews!
5. Attach the other end of the two dispense tubes to the tubing ports on the rear wall of the reader. They can go into either port.
6. Review the steps you just performed to make sure the components have been properly reassembled.
7. Slide the shroud onto the instrument.
8. Replace the two side screws to secure the shroud to the base.

Verify Performance

After reassembling the instrument, verify that the instrument is functioning properly:

- Connect power to the reader and turn it on. Allow the startup system test to complete. Run a System Test using Gen5; all tests should pass.
- Run any required OQ/PQ tests.

Chapter 6

As-Needed Maintenance

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

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 biohazards 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 2 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 or paper towels

Additional materials for models with the dispense module:

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

Procedure for Models without the Dispense Module

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

Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth. **Do not allow the cleaning solution to run into the interior of the instrument.** If this happens, contact the BioTek Service Department.

Turn off and unplug the instrument for all decontamination and cleaning operations.

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. Wet a cloth or paper towel with the bleach solution or alcohol, and then **thoroughly wring it out so that liquid does not drip from it.**
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* below when the equipment is functioning normally. If you are unable to perform a prime due to a system failure, perform the *Alternate Procedure* described on page 92.

ⓘ If using Gen5 version 2.05 or earlier, the **Prime** and **Purge** options are found under the **Dispenser** tab in the Reader Control dialog.

Routine Procedure

If disinfecting with sodium hypochlorite (bleach), flush repeatedly with deionized water to remove the bleach. After disinfecting with sodium hypochlorite, perform the rinse procedure provided on page 91.

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. Wet a cloth or paper towel with the bleach solution or alcohol, and then **thoroughly wring it out so that liquid does not drip from it**.
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 Fluid Path* on page 73) and detach the outlet tubes from 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.

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. 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 > Instrument Control**, and click the **Prime** 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 the next procedure, *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 µL, and keep the default dispense Rate.
5. Run five prime cycles, for a total of 25,000 µL.
6. Pause for 10 minutes and then run one prime cycle with 5000 µL. This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
7. Empty the beaker containing the outlet tubes.
8. Wipe all surfaces with deionized water.

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

Clean the Tubing and Injectors

Turn to *Clean the Internal Components* on page 75 for instructions on removing the reader's shroud and removing/cleaning the internal dispense tubes and injector heads.

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 system due to an equipment failure, decontaminate the instrument and the dispense module as follows:

1. Turn to *Clean the Internal Components* on page 75 for instructions on removing the reader's shroud and removing/cleaning the internal dispense tubes and injector heads.
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. Wet a cloth or paper towel with the bleach solution or alcohol, and then **thoroughly wring it out so that liquid does not drip from it**.
5. Use the cloth to wipe:
   - All exterior surfaces of the instrument
   - All surfaces of the plate carrier
   - The exposed surfaces of the dispense module, including the syringe valves

6. Remove the tubing and the syringes from the dispense module and soak them in the bleach or alcohol solution. Wait for 20 minutes.

   To remove a syringe: In Gen5, click **System > Instrument Control > Synergy 2**. On the Prime tab, select a dispenser and click **Maintenance**. The syringe bracket will move to its furthest-from-home position. Remove the metal thumbscrew from underneath the bracket. Unscrew the top of the syringe from the bottom of the syringe drive. Gently remove the syringe.

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 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 cables extend from the back of the lamp to plug into the reader.

	The 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.

3. Remove the EX filter wheel and set aside.
4. Grasp the light-blocking panel and slide the assembly toward you, out of the reader.

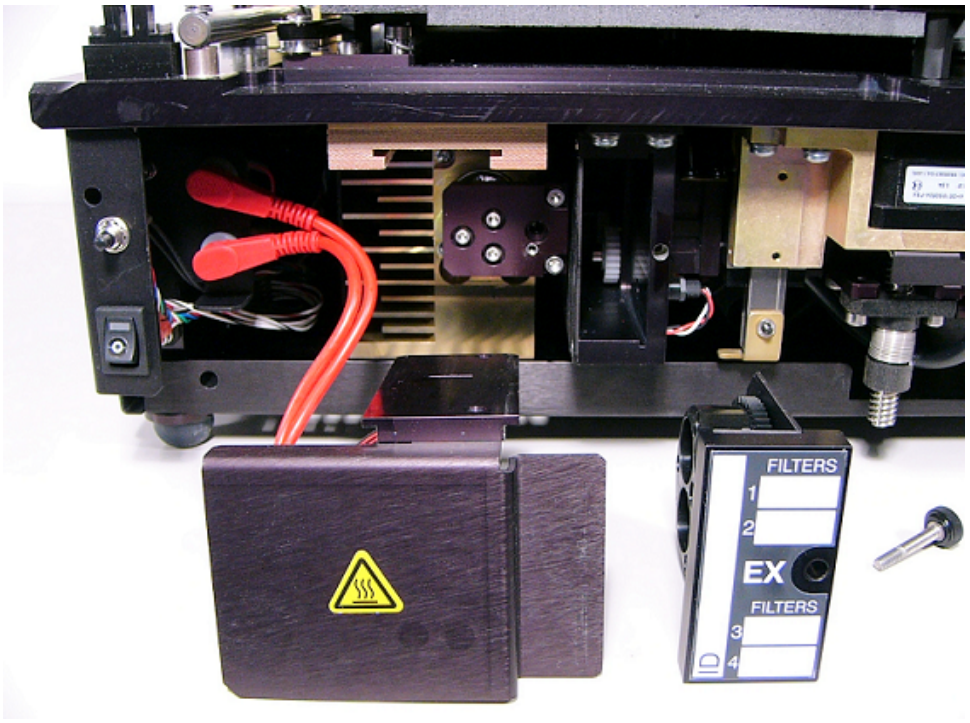


Figure 6-1: The light-blocking panel and excitation filter wheel removed for lamp replacement

5. Unplug the lamp's cables from the reader.
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.

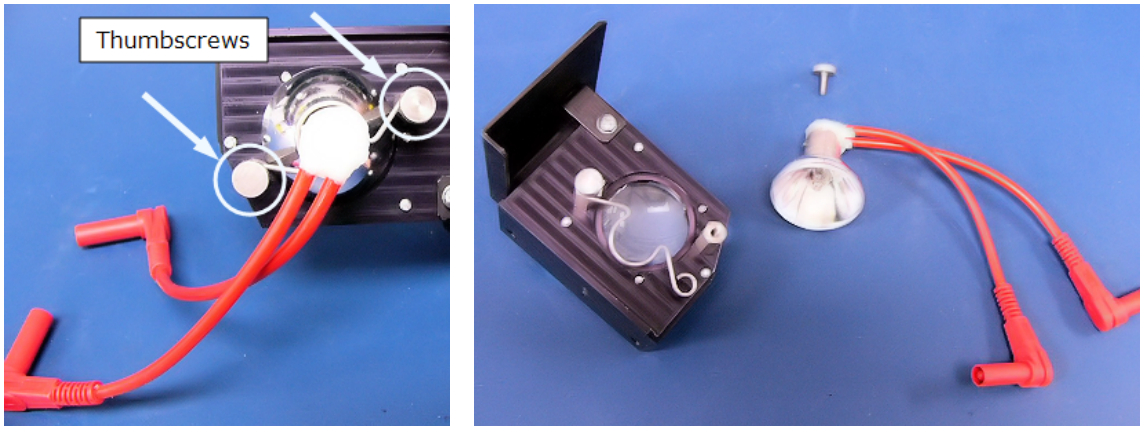


Figure 6-2: Removing the old bulb (with cables) from the bracket

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 **Error Codes** starting on page 163.

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 the Dispense Accuracy and Precision tests fail). If cleaning the injection system does not eliminate performance problems, or if a syringe is leaking, perform these instructions to replace a faulty syringe. Contact BioTek TAC to order replacement syringes.

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

Syringe Maintenance Position

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

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

1. In Gen5, select **System > Instrument Control > Synergy 2** and click the **Prime** tab (or **Dispenser** tab, if using Gen5 v2.05 or lower).
2. Select the appropriate Dispenser number (1 or 2) associated with the syringe.
3. Click **Maintenance**. The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve.

Replace the Syringe

Refer to Figure 2-13 on page 19.

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

1. Using your fingers, unscrew the bottom thumbscrew that secures the syringe, underneath the bracket. Retain this bottom thumbscrew; it is needed for the replacement syringe.
2. Unscrew the top thumbscrew to disengage the syringe from the valve.
3. Remove the new syringe from its protective box.
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 > Instrument Control > Synergy 2**.
8. Click the **Prime** tab and click **Initialize**.

Chapter 7

Instrument Qualification Process

This chapter describes the tests that BioTek Instruments, Inc. has developed for complete qualification of all models of the Synergy 2. This chapter introduces the various test methods, describes the materials and relevant Gen5 protocols used to execute the tests, explains how to analyze test results, and provides troubleshooting tips in the event of a failure.

Instrument Qualification Procedures starting on page 123 contains the actual step-by-step test procedures.

Instrument System Test

Each time the Synergy 2 is turned on, it automatically performs a series of tests on the reader's motors, lamp(s), the PMT, and various sub-systems. 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 will eject and the LED on the power switch will remain on and constant. The reader is then ready for use.

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 **Error Codes** starting on page 163 for information on error codes and troubleshooting tips.

Refer to **Sample Reports** on page 173 to see a sample System Test Report for Synergy 2.

Plate Shaker Test

This test verifies that the multi-speed plate shaker is operating properly. The test involves creating and running a protocol with shaking enabled for a duration of 30 seconds. The sound of the carrier shaking is all that needs to be confirmed to verify that the plate shaker is operating properly.

Absorbance Testing

For models with absorbance capability, BioTek developed a series of tests for the absorbance system using a combination of solid state Absorbance Test Plates and liquid plates. The test plates and the materials used for creating the liquid plates are available for purchase from BioTek.

To qualify the absorbance system for the Synergy 2, you should perform:

- Absorbance Liquid Test 1 *and* Absorbance Plate Test (using BTI #7260522) *or*
- Absorbance Liquid Test 2

Optionally, to qualify operation in the UV range, you should also perform:

- Absorbance Liquid Test 3 *or* Absorbance Plate Test at 340 nm (using BTI #7260551)

BioTek Absorbance Test Plates

Absorbance Test Plate PN 7260522 uses NIST-traceable neutral density filters to confirm absorbance specifications in the visible range (400–800 nm). This test plate also contains precision-machined holes to verify mechanical alignment, and a glass filter in position C6 to test the wavelength accuracy of the monochromator-based absorbance system. Absorbance Test Plate PN 7260551 uses NIST-traceable neutral density filters to confirm absorbance specifications in the UV range (340 nm).

Every test plate comes with a Test Plate Calibration Certificate, containing a table with Absorbance OD Standards for each filter at each wavelength supported by the plate. The certificate for test plate PN 7260522 also contains Wavelength Accuracy Standards tables with Expected Peak (nm) values with Test Ranges for the C6 glass filter.

Before the Absorbance Plate Test can be performed, the OD Standard values and Expected Peak/Test Range combinations must be entered into Gen5. Enter and save these values once initially, and then update them annually when the test plate is recertified by BioTek.

Test Methods

The Absorbance Plate Test is conducted using Gen5 software (System > Diagnostics > Test Plates) to confirm wavelength accuracy ("Peak Absorbance"); mechanical alignment; and optical density accuracy, linearity, and repeatability. When complete, Gen5 generates a results report displaying Pass or Fail for each individual test.

- **Peak Absorbance:** The BTI #7260522 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(s) of maximum absorbance are compared to the expected peak wavelength(s) supplied on the test plate's data sheet. The accuracy of the wavelength should be ± 3 nm (±2 nm instrument, ±1 nm filter allowance).

- **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:** The test plate contains NIST-traceable neutral-density glass filters of known OD values at one or more wavelengths. Actual measurements are compared against the expected values provided in the test plate's data sheet. 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. To further verify this, you can perform a linear regression analysis on the test plate OD values in a program such as Microsoft Excel; an R^2 value of at least 0.9900 is expected.
- **Repeatability:** This test ensures the instrument meets its repeatability specification by conducting repeated reads of each neutral-density filter on the test plate and comparing the results.

Sample Test Report

Refer to **Sample Reports** on page 173 to see a sample Absorbance Plate Test Report for Synergy 2.

Troubleshooting

If a test fails, try the troubleshooting tips below. If the test continues to fail, contact BioTek TAC.

Important! Do not remove filters from the Absorbance Test Plate. Do not use alcohol or other cleaning agents, and do not touch the filters with your bare fingers.

If a higher-OD well reports "#N/A" for Min/Max Limit and Result, the measured OD is beyond the specified range for Accuracy or Repeatability used with this test, and therefore no pass/fail determination is made. It does not indicate a test failure.

Peak Absorbance Test

- Check the filter in the C6 position to ensure it is clean. If needed, clean the filter with lens paper. Do not remove the filter, and do not use alcohol or other cleaning agents.
- Verify that the Peak wavelength information entered for the plate in Gen5 matches the information provided on the test plate's data sheet.
- Check the calibration due date on the test plate's label. If the test plate is overdue for

recalibration, contact BioTek to schedule service.

- Check the microplate carrier to ensure it is clear of debris.

Alignment Test

- Ensure that the test plate is properly 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 Test

- Check the neutral-density filters to ensure they clean (positions C1, D4, E2, F5, G3, H6). If needed, clean the filters with lens paper. Do not remove any filters, and do not use alcohol or other cleaning agents.
- Verify that the wavelength/expected OD values entered for the plate in Gen5 match the information provided on the test plate's data sheet.
- Check the calibration due date on the test plate's label. If the test plate is overdue for recalibration, contact BioTek to schedule service.

Repeatability Test

- Check the neutral-density filters 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.

Absorbance Liquid Tests

BioTek Instruments, Inc. has developed a series of liquid test procedures for testing your reader's absorbance system.

Test Methods

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. For the Repeatability portion of this test, two columns containing a color-absorbing solution are read five times at 405 nm. For each well, an "allowed deviation" is determined based on its Mean OD and the reader's repeatability specification. Each well's Standard Deviation must be less than its Allowed Deviation to pass. To confirm the reader’s mechanical alignment, the plate is rotated 180 degrees in the carrier (e.g., A1 is now in the H12 position) and the same two columns are read. The initial and new OD readings are compared, using the reader's accuracy specification. If the two readings in the same well do not meet specification, the reader may be out of alignment.

If an Absorbance Test Plate is not available, **Absorbance Liquid Test 2** may be conducted to test the instrument's alignment, repeatability, and accuracy by preparing a series of solutions of varying OD values as described on page 132.

Absorbance Liquid Test 3 is an optional test offered for those sites that must have proof of linearity at 340 nm. (Alternatively, the BioTek 340 nm Absorbance Test Plate may be used; see page 99.) This test is optional since the Synergy 2 has good "front-end" linearity throughout the specified wavelength range. While the absolute values of the OD cannot be determined by this test, the results will indicate if there is adequate repeatable absorbance and a linear slope. This method is dependent upon proper dye dilution and a skilled pipetting technique. It is expected that the first dilution (mid-level solution) will have an absorbance value near 75% of that of the stock (high-level) solution, and that the second dilution (low-level solution) will have an absorbance value near 50% of that of the stock solution.

Gen5 Protocol Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

ⓘ The Plate Type setting in each Gen5 protocol should match the actual plate in use.

Synergy 2 Abs Test 1.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Variable, 4 minutes
Two Read Steps	
Kinetic loop (one per Read step)	Set a Run time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromator
Read wells	First Read step: A1..H2 Second Read step: A11..H12
Wavelength	405 nm
Read Speed	Normal
Delay after plate movement	100 msec
Plate Out,In step between loops	Text "rotate the plate 180 degrees"

Synergy 2 Abs Test 2.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Variable, 4 minutes
Two Read Steps	
Kinetic loop (one per Read step)	Set a Run time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromator
Step labels	First Read step: "Normal" Second Read step: "Turnaround"
Read wells	Full plate
Wavelengths	2 (450 nm, 630 nm)
Read Speed	Normal
Delay after plate movement	100 msec
Data Reduction	Define two Delta OD transformations (450-630 nm), one per Read data set

Synergy 2 Abs Test 3.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Slow, 30 seconds
Kinetic loop	Set a Run time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromator
Read wells	A1..H6
Wavelength	340 nm
Read Speed	Normal
Delay after plate movement	100 msec

Results Analysis

The Absorbance Liquid Test procedures begin on page 131.

Absorbance specifications used with the liquid tests:

Accuracy:

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD
± 3.0% ± 0.010 OD from 2.000 OD to 3.000 OD

Repeatability:

± 1.0% ± 0.005 OD from 0.000 to 2.000 OD
± 3.0% ± 0.005 OD from 2.000 OD to 3.000 OD

Absorbance Liquid Test 1

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 (Mean OD x 0.010 + 0.005). For each well, its Standard Deviation should be less than its Allowed Deviation.

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

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

Absorbance Liquid Test 2

1. The plate is read five times at 450/630 nm ("Normal" position), resulting in five sets of Delta OD data. Calculate 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^2 value of at least 0.9900 is considered adequate.

2. Calculate the results for Repeatability:
   - Calculate the Mean and Standard Deviation for the five readings taken at each concentration. Only one row of data needs to be analyzed.
   - For each Mean below 2.000 OD, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate of ±1.0% ±0.005 OD. (If above 2.000 OD, apply the ±3.0% ±0.005 specification.)
   - The Standard Deviation for each set of readings should be less than the Allowed Deviation.

     Example: Readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a Mean of 1.951, and a Standard Deviation of 0.0026. The Mean (1.951) multiplied by 1.0% (1.951 x 0.010) = 0.0195, which, when added to the 0.005 (0.0195 + 0.005) = 0.0245 OD, which is the Allowed Deviation. Since the Standard Deviation is less than this value, the reader meets the test criteria.
3. After gathering data for the Linearity Test, the plate is read five more times with the A1 well in the H12 position ("Turnaround" position). This results in values for the four corner wells that can be used to assess alignment. Calculate results for the Alignment Test:
   - Calculate the means of the wells A1 and H1 in the Normal plate position (data from Linearity Test) and in the Turnaround position.
   - 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 96-well plates. If the four corner wells are within the accuracy range, the reader is in alignment.

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

Absorbance Liquid Test 3

1. The plate is read five times at 340 nm. 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 (Mean OD x 0.010 + 0.005). For each well, its Standard Deviation should be less than its Allowed Deviation.

   Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a Mean of 0.8004 and a Standard Deviation of 0.0018. The Mean multiplied by 1.0% (0.8004 * 0.010) equals 0.008, and when added to 0.005 equals 0.013; this is

the Allowed Deviation for well A1. Since the Standard Deviation for well A1 is less than 0.013, the well meets the test criteria.

3. Calculate 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^2 value of at least 0.9900 is considered adequate.

Troubleshooting

If an absorbance liquid test fails, try the following. If a test continues to fail, contact BioTek TAC.

- Check the microwells and plate carrier for debris that may have shifted and caused changes.
- Ensure the microplate is properly seated in the carrier.
- As applicable, confirm that the plate was properly oriented in the "Normal" and "Turnaround" positions.
- Liquid Test 1 can fail due to the meniscus effect, which can cause readings to decrease over time. If you suspect this may be the case, include a shake step between the read steps in the protocol.

Luminescence Testing

For models with luminescence capability, BioTek uses the Harta Luminometer Reference Microplate to test the luminescence system. The test plate is LED-based and NIST-traceable. Contact BioTek to purchase a plate (BTI #8030015; includes microplate carrier adapters) or visit www.HartaInstruments.com to learn more.

Test Method

The Harta Luminometer Reference Microplate is used to determine a detection limit by leveraging a known correlation of 35 photons per attomole of ATP. By using the NIST data provided with the Harta plate in photons/s, a conversion factor of 0.02884 attomole/photon is applied to determine an ATP concentration and subsequent limit of detection for the instrument under test.

Gen5 Protocol Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s).

Synergy 2 LumTest_Harta.prt

Parameter	Setting
Plate Type	"Costar 96 black opaque"
Delay Step	3 minutes
READ STEP 1	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Reference well A2
Read well	A2
Filter Set	1
Emission	Hole
Optics Position	Top
Gain	150
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	350 msec
Dynamic Range	Standard
Read Height	5.00 mm
READ STEP 2	

Parameter	Setting
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Background
Read wells	F1–G12
Filter Set	1
Emission	Hole
Optics Position	Top
Gain	150
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	350 msec
Dynamic Range	Standard
Read Height	4.00 mm
READ STEP 3	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Battery check
Read wells	A7–A8
Filter Set	1
Emission	Hole
Optics Position	Top
Gain	50
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	350 msec
Dynamic Range	Extended
Read Height	5.00 mm

Results Analysis

The Luminescence Test procedure is described on page 136.

1. Determine if the plate's battery is functioning properly. If A8 > (0.2 * A7), the battery is good. Otherwise, it requires replacement.

   A replacement battery is included with each new and recalibrated Harta Luminometer Reference Microplate.

2. On the Harta plate's calibration certificate, locate the NIST measurement for the A2 position. Convert it to attomoles: (A2 NIST measurement * 0.02884)
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

Pass/Fail Criteria

- 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 reader: #49984=low-noise PMT; #49721=red-shifted PMT

Troubleshooting

If a test fails, try the suggestions below. If a test continues to fail, contact BioTek's Technical Assistance Center (TAC).

- Ensure that the reading is performed through a hole in the EM filter wheel, not through a glass filter.
- Verify that the filter wheel definitions in Gen5 match the physical item.
- The optical probe(s) *may* need to be cleaned; contact BioTek TAC for guidance.

Fluorescence Testing

For models with fluorescence capability, BioTek has developed a series of liquid test procedures for testing your reader's fluorescence system.

Fluorescence Liquid Tests

Test Methods

- **Corners:** The Corners Test uses fluorescent compounds to verify that the plate carrier is properly aligned in relation to the fluorescence probe(s). This test may be conducted using either the top or bottom optics (as supported by your reader model).
- **Fluorescence Intensity (Sensitivity):** The Sensitivity Test measures a fluorescent compound (Sodium Fluorescein or Methylumbelliferone) and buffer solution to test the fluorescence reading capability of the instrument. The ability to detect specific compounds at the required limit of detection ensures that the filters, optical path, and PMT are all in working order. This test establishes the detection limit of the instrument, which is described as the lowest concentration that will create a signal that is statistically distinguishable from the buffer well. This test may be conducted using either the top or bottom optics (as supported by your reader model).
- **Linearity:** The Linearity Test is conducted in conjunction with the Sensitivity Test. It verifies that the system is linear, that is, signal changes proportionally with changes in concentration. This test is conducted using only the top optics.
- **Time-Resolved Fluorescence:** The optional TRF Test measures a fluorescent compound (Europium) and buffer solution to test the time-resolved fluorescence reading capability of the instrument. The ability to detect specific compounds at the required limit of detection ensures that the filters, optical path, and PMT are all in working order. This test establishes the detection limit of the instrument, which is described as the lowest concentration that will create a signal that is statistically distinguishable from the buffer well. This test is conducted using only the top optics.
- **Fluorescence Polarization:** The optional FP Test measures high- and low-polarized samples to verify the instrument's ability to measure polarization of a liquid fluorophore and confirm that the excitation and emission polarizers are properly oriented in the instrument. This test is conducted using only the top optics.

Gen5 Protocol Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

ⓘ The Plate Type setting in each Gen5 protocol should match the actual plate in use.

When performing the Fluorescence Intensity (FI) tests, if a test fails due to overranged well(s), reduce the Gain in the protocol by 1–5 counts and try the test again.

Synergy 2 FI_T.prt

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915)
Two Read Steps	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Labels	"Corners Read" "Sensitivity Read"
Read wells	Corners Read: Full plate Sensitivity Read: Wells C1–F12
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top 510 nm
Gain	75
Read Speed	Normal
Delay after plate movement	350 msec
Measurements per data point	40
Dynamic Range	Standard
Light Source	Tungsten
Read Height	5.00 mm

Synergy 2 FI_B.prt

Parameter	Setting
Plate Type	"Greiner SensoPlate" (#655892)
Two Read Steps	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Labels	"Corners Read" "Sensitivity Read"
Read wells	Corners Read: Full plate Sensitivity Read: Wells C1–F12
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Bottom
Gain	Corners Read: 80 Sensitivity Read: 100
Read Speed	Normal
Delay after plate movement	350 msec
Measurements per data point	40
Dynamic Range	Standard
Light Source	Tungsten

Synergy 2 FP.prt

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915)
Synchronized Mode	Plate mode with timing control
Detection Method	Fluorescence polarization
Read Type	Endpoint
Optics Type	Filters
Read wells	A5–H9
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top 510 nm

Parameter	Setting
Gain	100
Read Speed	Normal
Delay after plate movement	350 msec
Measurements per data point	60
Dynamic Range	Standard
Light Source	Tungsten
Read Height	5.00 mm

Synergy 2 TRF.prt

Parameter	Setting
Plate Type	"Costar 96 white opaque" (#3912)
Shake Step	Medium, 30 seconds
Delay Step	3 minutes
Detection Method	Time-resolved fluorescence
Read Type	Endpoint
Optics Type	Filters
Read wells	A5–H9
Filter Set	1
Excitation	360/40 nm
Emission	620/40 nm
Optics Position	Top 400 nm
Gain	125
Read Speed	Normal
Delay after plate movement	350 msec
Measurements per data point	20
Delay before collecting data:	300 µsec
Data collection time:	1000 µsec
Light Source	Xenon Flash
Read Height	5.00 mm

Synergy 2 FI_MUB.prt

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Read wells	C1–F12
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top 50%
Gain	90
Read Speed	Normal
Delay after plate movement	350 msec
Measurements per data point	40
Dynamic Range	Standard
Light Source	Tungsten
Read Height	5.00 mm

Results Analysis

The Fluorescence Liquid Test procedures begin on page 137.

Corners Test

1. Calculate the Mean of the twelve "corner" wells (A1–A3, A10–A12, H1–H3, and H10–H12).
2. Calculate the Standard Deviation of the same twelve wells.
3. Calculate the %CV: (Standard Deviation/Mean)*100

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

Sensitivity Test

Using Sodium Fluorescein

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/((MeanSF-MeanBuffer)/(3*STDBuffer))

Optic Probe	The Detection Limit must be:
Bottom 5 mm	<26 pM (10 pg/mL)
Bottom 3 mm	<53 pM (20 pg/mL)
Bottom 1.5 mm	<106 pM (40 pg/mL)
Top, with 510 nm dichroic mirror	<5 pM (2 pg/mL)

Using Methylumbelliferone

1. Calculate the Mean and Standard Deviation for the buffer wells (C10–F12).
2. Calculate the Mean for the 17.6 ng/mL (100 nM) MUB solution wells (C1–F1).
3. Calculate the Detection Limit, in ng/mL:

   17.6/((MeanMUB-MeanBuffer)/(3*STDBuffer)

   The Detection Limit must be **<0.16 ng/mL** to pass.

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:

Using Sodium Fluorescein	
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
Using Methylumbelliferone	
x	**y**
100	Mean of the 100 nM wells
50	Mean of the 50 nM wells
25	Mean of the 25 nM wells
12.5	Mean of the 12.5 nM wells
6.25	Mean of the 6.25 nM wells

3. Calculate the R^2 value; it must be **>=0.9500** to pass.

Time-Resolved Fluorescence (TRF) Test

1. Calculate the Mean and Standard Deviation of the wells containing deionized water (A6–H6).

2. Calculate the Mean and Standard Deviation of the wells containing the Europium solution (A8–B8).
3. Calculate the Detection Limit, in fM:

   20000/((MeanEu–MeanDIWater)/(3*STD DIWater))

   The Detection Limit must be **<=250 fM** to pass.

Fluorescence Polarization (FP) Test

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

   (Parallel LPR Signal*(1-0.02))/(Perpendicular LPR Signal*(1+0.02))
4. Calculate the Mean G-Factor.
5. Calculate the Polarization value in mP for each HPR well (“PHPR”):

   (Parallel HPR Signal-Mean G Factor*Perpendicular HPR Signal)/(Parallel HPR Signal+Mean G Factor*Perpendicular HPR Signal)*1000
6. Calculate the Mean PHPR, in mP.

   The Mean PHPR must be **>340 mP** to pass.
7. Calculate the Polarization value in mP for each LPR well (“PLPR”):

   (Parallel LPR Signal-Mean G Factor*Perpendicular LPR Signal)/(Parallel LPR Signal+Mean G Factor*Perpendicular LPR Signal)*1000
8. Calculate the Standard Deviation of the "PLPR", in mP.

   The Standard Deviation of the PLPR must be **<5 mP** to pass.

Troubleshooting

If a fluorescence liquid test fails, try the relevant suggestions below. If a test continues to fail, print the results and contact BioTek TAC.

- Are the solutions fresh? Discard open/unused buffer and stock solutions after seven days.
- Are the Excitation/Emission filters clean? Are they in the proper locations and in the proper orientation in the filter cube or wheel?
- Are you using new/clean plates? If the base of a clear-bottom plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster. If the test fails again, the optical probe(s) may need to be cleaned. Contact BioTek TAC for instructions.
- Review the pipetting instructions to verify the plate was correctly prepared.
- Does the Plate Type setting in the Gen5 protocol match the plate you used?
- For injector models, spilled fluid inside the reader may be fluorescing, which can corrupt your test results. If you suspect this is a problem, contact BioTek TAC for assistance.
- When testing Fluorescence Polarization capability using a solid black plastic microplate, if the standard deviation for the buffer wells is too high, try either moving the buffer wells to another column, or using the Greiner SensoPlate. 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.
- If the Corners Test continues to fail, the hardware may be misaligned. Contact BioTek TAC.

Injection System Testing

For models equipped with injectors and an external dispense module, BioTek has developed a set of tests to ensure that the injection system performs to specification.

Test Method

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.

The test uses a green dye test solution (available for purchase from BioTek, see page 5) and one 96-well microplate per injector to test the three different volumes. The balance is tared with the empty plate and the 80 µL dispense is performed for columns 1–4. The fluid is weighed and the balance is tared again with the plate. This process is repeated for the 20 µL and 5 µL dispenses.

It is assumed that one gram is equal to one milliliter and the solutions used are at room temperature. A three-place precision balance is used to weigh the plate.

The **Precision Test** is a measure of the variation among volumes dispensed to multiple wells, and uses the green test dye solution. For each volume dispensed (80 µL, 20 µL, and 5 µL) to four columns, the %CV 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. Columns 1–4 are read at 405/750 nm and columns 5–12 at 630/750 nm.

The Accuracy and Precision tests are performed simultaneously and use the same plate.

Gen5 Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

ⓘ The Plate Type setting in each Gen5 protocol should match the actual plate in use.

Synergy 2 Disp 1 Test.prt and **Synergy 2 Disp 2 Test.prt** (for use with models with Absorbance capability)

Parameter	Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Wells A1–H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at 275 µL/sec

Parameter	Setting
Plate Out,In	Comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A5–H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at 250 µL/sec
Plate Out,In	Comment: Weigh the plate (20 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A9–H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at 225 µL/sec
Plate Out,In	Comment: Weigh the plate (5 uL test). RECORD the weight, TARE the balance. PIPETTE 150 µL/well of DI water into all 12 columns. Place the plate back on the carrier. Click OK to perform the Read steps.
Shake Step	Linear, 15 seconds, default frequency
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: Monochromator Step label: 80 ul Read_Disp <1 or 2> Wells: A1–H4 Wavelengths, 2: 405 nm, 750 nm Speed: Normal
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: Monochromator Step label: 20 and 5 ul Read_Disp <1 or 2> Wells: A5–H12 Wavelengths, 2: 630 nm, 750 nm Speed: Normal
Data Reduction	Define two Delta OD transformations: 405–750 nm for the 80 uL Read step, columns 1–4 630–750 nm for the 20 and 5 uL Read step, columns 5-12

Synergy 2 Disp 1 Test No Read.prt and **Synergy 2 Disp 2 Test No Read.prt** (for use with models without Absorbance capability)

Parameter	Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at 275 µL/sec
Plate Out,In	Comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A5..H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at 250 µL/sec
Plate Out,In	Comment: Weigh the plate (20 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A9..H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at 225 µL/sec
Plate Out,In	Comment: Weigh the plate (5 uL test). RECORD the weight, TARE the balance. PIPETTE 150 µL/well of DI water into all 12 columns. Set the plate aside and click OK.
Read Step	*Define a brief Read step for a single well. The measurement value will not be used. The step is only necessary because Gen5 requires a Read step with dispense protocols.*

Synergy 2 Disp Test Other Reader.prt (for use with a BioTek absorbance-capable reader other than Synergy 2)

Parameter	Setting
Shake Step	<medium intensity> for 15 seconds
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: <as appropriate for the reader type> Step label: 80 ul Read Wells: A1..H4 Wavelengths, 2: 405 nm, 750 nm Speed: Normal

Parameter	Setting
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: <as appropriate for the reader type> Step label: 20 and 5 ul Read Wells: A5..H12 Wavelengths, 2: 630 nm, 750 nm Speed: Normal
Data Reduction	Define two Delta OD transformations: 405-750 nm for the 80 ul Read step, columns 1-4 630-750 nm for the 20 and 5 ul Read step, columns 5-12

Results Analysis

The Injection System Test procedures begin on page 149.

When the experiment for one injector is complete, 32 delta OD values are reported for each of the three dispense volumes. 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 injector (1, 2):

1. Calculate the Standard Deviation of the 32 wells
2. Calculate the Mean of the 32 wells
3. Calculate the %CV: (Standard Deviation / Mean) x 100
4. Calculate the Accuracy % Error:

   ((ActualWeight–ExpectedWeight)/ExpectedWeight)* 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%

If any tests fail, prime the fluid lines and rerun the tests. If the tests fail again, the injectors may require cleaning; see *Clean the Internal Components* starting on page 75. If tests continue to fail, contact BioTek TAC.

Chapter 8

Instrument Qualification Procedures

This chapter contains the step-by-step procedures for verifying that the Synergy 2 and its various sub-systems are performing to specification.

Instrument Qualification Process starting on page 97 introduces the various test methods, describes the materials and relevant Gen5 protocols used to execute the tests, explains how to analyze test results, and provides troubleshooting tips in the event of a failure.

Overview

This chapter contains BioTek Instrument's recommended qualification procedures for all Synergy 2 models.

Every Synergy 2 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.

See the *Recommended Qualification Schedule* on page 126 to determine which qualification tests shall be conducted for your Synergy 2 model and to meet your site's regulatory requirements.

A Product Qualification Package (BTI #7130566) for the Synergy 2 is available for purchase. The package contains test procedures, Gen5 protocols, checklists, and logbooks for performing Installation Qualification, Operational Qualification, Performance Qualification, and Preventive Maintenance. Contact your BioTek dealer for more information.

IQ/OQ/PQ Description

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

- The recommended IQ procedure consists of setting up the instrument and its components as described in the Installation chapter, and performing the System Test. For models with injectors, a quick test with fluid is also performed, to ensure that the dispense module is properly installed and there are no leaks.
- The IQ procedure should be performed before the reader is used for the first time. The successful completion of the IQ procedure verifies that the instrument is installed correctly.

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 Fluorescence Tests, and, if the external dispense module is used, Dispense Accuracy and Precision Tests.
- The OQ procedure should be performed initially (before first use) and then routinely; the recommended interval is annually. It should also be performed after any major repair or upgrade to the hardware or software.
- Although out-of-tolerance failures will be detected by the OQ tests, results should be compared with those from the routine Performance Qualification tests and previous OQ tests to monitor for trends.
- The successful completion of the OQ procedure, in combination with results that are comparable to previous PQ and OQ tests, confirms that the equipment is operating according to specification initially and over time.

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

- The recommended PQ procedure consists of performing the System Test, Absorbance Plate Test, a series of Fluorescence Tests, and, if the external dispense module is used, 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 2 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.

	IQ	OQ	PQ	
Tasks/Tests	Initially	Initially/ Annually	Monthly	Quarterly
All models:				
Installation, setup, and configuration of the reader, host computer, and Gen5	✓			
System Test	✓	✓	✓	
Models with absorbance capability:				
Absorbance Plate Test		✓	✓	
Absorbance Liquid Test 1 or Liquid Test 2*		✓		✓
(Optional) Absorbance Liquid Test 3 *or* 340 nm Absorbance Plate Test (using BTI #7260551)		✓		✓
Models with fluorescence capability:				
Corners, Sensitivity, Linearity (FI) Tests		✓	✓	
Fluorescence Polarization (FP) Test		✓		✓
Time-Resolved Fluorescence (TRF) Test		✓		✓
Models with luminescence capability:				
Luminescence Test		✓	✓	
Models with injectors and an external dispense module:				
Installation and setup of external dispense module	✓			
Injection System Test	✓			
Dispense Accuracy and Precision Test		✓		✓

* If you have Absorbance Test Plate BTI #7260522, perform Liquid Test 1. Otherwise, perform Liquid Test 2.

System Test

Instrument System Test starting on page 98 describes this test and explains where to find information on error codes and troubleshooting tips, as well as sample test reports for Synergy 2.

Setup

- If your assays use incubation, we recommend enabling temperature control for at least 37°C and allowing the incubator to reach its set point before running the System Test. To access this feature, select **System > Instrument Control** and click the **Pre-Heating** tab.

Test Procedure

1. From the Gen5 main screen, select **System > Diagnostics > Run System Test**.

   The duration of the test depends on the reader model; it can take a few minutes to complete.

   If the test fails during execution, a message box will appear in Gen5. Close the box; the System Test Report will contain the error code that was generated by the failure.

2. When the test is complete, a dialog will appear, requesting additional information. Enter any required information and then click **OK**.
3. The test report will appear; scroll to the bottom, it will show either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL *** ERROR (error code) DETECTED."

   If the test failed, go to page 163 to look up the error code and 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 TAC.
4. If required, print, sign, and date the report, and store it with your test documentation.
5. If applicable, turn off the incubator.

Plate Shaker Test

Refer to the Gen5 Help system for complete instructions for defining a protocol and setting the shake parameters.

1. Create a protocol with a Shake step and a Read step in its procedure.
   - In the Shake step, set the Intensity to Variable and the Duration to 30 seconds. Alternatively, choose shake parameters that mostly closely align with your assays.
   - In the Read step, define an endpoint read. You can select any detection method; it does not affect the result of the Shake test.
2. Create an experiment based on this protocol and then read a plate. If you can hear the plate shaking, the shaker is operating properly. If you do not hear the plate shaker, contact BioTek.

Absorbance Plate Tests

BioTek Absorbance Test Plates starting on page 99 describes the test methods and provides troubleshooting tips in the event of test failure.

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

Requirements

To perform this test, you will need:

- Absorbance Test Plate, BTI #7260522
- (Optional) 340 nm Absorbance Test Plate, BTI #7260551
- Current Absorbance Test Plate Calibration Certificate(s)

Setup

Before an Absorbance Test Plate can be used for qualification, you must enter information from its Calibration Certificate into Gen5. Perform these steps initially, and then repeat them annually after the test plate is recertified by BioTek:

1. Obtain the current Test Plate Calibration Certificate.
2. Start Gen5 and select **System > Diagnostics > Test Plates > Add/Modify Plates**.
3. Click **Add**. The Absorbance Test Plate dialog appears.
4. Select the appropriate Plate Type and then enter the plate’s serial number.
5. Enter the Last Certification and Next Certification dates from the calibration label on the Test Plate.
6. If the wavelength values in the top row of the grid in Gen5 are appropriate for your tests, enter the OD Standard values from the Calibration 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**. Add, change, or delete the values as needed and click **OK**.

7. If applicable: Select the number of Peak Wavelength tests to run (up to 4), based on the desired Expected Peak wavelengths provided on the Calibration Certificate. Enter the Expected Peak value(s) from the Certificate and set the Test Range – and + values.

Depending on the manufacture date of the test plate, the glass type may be Erbium, Holmium, or Didymium. Contact BioTek TAC if you are not sure which glass type is used in your plate.

- If the C6 filter is Erbium or Holmium glass, the certificate contains two Spectral Bandpass tables.

  Use the values in the **2.4 nm** table with Synergy 2.

  Erbium: Any peak value in the table can be used.

  Holmium: For best results use the expected peak values *closest to* 242, 279, 362, 417, and 538 nm.

- If the C6 filter is Didymium glass, the certificate provides a single peak wavelength value. Enter this value into Gen5 and set the Test Range – and + values so the range displayed in parenthesis is "(580 to 590)".

8. Review all of the values that you entered. When finished, click **OK** to save the information.

Test Procedure

1. In Gen5, select **System > Diagnostics > Test Plates > Run**. If prompted, select the desired Test Plate and click **OK**.
2. When the Absorbance Test Plate Options dialog appears, enter any required information.
3. If applicable, check the **Perform Peak Wavelength Test box**.
4. Highlight the wavelength(s) to be included in this test. Select only those wavelengths most appropriate for your use of the reader.
5. (Optional) Enter a comment.
6. Click **Start Test**.
7. Place the Absorbance Test Plate on the microplate carrier, with A1 in the proper location.
8. Click **OK** to run the test.
9. When the test completes, the results report will appear. Scroll down through the report; every result should show "PASS".
   - Troubleshooting tips are provided on page 100.
   - Test descriptions are provided on page 99.

Absorbance Liquid Tests

Absorbance Liquid Tests starting on page 101 describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Absorbance Liquid Test 1

The tests in this section require specific microplates, solutions, and filters or wavelengths. Your laboratory may require a deviation from some of these tests. For example, you may wish to use a different plate or test solution. If deviation from the tests as presented in this section is required, perform the following steps the first time each test is run:

- Perform the tests exactly as described here.
- Rerun the tests using your particular plates, solutions, and so on.
- If results are comparable, then the results from these tests will be your baseline for future tests. Document your new test procedure and save all test results.

Materials

Manufacturer part numbers are subject to change.

- 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 materials listed below (B)
- Gen5 protocol **Synergy 2 Abs Test 1.prt** described on page 102

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 µL in a flat-bottom microwell.

Test Procedure

ⓘ Be sure to use a new microplate. Debris, fingerprints, or scratches may cause variations in readings.

1. Using freshly prepared stock solution (Solution A or B), prepare a 1:2 dilution using deionized water (one part stock, one part deionized water; the resulting solution is a 1:2 dilution).
2. Pipette 200 µL/well of the stock solution into column 1.
3. Pipette 200 µL/well of the diluted solution into column 2.
4. Create a Gen5 experiment based on the **Synergy 2 Abs Test 1** protocol and read the plate. When prompted, rotate the plate 180 degrees and continue.
5. When the experiment is finished:
   - Save the experiment. Refer to the instructions on page 104 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 106.
   - Test descriptions are provided on page 101.

Absorbance Liquid Test 2

The recommended method for testing the instrument's alignment, repeatability, and accuracy is to use Absorbance Test Plate BTI #7260522 (see page 129). If the test plate is not available, however, Liquid Test 2 can be used for these tests.

Materials

Manufacturer part numbers are subject to change.

- New 96-well clear flat-bottom microplate (Corning Costar #3590 recommended)
- Ten test tubes, numbered consecutively, set in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Stock Solution A or B (see instructions for Liquid Test 1)
- 0.05% solution of deionized water and Tween 20
- Gen5 protocol **Synergy 2 Abs Test 2.prt** described on page 103

Test Procedure

1. 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.

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 2.000 OD 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.

2. 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.
3. 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).
4. Create a Gen5 experiment based on the **Synergy 2 Abs Test 2** protocol and read the plate. When prompted, rotate the plate 180 degrees.
5. When finished:
   - Save the experiment. Refer to the instructions on page 104 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 106.
   - Test descriptions are provided on page 99.

Absorbance Liquid Test 3

Absorbance Liquid Test 3 is provided for sites requiring proof of linearity at 340 nm. This test is optional because the Synergy 2 has good "front end" linearity throughout its wavelength range. As an alternative, the 340 nm Absorbance Test Plate (BTI #7260551) may be used for this test.

Materials

Manufacturer part numbers are subject to change.

- New 96-well clear flat-bottom microplate (Corning Costar #3590 recommended)
- Calibrated hand pipette(s)
- Beakers and graduated cylinder
- Precision balance with readability to 0.010 g
- Buffer solution described below
- Gen5 protocol **Synergy 2 Abs Test 3.prt** described on page 103

Buffer Solution

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

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

Test Procedure

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

4. Create a Gen5 experiment based on the **Synergy 2 Abs Test 3** protocol and read the plate.
   - Save the experiment. Refer to the instructions on page 105 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 106.
   - Test descriptions are provided on page 101.

Luminescence Test

Luminescence Testing starting on page 107 describes the test method, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Requirements

To perform this test, you will need:

- Harta Luminometer Reference Microplate, BioTek PN 8030015 (which includes adapter PN 8032028 for this reader)
- Gen5 protocol described on page 107:
  - **Synergy 2 LumTest_Harta**
- A Plug in the Excitation filter wheel
- An open position (Hole) in the Emission filter wheel

Test Procedure

1. Turn on the Harta reference plate using the I/O switch on the back of the plate.
2. Check the battery by pressing the test button on the back of the plate and ensuring that the test light turns on. The test light may be difficult to see in bright light; change your angle of view or move to a darker environment if necessary. If the light does not turn on, replace the battery.
3. Place the adapter on the reader's microplate carrier and then place the Harta reference plate on top of the adapter.
4. In Gen5, create an experiment based on the **Synergy 2 LumTest_Harta** protocol and initiate a plate read.

   The experiment begins with a three-minute Delay step.

5. When the experiment is complete, calculate and evaluate results as described under *Results Analysis* on page 109.
6. When finished, turn off the Harta reference plate to preserve battery life.

Fluorescence Liquid Tests

Fluorescence Liquid Tests starting on page 110 describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

The tests in this section require specific microplates, solutions, and filters or wavelengths. Your laboratory may require a deviation from some of these tests. For example, you may wish to use a different plate or test solution. If deviation from the tests as presented in this section is required, perform the following steps the first time each test is run:

- Perform the tests exactly as described here.
- Rerun the tests using your particular plates, solutions, and so on.
- If results are comparable, then the results from these tests will be your baseline for future tests. Document your new test procedure and save all test results.

Materials

	Kits containing the microplates and solutions required by the Liquid Tests are available for purchase; see *Materials for Conducting Liquid Tests* on page 5.

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

Manufacturer part numbers are subject to change.

All tests:

- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- 95% ethanol (for cleaning clear-bottom plates)
- Aluminum foil
- (Optional, but recommended) 0.45-micron filter
- (Optional) Black polyethylene bag(s) to temporarily store plate(s)
- Gen5 protocols listed below (as applicable for your reader model) and described in detail under *Gen5 Protocol Parameters* starting on page 111

Synergy 2 FI_B.prt	Corners, Sensitivity, Linearity tests, Bottom optics, Sodium Fluorescein (SF)
Synergy 2 FI_T.prt	Corners, Sensitivity, Linearity tests, Top optics, Sodium Fluorescein (SF)
Synergy 2 FP.prt	Fluorescence Polarization (FP) test
Synergy 2 TRF.prt	Time-Resolved Fluorescence (TRF) test
Synergy 2 FI_MUB.prt	Corners, Sensitivity, Linearity tests, Top optics, Methylumbelliferone (MUB, alternate/supplemental test for Top optics)

Corners/Sensitivity/Linearity (FI) Tests:

The materials listed here are for use with Sodium Fluorescein. Methylumbelliferone (MUB) may be used as an alternate or supplemental method for conducting the FI tests for the Top optics; see page 145.

If using test kit BTI #7160010 or #7160013 (see page 5) the buffer and SF are pre-diluted.

- 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 range 4–10
- Sodium Fluorescein Powder (1-mg vial, BioTek PN 98155)
- Bottom optics:
  - 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
- Top optics:
  - A new, clean 96-well solid black microplate, such as Corning Costar #3915. The Greiner SensoPlate mentioned above can also be used.
- Excitation filter 485/20 nm installed
- Emission filter 528/20 nm installed
- 510 nm dichroic mirror installed

Fluorescence Polarization (FP) Test:

The FP Test may be conducted using the same plate as for the **Top** Corners/Sensitivity/Linearity (FI) Tests.

- The recommended test solutions are available from Invitrogen Corporation in their "FP One-Step Reference Kit" (#P3088) or BioTek (#7160014; see page 5). This kit includes:
  - (Green) Polarization Reference Buffer, 15 mL
  - Green Low Polarization Reference, 4 mL
  - Green High Polarization Reference, 4 mL
  - The Invitrogen kit also includes two red polarization solutions, not used
- A new, clean, 96-well solid black microplate, such as Corning Costar #3915. A Greiner SensoPlate can also be used.
- Excitation filter 485/20 nm installed

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

Time-Resolved Fluorescence (TRF) Test:

- The recommended test solution (FluoSpheres carboxylate-modified microspheres, 0.2 µm europium luminescent, 2 µL) is available from Invitrogen Corporation (#F20881) or BioTek (#7160011; see page 5)
- A new, clean 96-well solid white microplate, such as Corning Costar #3912
- 15-mL conical-bottom, polypropylene sample tube
- Excitation filter 360/40 nm installed
- Emission filter 620/40 nm installed
- 400 nm dichroic mirror installed

Test Solutions

Determine which tests to run for your reader model. Prepare the necessary test solutions using the materials described on the previous pages.

Corners/Sensitivity/Linearity (FI) Tests:

If using BioTek's sodium fluorescein powder (BTI #98155), be sure to hold the vial upright and open it carefully; the material may be concentrated at the top. If a centrifuge is available, spin down the tube before opening.

When diluting the sodium fluorescein powder in buffer, it takes time for the powder to completely dissolve. Allow the solution to dissolve for five minutes, with intermittent vortexing, before preparing the titration dyes.

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

1. The Sodium Borate solution does not require further preparation; proceed to step 2. If you are using PBS, prepare the solution:
   - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
   - Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
   - Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
   - Check the pH; it should be between 7.2 and 7.6 at 25°C.

2. Prepare the sodium fluorescein stock solution:
   - Add 2.0 mL of the buffer solution to the 1 mg Sodium Fluorescein (SF) vial. This yields a 1.3288 mM stock solution.
   - Ensure that the dye has completely dissolved and is well mixed.
3. Carefully prepare the dilutions. Label each with "SF" and the concentration:

Mix this SF solution:	with buffer:	to make:	
0.53 mL of 1.3288 mM stock solution	13.47 mL	50.2 µM	
110 µL of 50.2 µM SF	13.89 mL	400 nM	
3.5 mL of 400 nM SF	10.50 mL	100 nM	
0.46 mL of 100 nM SF	13.54 mL	**3.3 nM**	*Corners Test*
4.24 mL of 3.3 nM SF	9.76 mL	**1 nM**	*Sensitivity/Linearity Tests*

Fluorescence Polarization (FP) Test:

As described in the *Materials* section, the recommended test solutions are available from Invitrogen Corporation (or BioTek). They do not require additional preparation.

Time-Resolved Fluorescence (TRF) Test:

As described in the *Materials* section, the recommended test solutions are available from Invitrogen Corporation (or BioTek).

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

> 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.

Test Procedure

1. If you have not already done so, prepare the solutions for the tests you plan to perform. See instructions starting on page 140.

Refer to the pipette maps on the next few pages for the remaining steps.

2. Perform the Corners/Sensitivity/Linearity tests using the Bottom optics:
   - Pipette the solutions into a clean 96-well glass-bottom or quartz microplate.
   - Create an experiment based on **Synergy 2 FI_B.prt** and read the plate.
3. Perform the Corners/Sensitivity/Linearity tests using the Top optics:
   - Pipette the solutions into a new 96-well solid black or glass-bottom plate.
   - Create an experiment based on **Synergy 2 FI_T.prt** and read the plate.
4. If your reader is equipped with Fluorescence Polarization capability:
   - Pipette the solutions for the "FP" test into the same plate as used in step 3.
   - Create an experiment based on **Synergy 2 FP.prt** and read the plate.
5. If your reader is equipped with Time-Resolved Fluorescence capability:
   - Pipette the solutions for the "TRF" test into a new 96-well solid white plate.
   - Create an experiment based on **Synergy 2 TRF.prt** and read the plate.
6. Save the experiments. Refer to the instructions starting on page 114 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 116.
   - Test descriptions are provided on page 110.

Pipette Maps

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

Corners, Sensitivity, and Linearity (FI) Tests:

Using a single-channel pipette:

- Pipette **200 µL** of the **3.3 nM SF** solution into the twelve "corner" wells.
- Pipette 200 µL of the buffer in the wells surrounding the SF. (Omit if using a solid black plate or Greiner SensoPlate.)

Using a multi-channel pipette with just four tips installed:

- 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
	B	BUF	BUF	BUF	BUF					BUF	BUF	BUF	BUF
	C	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
Sensitivity/	D	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
Linearity	E	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
	F	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM					BUF	BUF	BUF
	G	BUF	BUF	BUF	BUF					BUF	BUF	BUF	BUF
Corners	H	3.3 nM	3.3 nM	3.3 nM	BUF					BUF	3.3 nM	3.3 nM	3.3 nM

Fluorescence Polarization (FP) Test:

The plate used for testing Corners/Sensitivity/Linearity of the Top optics can also be used for this 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 (DI) 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						

Alternate/Supplemental Tests Using Methylumbelliferone (MUB)

(Optional) As an alternative to using Sodium Fluorescein, Methylumbelliferone (MUB) can be used to perform the Sensitivity/Linearity tests for the top optics.

Materials

Kits containing the microplates and solutions required by the Liquid Tests are available for purchase; see *Materials for Conducting Liquid Tests* on page 5.

Manufacturer part numbers are subject to change.

- Methylumbelliferone (MUB) (10-mg vial, BTI #98156)
- Carbonate-Bicarbonate buffer (CBB) capsules (Sigma #3041)
- 100% methanol (BTI #98161)
- A new, clean 96-well solid black plate, such as Corning Costar #3915 (or equivalent)
- Excitation filter 360/40 nm, 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 protocol **Synergy 2 FI_MUB.prt**, described in detail under *Gen5 Protocol Parameters* starting on page 111

Test Solutions

	Filter solutions to remove particulates that could cause erroneous readings. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate. Wrap the vial containing the MUB stock solution in foil to prevent exposure to light. Discard any open, unused solutions after seven days.

1. Prepare the buffer (CBB) solution:
   - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
   - Open and dissolve the contents of two CBB capsules (do not dissolve the outer gelatin capsule) into 200 mL of the water.
   - Stir the solution (preferably using a stir table) until the CBB is completely dissolved.
2. Prepare the MUB stock solution:
   - Add 1 mL of 100% methanol to the 10-mg vial of MUB.
   - Make sure all of the dye has completely dissolved and is well mixed. This yields a 10 mg/mL stock solution.
   - Wrap the solution in aluminum foil to prevent exposure to light.
3. Prepare the dilutions. Label each with "MUB" and the concentration.

Mix this MUB solution:	with:	to make:
0.5 mL of 10 mg/mL stock solution	4.5 mL of 100% methanol	1 mg/mL
0.88 mL of 1 mg/mL solution	4.12 mL of CBB	176 µg/mL
0.1 mL of 176 µg/mL solution	9.9 mL of CBB	1.76 µg/mL
0.5 mL of 1.76 µg/mL solution	4.5 mL of CBB	176 ng/mL
1 mL of 176 ng/mL solution	9 mL of CBB	**17.6 ng/mL (100 nM)**

Test Procedure

1. If you have not already done so, prepare the test solutions; see page 146.

   Refer to the pipette map on the next page for the remaining steps.

2. Perform the Sensitivity/Linearity tests using the Top optics:
   - Pipette the solutions into a new 96-well solid black plate.
   - Create an experiment based on **Synergy 2 FI_MUB.prt** and read the plate.
3. Save the experiment. Refer to the instructions starting on page 114 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 116.
   - Test descriptions are provided on page 110.

Pipette Map

Seal the plate with foil or store it in a black polyethylene bag until use.

Use a multi-channel pipette with just four tips installed:

- Pipette 150 µL of the buffer into wells **C2–F5** and **C10–F12**. Discard the tips.
- Pipette 150 µL of the **17.6 ng/mL (100 nM) MUB** solution into column 1.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) MUB 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
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												

Injection System Tests

Injection System Testing starting on page 118 describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Materials

Manufacturer part numbers are subject to change.

- Absorbance reader with capability of reading at 405, 630, and 750 nm. The reader must have an accuracy specification of ±1.0% ±0.010 OD or better and a repeatability specification of ±1.0% ±0.005 OD or better.

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

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200 µL hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250-mL beaker
- New 96-well, clear, flat-bottom microplates
- Green Test Dye Solution (BTI #7773003) undiluted, or one of the alternate test solutions provided on the next page
- 100-mL graduated cylinder and 10-mL pipettes (if not using BioTek's Green Test Dye Solution)
- Gen5 protocols listed below (as applicable for your reader model) and described in detail under *Gen5 Parameters* starting on page 118:

  For models with Absorbance capabilities:

  Synergy 2 **Disp 1 Test.prt**
  Synergy 2 **Disp 2 Test.prt**

  For models without Absorbance capabilities:

  Synergy 2 **Disp 1 Test No Read.prt**
  Synergy 2 **Disp 2 Test No Read.prt**

  and, if you will use Gen5 with another BioTek absorbance-capable reader:

  Synergy 2 **Disp Test Other Reader.prt**

Alternate Test Solutions

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

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.

Using BioTek's Blue and Yellow Concentrate Dye Solutions:

Item	Quantity
Concentrate Blue Dye Solution (BTI #7773001, 125 mL)	4.0 mL
QC (Yellow) Solution (BTI #7120782, 125 mL)	5.0 mL
Deionized water	90.0 mL

Using FD&C Blue and Yellow Dye Powder:

Item	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_3Na	0.100 gram
Deionized water	make to 1 liter

Test Procedure for Models with Absorbance Capability

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. 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.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. Create an experiment based on the **Synergy 2 Disp 1 Test** protocol.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

Running a dispense protocol with no plate on the carrier will contaminate the reading chamber with spilled fluid.

When each dispense step is finished, you will weigh the plate, record the weight, tare the balance with the plate on it, and then place the plate back on the carrier for the next step.

7. Initiate a plate read. Gen5 will prompt you to empty the tip priming trough.
8. When ready, proceed with the experiment. The sequence is as follows:
   - 80 µL/well is dispensed to columns 1–4.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 20 µL/well is dispensed to columns 5–8.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 5 µL/well is dispensed to columns 9–12.
   - When prompted, remove the plate and weigh it. Record the weight.
   - Manually pipette **150 µL** of deionized or distilled water into all 12 columns, on top of the green test dye solution.
   - Place the plate on the carrier for the shake and read steps.
9. When the experiment is complete, save the file with an identifying name.
10. Remove the plate from the carrier and set it aside.
11. Repeat the procedure using the **Synergy 2 Disp 2 Test** protocol and a new microplate.
12. When the tests are complete:
    - Prime both dispensers with at least 5000 µL of deionized water to flush out the dye solution.
    - Refer to the instructions on page 121 to perform calculations and determine pass/fail.
    - Test descriptions are provided on page 118.

Test Procedure for Models without Absorbance Capability

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

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

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. 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.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. Create an experiment based on the **Synergy 2 Disp 1 Test No Read** protocol.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

Running a dispense protocol with no plate on the carrier will contaminate the reading chamber with spilled fluid.

When each dispense step is finished, you will weigh the plate, record the weight, tare the balance with the plate on it, and then place the plate back on the carrier for the next step.

7. Initiate a plate read. Gen5 will prompt you to empty the tip priming trough.
8. When ready, proceed with the experiment. The sequence is as follows:
   - 80 µL/well is dispensed to columns 1–4.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 20 µL/well is dispensed to columns 5–8.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 5 µL/well is dispensed to columns 9–12.
   - When prompted, remove the plate and weigh it. Record the weight.
   - Manually pipette **150 µL** of deionized or distilled water into all 12 columns, on top

of the green test dye solution.

- Carefully set the plate aside.

9. Close the experiment without saving it.

> If you are not using a BioTek absorbance reader, read the plate using the parameters described in the table above. Perform the calculations and dettermine pass/fail according to the instructions on page 121.

10. If you are using a BioTek absorbance reader, configure Gen5 to communicate with the reader.
11. Create an experiment based on the **Other Reader** protocol and read the plate.
12. When the experiment is complete, save the file with an identifying name.
13. Remove the plate from the carrier and set it aside.
14. Repeat the procedure using the **Synergy 2 Disp 2 Test No Read** protocol and a new microplate.
15. When the tests are complete:
    - Prime both dispensers with at least 5000 µL of deionized water to flush out the dye solution.
    - Refer to the instructions on page 121 to perform calculations and determine pass/fail.
    - Test descriptions are provided on page 118.

Dispense Accuracy & Precision Tests — Dispenser #____

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

80 µL weight: ____ g

Expected weight: 2.5600 g

Accuracy % Error: ____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 2.0% ☐ P ☐ F

	20 µL Dispense Delta ODs @630/750 nm			
	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: ____ g

Expected weight: 0.6400 g

Accuracy % Error: ____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 7.0% ☐ P ☐ F

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

5 µL weight: ____ g

Expected weight: 0.1600 g

Accuracy % Error: ____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/
Approved By: _____

Signature: _____

Dispense Accuracy & Precision Tests — Dispenser #____

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

80 µL weight: ____ g

Expected weight: 2.5600 g

Accuracy % Error: ____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 2.0% ☐ P ☐ F

	20 µL Dispense Delta ODs @630/750 nm			
	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: ____ g

Expected weight: 0.6400 g

Accuracy % Error: ____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 7.0% ☐ P ☐ F

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

5 µL weight: ____ g

Expected weight: 0.1600 g

Accuracy % Error: ____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: ____

Mean: ____

%CV: ____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/
Approved By: _____

Signature: _____

Appendix A

Specifications

This appendix contains BioTek's published specifications for the Synergy 2.

General Specifications

Microplates

The Synergy 2 accommodates standard 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with 128 x 86 mm geometry, and the BioTek Take3 Micro-Volume Plate.

Note: 1536-well microplates are not supported for luminescence measurements.

Maximum Plate Height:

- Absorbance mode: plates up to 0.8" (20.30 mm) high
- Fluorescence and luminescence modes: plates up to 1.25" (31.75 mm) high
- PCR tube trays up to 1.25" (31.75 mm) high (may require an adapter)
- Injector models dispense to standard height 6-, 12-, 24-, 48-, and 96-well microplates.

Hardware and Environmental

Light Source	Absorbance: Xenon flash light source, 10W maximum average power, not user-changeable Fluorescence: Tungsten quartz halogen, 20W power, user-changeable TRF (and optionally for FI and FP): Xenon flash light source, 60W maximum average power, not user-changeable
Dimensions	Approximately 17.5" D x 15" W x 17.5" H (44.5 cm D x 38.5 cm W x 44.5 cm H) Note: For dimensions that include installation with a BioStack, refer to the *BioStack Operator's Manual*
Weight	For a model equipped with all available modules, excluding the power supply and dispense module, approximately 57 lbs. (25.8 kg)
Environment	Operational temperature, 64° to 104°F (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 4º over ambient to 65ºC. Temperature variation ± 0.50°C across the plate @ 37°C (250 µL per well with the plate sealed).

Absorbance Specifications

Optics

Wavelength Range	200 to 999 nm
Wavelength Bandpass	2.4 nm
Measurement Range	0.000 to 4.000 OD
Wavelength Accuracy	±2 nm
Wavelength Repeatability	± 0.2 nm
Detector	Photodiodes (2) Measurements are reference channel-corrected for light source fluctuation

Performance

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

Accuracy

0.000 to 2.000 OD ±1.0% ±0.010 OD Normal/Rapid modes, 96-well plates
0.000 to 2.000 OD ±2.0% ±0.010 OD Normal/Rapid modes, 384-well plates
2.000 to 2.500 OD ±3.0% ±0.010 OD Normal/Rapid modes, 96-/384-well plates
2.500 to 3.000 OD ±3.0% ±0.010 OD Normal mode, 96-well plates
0.000 to 1.000 OD ±1.0% ±0.010 OD Sweep mode, 96-/384-well plates

Linearity

0.000 to 2.000 OD ±1.0% Normal/Rapid modes, 96-well plates
0.000 to 2.000 OD ±2.0% Normal/Rapid modes, 384-well plates
2.000 to 2.500 OD ±3.0% Normal/Rapid modes, 96-/384-well plates
2.500 to 3.000 OD ±3.0% Normal mode, 96-well plates
0.000 to 1.000 OD ±1.0% Sweep mode, 96-/384-well plates

Repeatability

0.000 to 2.000 OD ±1.0% ±0.005 OD Normal/Rapid modes, 96-/384-well plates
2.000 to 2.500 OD ±3.0% ±0.005 OD Normal/Rapid modes, 96-/384-well plates
2.500 to 3.000 OD ±3.0% ±0.005 OD Normal mode, 96-/384-well plates
0.000 to 1.000 OD ±2.0% ±0.010 OD Sweep mode, 96-/384-well plates

Read Timing

Endpoint read time is from plate start to plate stop. Kinetic read time is from A1 to A1 read positions.

		Single 630 nm	Dual 630/450 nm
Endpoint 96-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	34 sec 43 sec 22 sec	53 sec 73 sec 35 sec
Endpoint 384-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	77 sec 115 sec 32 sec	143 sec 220 sec 56 sec
Endpoint 1536-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	231 sec 385 sec 53 sec	450 sec 757 sec 97 sec
Kinetic 96-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	21 sec 31 sec 11 sec	
Kinetic 384-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	66 sec 104 sec 22 sec	
Kinetic 1536-well plate	Normal 0 ms delay: Normal 100 ms delay: Sweep:	219 sec 373 sec 42 sec	

Fluorescence Specifications

Read Timing

Because of the possible wide variations in setup, the following benchmark conditions are specified: Excitation Filter 485/20 nm, Emission Filter 528/20 nm; 10 Samples per well; 100 ms Delay before sampling; 1 ms Delay between samples.

96-well read: 99 seconds, 384-well read: 339 seconds, 1536-well read: 1256 seconds

Optical Probes

Bottom position: 1.5, 3, or 5 mm diameter probes can be installed

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

Sensitivity

The following specifications apply to 96-well read formats using the Tungsten bulb.

5 mm optical probe, bottom reading

10 pg/mL solution of Sodium Fluoroscein in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 528/20
Hellma 96-well quartz plate or Greiner SensoPlate

62.5 ng/mL solution of Propidium Iodide in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 645/40
Corning Costar 96-well plate with black sides, clear bottom

3 mm optical probe, bottom reading

20 pg/mL solution of Sodium Fluoroscein in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 528/20
Hellma 96-well quartz plate

125 ng/mL solution of Propidium Iodide in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 645/40
Corning Costar 96-well plate with black sides, clear bottom

1.5 mm optical probe, bottom reading

40 pg/mL solution of Sodium Fluoroscein in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 528/20
Hellma 96-well quartz plate

250 ng/mL solution of Propidium Iodide in PBS

40 reads per location averaged, 350 ms delay before read
150 µL per well signal-to-noise ratio greater than 2
Excitation 485/20, Emission 645/40
Corning Costar 96-well plate with black sides, clear bottom

3 mm fixed optical probe with movable apertures, top reading

2 pg/mL solution of Sodium Fluoroscein in PBS

40 reads per location averaged, 5 mm Z-axis 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

0.16 ng/mL solution of Methylumbelliferone in CBB

40 reads per location averaged, 5 mm Z-axis 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

Time-Resolved Fluorescence

96/384-well plates: 250 fM Europium (plate and well modes), 20 reads per location, 5 mm Z-axis 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 Z-axis offset 350 ms delay before read

Luminescence Specifications

50 amol/well flash ATP in a 96-well plate (low-noise PMT)

500 amol/well flash ATP in a 96-well plate (red-shifted PMT)

Dispense/Read Specifications

Applies only to models equipped with injectors

Plate Type	Dispenses to standard height 6-, 12-, 24-, 48-, and 96-well microplates
Detection Method	Absorbance, Fluorescence, Luminescence
Volume Range	5–1000 µL with a 5–20 µL tip prime
Accuracy	±1 µL at 5–50 µL ±2% at 51–1000 µL
Precision	<2.0% for volumes of 50–200 µL <4.0% for volumes of 25–49 µL <7.0% for volumes of 10–24 µL <10.0% for volumes of 5–9 µL

Appendix B

Error Codes

This appendix lists and describes Synergy 2 error codes that may appear in Gen5.

Overview

When a problem occurs during operation with the Synergy 2, an error code appears in Gen5. Error codes typically contain four characters, such as "4168," and in most cases are accompanied by descriptive text, such as "PMT overload error." With many errors, the instrument will beep repeatedly; press the carrier eject button to stop this alarm.

Some problems can be solved easily by the user, 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**.

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.) or 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 2 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
0202/0203	**EX/EM filter wheel (respectively) did not home** Generally, this error indicates the Excitation or Emission filter wheel is not seated properly in the reader. Remove the wheel, ensure each filter or plug is properly positioned, and reinstall it securely. Restart the reader.
020B/020C	**Dispenser syringe 1 or 2 (respectively) did not home** 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.
0402/0403	**EX/EM filter wheel (respectively) failed positional verify** Generally, this error indicates the Excitation or Emission filter wheel is not seated properly in the reader. Remove the wheel, ensure each filter or plug is properly positioned, and reinstall it securely. Restart the reader.
040B/040C	**Dispenser syringe 1 or 2 failed position verify** 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.

Code	Description and possible remedy
050x	**Light beam saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).** 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. See also the description for 0E01. Verify that the microplate carrier access door is closing properly and no ambient light is getting into the read chamber. If you are manually setting the Gain in your Gen5 procedure, try using the Automatic Gain Adjustment feature. Verify that the Gen5 Fluorescence/Luminescence filter table matches the contents of the filter wheels. Verify that the EX filter in position #2 does not overlap with the EM filter in position #3. For models with injectors, the internal chamber may require cleaning (see the **Preventive Maintenance** chapter).
0700/0800/ 0900	**Noise Test Errors, Offset Test Errors, Dark Range Errors** This series of System Test errors may indicate that there is 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 injectors, 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.
0E01-6	**Fluorescence wavelength not found in table** This error indicates that the specified wavelength is not detected in the reader's filter table. The last number is the filter set number in the assay protocol. 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.
0F01	**Filter is defined in the wrong location** Ensure that Gen5's Fluor/Lum wavelengths table matches the actual filters installed in the filter wheels.

Code	Description and possible remedy
1306-7/ 1406-7	**<Motor> not homed successfully** This error indicates that the <motor> is not at home. Make sure the Plate Type described in the Gen5 Protocol matches the plate you are using. Check for any obstructions that may prevent the carrier, syringes, or filter wheels from moving normally. Restart the reader.
2901	**Tungsten Lamp reference voltage out of range** A test of the tungsten lamp is performed when the instrument is 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.
2A01	**Plate jam error** Make sure the Plate Type described 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 and be sure to enter the correct Plate Height. Models with injectors: Verify that the tip prime trough and priming plate are not stuck in the reading chamber.
2B01-04	**Syringe motor axis did not find the home opto sensor transition** 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.
2B0A	**Priming plate not detected** Applies to models with injectors.
3306	**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 and restart the experiment.

Code	Description and possible remedy
4xxx	**PMT overload well error at <well #>** This error typically means that the fluid in a well has oversaturated the PMT (i.e., the well is too "bright"). Try lowering the gain value in the read step. Wells are counted starting at A1, moving left-to-right, row-by-row. The row and column of the well can be extracted from the well number code by applying the following formula (example uses 8 x 12 geometry, 96-well plate): 1. Convert the ASCII hex string to a decimal equivalent. Ex: "057" indicates 57 hex, yielding a well code of 87 decimal. 2. Row = (well code) / (columns in plate), rounded up to a whole number. Ex: 87/12 = 7.25, indicating row 8 (or H). 3. Column = (well code) - ((row-1) * (columns in plate)). Ex: 87 - ((8 - 1) * 12) = column 3. NOTE: If this code is returned during an area scan, it indicates the scan point corresponding to the row/column equivalent in the currently defined scan map, NOT the actual well where the error occurred.

Appendix C

Instrument Dimensions for Robotic Interface

This appendix shows the location of the microplate carrier in reference to the exterior surfaces of the Synergy 2 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.

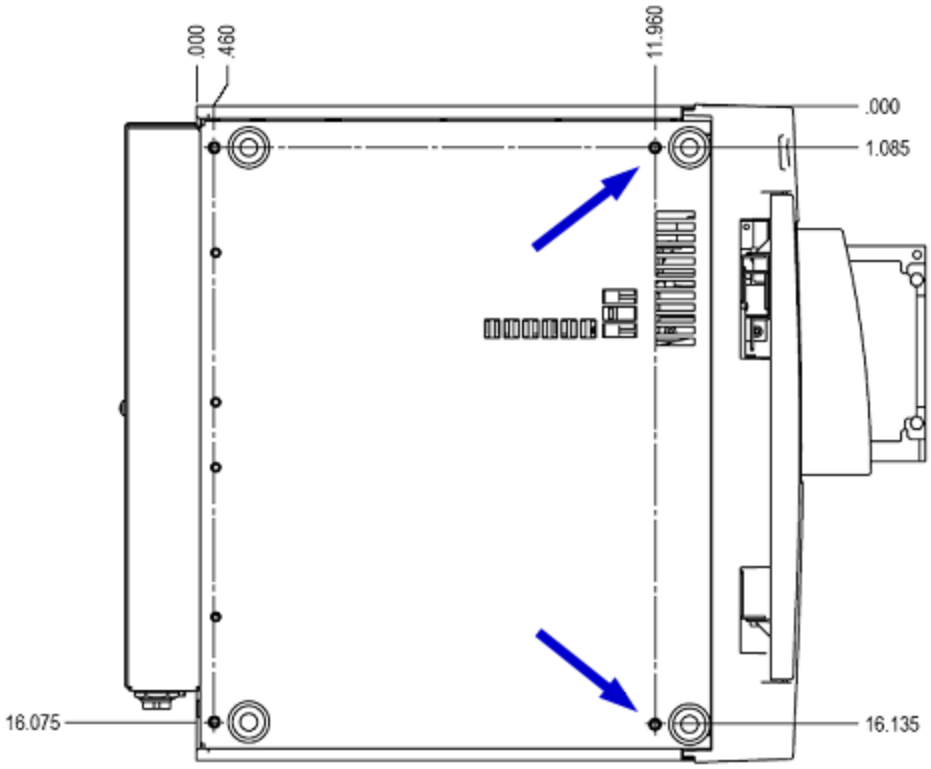


Figure C-1: Bottom view

The two arrows point to special mounting holes for alignment caps for operation with the BioStack.

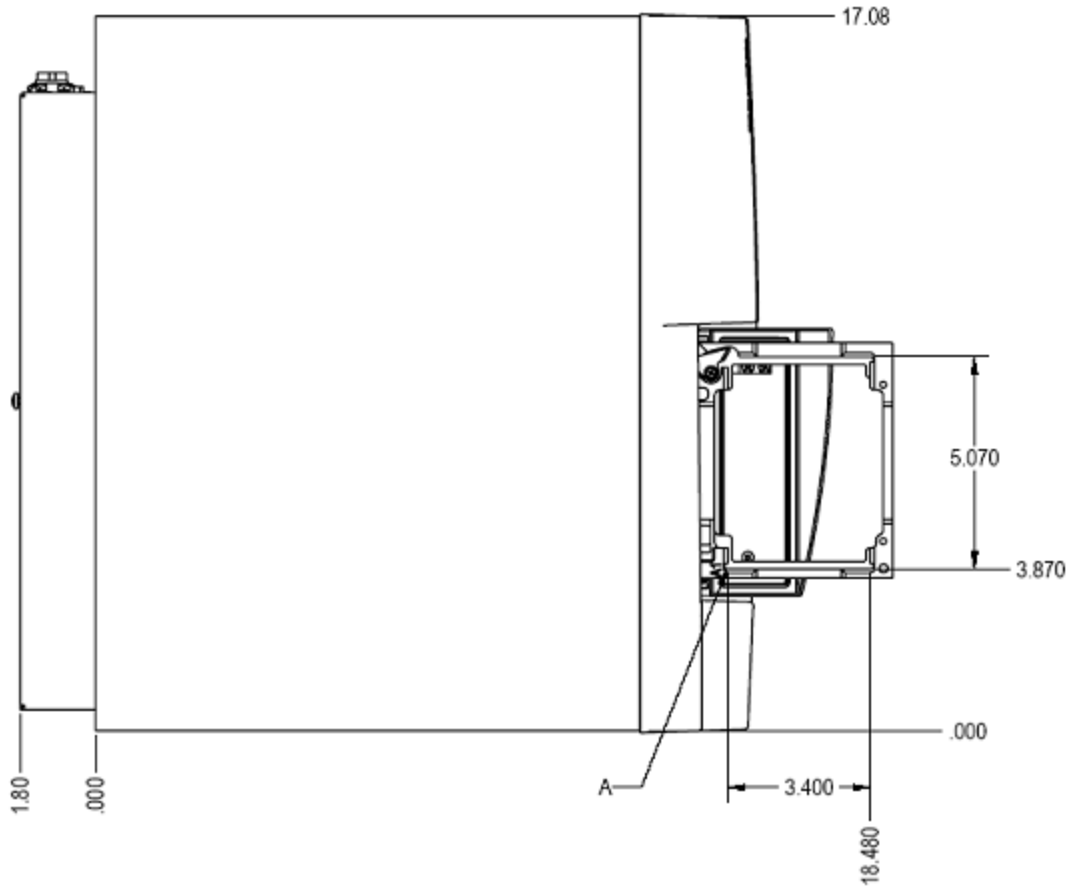


Figure C-2: Top view

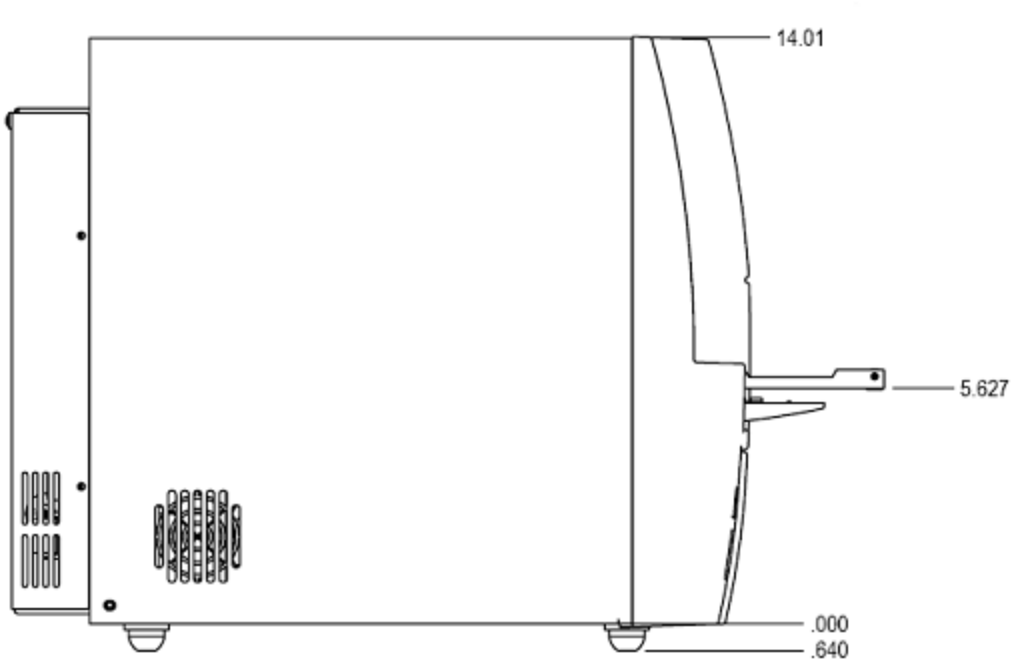


Figure C-3: Side view

BioStack users: Special alignment hardware is included in the BioStack's alignment kit for correct positioning with the Synergy 2. Refer to the **Installation** chapter in the *BioStack Operator's Manual* for instructions.

Appendix D

Sample Reports

This appendix contains sample System Test and Absorbance Plate Test reports for the Synergy 2.

Gen5 System Test Report

Reader:	Synergy 2 (Serial Number: 15060819)
Basecode:	P/N 7160204 (v1.14)
Gen5 Version:	2.06.10
Date and Time:	6/10/2015 9:15:35 AM
User:	7358
Company:	BioTek
Comments:	

Test Results

Operator ID:_____

Notes:_____

_____

SYSTEM SELF TEST

7160204 Version 1.14 15060819 1111 1100 0010 0000

Voltage Reference Test	Min	Low	High	Max
Fluorescence Flash	666	836	1089	1260
Absorbance Flash	1323	1605	1992	2275
Motors	3728			
Tungsten Lamp	21			3419

ABSORBANCE

Optics Test	Ref	Meas	Gain	Resets
#1:200			2.12	4
Light	13419	38608		
Dark	9870	9845		
Delta	3549	28763		
#2:352			1.51	4
Light	12607	39140		
Dark	9872	9855		
Delta	2735	29285		
#3:620			2.88	2
Light	12309	39704		
Dark	9872	9821		
Delta	2437	29883		
#4:790			2.33	2
Light	12234	39677		
Dark	9873	9832		
Delta	2361	29845		
#5:860			1.63	1
Light	12219	39399		
Dark	9881	9832		
Delta	2338	29567		
#6:962			2.02	1
Light	12223	39712		
Dark	9881	9822		

Delta	2342	29890	

Noise Test	Ref	Meas
Max	9827	9745
Min	9826	9743
Delta	1	2

FLUORESCENCE/LUMINESCENCE

Filter PCB

Bias current offset	-0.4	counts	PASS
Offset voltage	1489	counts	PASS
750V measurement	25.2	counts	PASS
750V noise	13	counts	
750V offset	1492	counts	
500V measurement	11.7	counts	
500V noise	1	counts	
500V offset	1491	counts	
Reset offset	1504	counts	
Reference bias	1.6	counts	PASS
Reference offset	10817	counts	PASS
Reference noise	0.3	counts	PASS

Excitation Wheel	500V	650V	750V
#1:360/40			
Gain	1.00	1.00	1.00
Light	15084	17238	18830
Dark	10817	10817	10817
Delta	4267	6421	8013
#2:485/20			
Gain	1.00	1.00	1.00
Light	15825	17454	18588
Dark	10817	10817	10817
Delta	5008	6637	7771
#3:540/25			
Gain	1.00	1.00	1.00
Light	15908	17589	18730
Dark	10817	10817	10817
Delta	5091	6772	7913
#4:PLUG			

Emission Wheel

- #1:460/40
- #2:528/20
- #3:620/40
- #4:HOLE

CALIBRATION

Carrier - Bottom Fluorescence Probe

Upper Left	x= 9744	y= 4564
Lower Left	x= 9740	y=10088
Lower Right	x= 1052	y=10088
Upper Right	x= 1056	y= 4568
Delta 1	9744 - 9740=	+4

```
   Delta 2          1056 - 1052=   +4
   Delta 3          4568 - 4564=   +4
   Delta 4         10088 -10088=   +0

Carrier - Top Fluorescence Probe (Filters)
   Upper Left       x=10628   y=  228
   Lower Left       x=10620   y= 5748
   Lower Right      x= 1932   y= 5752
   Upper Right      x= 1936   y=  232
   Delta 1         10628 -10620=   +8
   Delta 2          1936 - 1932=   +4
   Delta 3           232 -  228=   +4
   Delta 4          5752 - 5748=   +4

Carrier - Luminescence Probe
   Upper Left       x= 9740   y= 3000
   Lower Left       x= 9736   y= 8524
   Lower Right      x= 1044   y= 8528
   Upper Right      x= 1052   y= 3004
   Delta 1          9740 - 9736=   +4
   Delta 2          1052 - 1044=   +8
   Delta 3          3004 - 3000=   +4
   Delta 4          8528 - 8524=   +4

Carrier - Absorbance Probe
   Upper Left       x=11312   y= 4564
   Lower Left       x=11308   y=10092
   Lower Right      x= 2616   y=10096
   Upper Right      x= 2616   y= 4572
   Delta 1         11312 -11308=   +4
   Delta 2          2616 - 2616=   +0
   Delta 3          4572 - 4564=   +8
   Delta 4         10096 -10092=   +4

Carrier - Injectors
   Upper Left       x= 9740   y= 4560
   Lower Left       x= 9732   y=10080
   Lower Right      x= 1044   y=10084
   Upper Right      x= 1048   y= 4564
   Delta 1          9740 - 9732=   +8
   Delta 2          1048 - 1044=   +4
   Delta 3          4564 - 4560=   +4
   Delta 4         10084 -10080=   +4

Carrier - Test Sensors
   Middle Sensor    y=11936
   Tested             11936
   Delta                 +0
   Back Sensor      x=11592   y=10624
   Tested             11592     10624
   Delta                 +0        +0

Probe Height        34.06 mm
Plate Sensor        336

Probe Changer       444
```

Mirror 3908

Lamp Reflector 640

Aperture 2180

Absorbance Monochromator A=+0.000000 B=-0.000936 C=-0.279281

INCUBATION

Temperature Setpoint: 0.0 Current Average: 43.6 A/D Test: PASS

Zone 1: 43.9	Min: 43.9	Max: 44.5	Range: PASS	Thermistor: PASS
Zone 2: 42.5	Min: 42.5	Max: 43.3	Range: PASS	Thermistor: PASS
Zone 3: 44.4	Min: 44.4	Max: 45.1	Range: PASS	Thermistor: PASS
Zone 4: 43.4	Min: 43.4	Max: 44.4	Range: PASS	Thermistor: PASS

SYSTEM TEST PASS
0000

Dispenser 1: 005.1,009.8,019.7,039.3,079.6,199.5
Dispenser 2: 004.9,009.8,019.7,039.3,079.1,199.0

Mirror 1: 50% Mirror, 0200, 0850, 0200, 0850
Mirror 2: Dichroic , 0320, 0390, 0410, 0800
Mirror 3: Dichroic , 0440, 0505, 0515, 0640
Polarizers present.

Reviewed/Approved By: _____

Date: _____

Absorbance Test Plate Results

Reader:	Synergy 2 (Serial Number: 15060819)
Basecode:	P/N 7160204 (v1.14)
Date and Time:	6/9/2015 1:01:47 PM
Absorbance Plate:	7 Filter Test Plate (P/N 7260522) - S/N 210505
Last Plate Certification:	April 2015
Next Plate Certification Due:	April 2016
User:	7358
Comments:	

Peak Absorbance Results

Well	C6
Reference	279
Tolerance	3
Read	280
Result	PASS

Alignment Results

Wells	A1	A12	H1	H12
Read	0.000	0.000	0.000	0.000
Tolerance	0.015	0.015	0.015	0.015
Result	PASS	PASS	PASS	PASS

Wavelength = 630 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.172	0.589	1.083	1.666	1.804	2.338
Min Limit	0.149	0.557	1.041	1.613	1.748	2.224
Max Limit	0.195	0.621	1.125	1.719	1.860	2.452
Read 1	0.174	0.590	1.083	1.668	1.805	2.333
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.174	0.590	1.083	1.668	1.805	2.333
Min Limit	0.168	0.579	1.067	1.647	1.781	2.258
Max Limit	0.181	0.601	1.099	1.690	1.828	2.408
Read 2	0.174	0.589	1.083	1.668	1.805	2.330
Result	PASS	PASS	PASS	PASS	PASS	PASS

Reviewed/Approved By: _____

Date: _____