

Multi-Mode Microplate Reader

# Synergy<sup>™</sup> HTX

## Operator's Manual





# **Synergy HTX**

## **Multi-Mode Reader**

### ***Operator's Manual***

**BioTek® Instruments, Inc.**

**Part Number 1341000**

**Revision D**

**May 2016**

**© 2016**

---

## Notices

### **BioTek® Instruments, Inc.**

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

© 2016, BioTek® Instruments, Incorporated. No part of this publication may be reproduced, transcribed, or transmitted in any form, or by any means electronic or mechanical, including photocopying and recording, for any purpose other than the purchaser's use without written permission of BioTek Instruments, Inc.

## Trademarks

BioTek® is a registered trademark and Synergy™, Gen5™, BioStack™, BioCell™, and Take3™ are trademarks of BioTek Instruments, Inc. Harta™ is a trademark of Harta Instruments.

Microsoft®, Windows®, Windows 7, Windows 8, and Excel® are either registered trademarks or trademarks of Microsoft Corporation in the United States and/or other countries.

All other trademarks are the property of their respective holders.

## Restrictions and Liabilities

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

---

# Contents

Notices .....	ii
Trademarks .....	ii
Restrictions and Liabilities .....	ii
Contents .....	iii
Contact Information .....	x
Global Service and Support .....	x
Customer Service and Sales .....	x
Service/Technical Assistance Center (TAC) .....	x
European Coordination Center/Authorized European Representative .....	x
Revision History .....	xi
Document Conventions .....	xii
Intended Use Statement .....	xii
Quality Control .....	xiii
Warranty and Product Registration .....	xiii
Repackaging and Shipping .....	xiii
Warnings .....	xiv
Hazards .....	xiv
Precautions .....	xvi
CE Mark .....	xvii
Directive 2014/30/EU: Electromagnetic Compatibility .....	xvii
Emissions—Class A .....	xvii
Immunity .....	xvii
Directive 2014/35/EU Low Voltage (Safety) .....	xvii
Directive 2012/19/EU: Waste Electrical and Electronic Equipment .....	xviii
Directive 98/79/EC: In Vitro Diagnostics (if labeled for this use) .....	xviii
Electromagnetic Interference and Susceptibility .....	xviii
USA FCC CLASS A .....	xviii
Canadian Department of Communications Class A .....	xviii
User Safety .....	xix
Safety Symbols .....	xx
<b>Introduction .....</b>	<b>1</b>
Product Description .....	2
Package Contents & Accessories .....	3
Optional Accessories .....	4
Materials for Conducting Liquid Tests .....	5

Product Support & Service .....	6
Technical Assistance Center (TAC) .....	6
Applications Support .....	6
<b>Installation .....</b>	<b>7</b>
Product Registration .....	8
Important Information .....	8
1: Unpack and Inspect the Synergy HTX .....	9
2: Select an Appropriate Location .....	12
3: Remove the Shipping Hardware .....	13
4: Install the Fluorescence Lamp Assembly .....	14
5: Install the Power Supply .....	15
6: Unpack and Inspect the Dispense Module .....	15
7: Install the Dispense Module .....	16
8: Connect the Host Computer .....	17
9: Install Gen5 Software .....	18
10: Turn on the Reader .....	18
11: Establish Communication .....	18
12: Set Dispenser Calibration Values .....	19
13: Run a System Test .....	20
14: Test the Injection System .....	21
Operational/Performance Qualification .....	22
Repackaging and Shipping Instructions .....	23
Prepare the Dispense Module for Shipment .....	24
<b>Getting Started .....</b>	<b>29</b>
Key Components .....	30
Lamp Assembly and Filter Wheel Access .....	31
Excitation and Emission Filter Wheels .....	32
Removing the Filter Wheels, Changing Filters .....	33
Installing the Time-Resolved Cartridge .....	34
Configuring the System for Luminescence Measurements .....	35
Injection System .....	35
External Dispense Module .....	35
Internal Tubing .....	37
Priming the Injection System .....	37
Gen5 Software .....	38
Viewing and Modifying Filter Wheel Information .....	38
Protocols and Experiments .....	39
Dispense Module Control .....	40

Prime .....	40
Purge .....	40
Plate Shaking Options .....	41
Recommendations for Optimum Performance .....	42
General .....	42
Luminescence Measurements .....	43
Models with Injectors .....	43
Incubation and Partial Plates .....	43
<b>Preventive Maintenance .....</b>	<b>45</b>
Overview .....	46
Daily Cleaning for the Dispense Module .....	46
Recommended Maintenance Schedule .....	47
Warnings and Precautions .....	48
Clean Exposed Surfaces .....	49
Inspect/Clean Excitation and Emission Filters .....	50
Flush/Purge Fluid Path .....	51
Run a Dispense Protocol (Optional) .....	52
Empty/Clean the Tip Priming Trough .....	53
Clean the Priming Plate .....	53
Clean the Internal Components .....	53
Required Materials .....	54
Procedure .....	55
Remove the Reader's Shroud .....	55
Remove the Internal Dispense Tubes and Injector Heads .....	56
Clean the Dispense Tubes and Injector Heads .....	60
Clean the Optical Probes .....	61
Clean the Reader's Internal Surface .....	69
Reassemble the Components .....	71
Performance Check .....	72
<b>As-Needed Maintenance .....</b>	<b>73</b>
Decontamination .....	74
Required Materials .....	74
Procedure for Models without the Dispense Module .....	75
Procedure for Models with the Dispense Module .....	76
Routine Procedure .....	76
Clean Exposed Surfaces .....	76
Decontaminate the Fluid Lines .....	76
Rinse the Fluid Lines .....	77

Clean the Tubing and Injectors .....	78
Decontaminate the Tip Priming Trough and Priming Plate .....	78
Alternate Procedure .....	78
Dispense Module, Syringe Replacement .....	79
Syringe Maintenance Position .....	79
Replace the Syringe .....	80
<b>Instrument Qualification Process .....</b>	<b>81</b>
Instrument System Test .....	82
Plate Shaker Test .....	82
Absorbance Testing .....	83
BioTek Absorbance Test Plates .....	83
Test Methods .....	83
Sample Test Report .....	84
Troubleshooting .....	84
Peak Absorbance Test .....	84
Alignment Test .....	85
Accuracy Test .....	85
Repeatability Test .....	85
Absorbance Liquid Tests .....	85
Test Methods .....	85
Gen5 Protocol Parameters .....	86
Results Analysis .....	88
Absorbance Liquid Test 1 .....	88
Absorbance Liquid Test 2 .....	88
Absorbance Liquid Test 3 .....	89
Troubleshooting .....	90
Luminescence Testing .....	91
Test Method .....	91
Gen5 Protocol Parameters .....	91
Results Analysis .....	93
Pass/Fail Criteria .....	93
Troubleshooting .....	93
Fluorescence Testing .....	94
BioTek Fluorescence Test Plate .....	94
Results Analysis .....	94
Fluorescence Liquid Tests .....	95
Test Methods .....	95
Gen5 Protocol Parameters .....	95

Results Analysis .....	99
Corners Test .....	99
Sensitivity Test .....	99
Linearity Test .....	100
Troubleshooting .....	101
Injection System Testing .....	102
Test Method .....	102
Gen5 Parameters .....	102
Results Analysis .....	105
<b>Instrument Qualification Procedures .....</b>	<b>107</b>
Overview .....	108
IQ/OQ/PQ Description .....	109
Recommended Qualification Schedule .....	110
System Test .....	111
Setup .....	111
Test Procedure .....	111
Plate Shaker Test .....	112
Absorbance Plate Tests .....	113
Requirements .....	113
Setup .....	113
Test Procedure .....	114
Absorbance Liquid Tests .....	115
Absorbance Liquid Test 1 .....	115
Materials .....	115
Solution A .....	115
Solution B .....	116
Test Procedure .....	116
Absorbance Liquid Test 2 .....	116
Materials .....	117
Test Procedure .....	117
Absorbance Liquid Test 3 .....	118
Materials .....	118
Buffer Solution .....	118
Test Procedure .....	118
Luminescence Test .....	120
Requirements .....	120
Test Procedure .....	120
Fluorescence Plate Tests .....	121

Requirements .....	121
Test Procedure .....	121
Fluorescence Liquid Tests .....	122
Materials .....	123
Test Solutions .....	124
Test Procedure .....	125
Pipette Map .....	126
Corners, Sensitivity, and Linearity (FI) Tests: .....	126
Alternate/Supplemental Tests Using Methylumbellifерone (MUB) .....	128
Materials .....	128
Test Solutions .....	129
Test Procedure .....	130
Pipette Map .....	131
Injection System Tests .....	132
Materials .....	132
Alternate Test Solutions .....	133
Test Procedure for Models with Absorbance Capability .....	133
Test Procedure for Models without Absorbance Capability .....	135
<b>Specifications .....</b>	<b>137</b>
General Specifications .....	138
Microplates .....	138
Hardware and Environmental .....	138
Absorbance Specifications .....	139
Optics .....	139
Performance .....	139
Accuracy .....	139
Linearity .....	139
Repeatability .....	140
Read Timing .....	140
Fluorescence Specifications .....	141
Optics .....	141
Sensitivity .....	141
Read Timing .....	141
Time-Resolved Fluorescence .....	141
Luminescence Specifications .....	142
Dispense/Read Specifications .....	142
<b>Error Codes .....</b>	<b>143</b>
Overview .....	144

Error Codes .....	145
<b>Sample Reports .....</b>	<b>155</b>

## Contact Information

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

### 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.bioteck.com](http://www.bioteck.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.bioteck.com](http://www.bioteck.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@bioteck.com](mailto:customercare@bioteck.com)

### Service/Technical Assistance Center (TAC)

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@bioteck.com](mailto:tac@bioteck.com)

### European Coordination Center/Authorized European Representative

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

## Revision History

Rev	Date	Changes
A	7/2014	First issue
B	4/2015	<p>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 <a href="http://www.bioteck.com">www.bioteck.com</a> for the most up-to-date information.</p> <p>CE Mark: Updated Directive headings.</p> <p>Chapter 1, Introduction: Removed information regarding plate heights and PCR tubes that conflicted with Appendix A, Specifications.</p> <p>Chapter 3, Getting Started: To 'Recommendations for Optimum Performance' added information on the use of acids, corrosives, and solvents.</p> <p>Chapter 6, Instrument Qualification, Luminescence Tests: Added the luminescence test to the Recommended Qualification Schedule. Modified the formula for verifying the Harta test plate battery (<math>A_8 &gt; (0.2 * A_7)</math>). In the Gen5 protocol parameters table, changed the Dynamic Range for the Battery Check read step to Extended.</p>
C	10/2015	<p><i>General:</i> Added information for purchasing and using the BioTek 340 nm Absorbance Test Plate (BTI #7260551).</p> <p><i>Preface, CE Mark section:</i> Updated Directive headings.</p> <p><i>Chapter 1, Introduction.</i> Corrected the priming plate part number (8042202).</p> <p>Created a new Chapter 6, <i>Instrument Qualification Process</i> to describe the tests designed to qualify the Synergy HTX. Renamed the former Chapter 6, Instrument Qualification as <i>Chapter 7, Instrument Qualification Procedures</i> and moved the description content to the aforementioned new chapter.</p> <p><i>Chapter 7, Instrument Qualification Procedures:</i> In the Pipette Map instructions for fluorescence intensity tests with Methylumbelliferone (MUB) changed the volume for the Sensitivity Test from 150 µL/well to 200 µL.</p> <p><i>Appendix A, Specifications:</i> Added a specification for sodium fluorescein for the top 3 mm probe.</p>
D	5/2016	Added information on using the BioTek Fluorescence Test Plate to qualify the Synergy HTX fluorescence system.

---

## Document Conventions

	This icon identifies information that protects the <b>safety</b> of the operator and the integrity of data.
<b>Warning!</b>	A <b>Warning</b> indicates the potential for bodily harm and tells you how to avoid the problem.
<b>Caution</b>	A <b>Caution</b> indicates the potential for damage to the instrument and tells you how to avoid the problem.
<b>Note:</b>	<b>Bold text</b> is primarily used for emphasis.
	This icon calls attention to <b>important</b> 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 HTX is a single-channel absorbance, fluorescence, and luminescence microplate reader that uses a dual-optics design to perform measurements of samples in a microplate format. 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.bioteck.com](http://www.bioteck.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 weighs up to 38 pounds (17 kg). Use two people when lifting and carrying the instrument.

	<p><b>Warning! Liquids.</b> 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.</p> <p><b>Warning! Unspecified Use.</b> Failure to operate the equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.</p> <p><b>Warning! Software Quality Control.</b> The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading or dispensing methods. <b>Failure to conduct quality control checks could result in erroneous test data.</b></p> <p><b>Warning! Reader Data Reduction Protocol.</b> No limits are applied to the raw measurement data. All information exported via computer control must be thoroughly analyzed by the operator.</p>
	<b>Warning! Internal Voltage.</b> Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument or removing its top case.
	<b>Warning! Hot Surface.</b> 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.
	<b>Warning! Potential Biohazards.</b> 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.
	<b>Warning! Pinch Hazard.</b> 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.

**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 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



**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 2014/30/EU: 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 2014/35/EU 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 présent appareil numerique n'émet pas de bruits radioélectriques dépassant les limites applicables aux appareils numériques de la Class A prescrites dans le Réglement sur le brouillage radioélectrique édicté par le ministère des Communications du Canada.

---

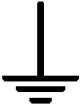
## User Safety

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 xvii.

## 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

	<p>Warning, risk of electric shock Attention, risque de choc électrique Gefährliche elektrische schlag Precaución, riesgo de sacudida eléctrica Attenzione, rischio di scossa elettrica</p>		<p>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</p>
	<p>Warning, hot surface Attention, surface chaude Warnen, heiße Oberfläche Precaución, superficie caliente Attenzione, superficie calda</p>		<p>Warning, potential biohazards Attention, risques biologiques potentiels Warnung! Mögliche biologische Giftstoffe Atención, riesgos biológicos Attenzione, rischio biologico</p>
	<p>In vitro diagnostic medical device Dispositif médical de diagnostic in vitro Medizinisches In-Vitro-Diagnostikum Dispositivo médico de diagnóstico in vitro Dispositivo medico diagnostico in vitro</p>		<p>Separate collection for electrical and electronic equipment Les équipements électriques et électroniques font l'objet d'une collecte sélective Getrennte Sammlung von Elektro- und Elektronikgeräten Recogida selectiva de aparatos eléctricos y electrónicos Raccolta separata delle apparecchiature elettriche ed elettroniche</p>
	<p>Consult instructions for use Consulter la notice d'emploi Gebrauchsanweisung beachten Consultar las instrucciones de uso Consultare le istruzioni per uso</p>		



## *Chapter 1*

# Introduction

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

Product Description .....	2
Package Contents & Accessories .....	3
Optional Accessories .....	4
Materials for Conducting Liquid Tests .....	5
Product Support & Service .....	6
Technical Assistance Center (TAC) .....	6
Applications Support .....	6

---

## Product Description

The Synergy HTX is a single-channel microplate reader available with absorbance, fluorescence, and luminescence detection. It is computer-controlled using BioTek's Gen5 software for all operations including data reduction and analysis. Synergy HTX is robot accessible and compatible with BioTek's BioStack Microplate Stacker.

When making fluorescence determinations, the Synergy HTX uses a tungsten quartz halogen lamp with interference filters for wavelength specificity in conjunction with a photomultiplier (PMT) tube detector. The Synergy HTX has both top and bottom probes for fluorescence measurements. The top probe can be adjusted vertically for the correct reading height, via Gen5's Read Height reading parameter.

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

Absorbance measurements are made by switching to a xenon flash lamp and a monochromator for wavelength selection. The use of a xenon flash lamp allows for both UV and visible light absorbance measurements. The monochromator provides wavelength selection from 200 to 999 nm in 1-nm increments.

The Synergy HTX has a 4-Zone temperature control from 4°C over ambient to 50°C, controlled via a software-adjustable gradient. Internal plate shaking, with both linear and orbital modes, is supported to ensure that reagents are properly mixed prior to reading.

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

For models with time-resolved fluorescence (TRF) capability, the TRF option allows measurements by using the xenon flash light source in conjunction with the PMT measurement detector. A special cartridge installed in the Excitation filter wheel location is required.

Models with injectors support dual-reagent dispensing to 6-, 12-, 24-, 48-, 96-, and 384-well microplates with standard 128 x 86 mm geometry. 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.

<b>Item</b>	<b>BTI Part #</b>
Synergy HTX Operator's Manual (on USB flash drive)	1341000
Power supply	76061
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	7772028
Fluorescence lamp assembly, if applicable to your reader model (Note: The replacement lamp assembly is PN 7080500)	7080501
Filter "plugs" (2) (also referred to as "dummy filters" or "blanks")	7082073
Storage bag and fastener strips	--
Time-Resolved Fluorescence cartridge assembly ("T" models only)	7090523
Models with injectors, 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	8042202
Injector tip priming trough	1342017
Dispense module communication cable	75107
Dispense module front cover	7082137
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 microplate carrier adapter BTI #8042028 for Synergy HTX)	8030015
Fluorescence Test Plate**	1400006
Take3 Micro-Volume Plate	TAKE3
Take3 Trio Micro-Volume Plate	TAKE3TRIO
Terasaki Adapter Plate	7330531
BioCell Adapter Plate	7270512
BioCell Quartz Vessel	7272051
Synergy HTX Qualification and Maintenance (IQ/OQ/PQ) package	1340508
Additional fluorescence filters; contact BioTek Customer Care for availability and part numbers	
The Synergy HTX 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.

\*\* Requires Gen5 version 2.06 or higher.

## Materials for Conducting Liquid Tests

Manufacturer part numbers are subject to change.

Item	Part Number
<b>Absorbance Liquid Tests</b>	
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
<b>Fluorescence Liquid Tests</b>	
<i>Test Kits</i>	
Kit for FI tests using Sodium Fluorescein	BTI #7160013
Kit for FI tests using Methylumbellifерone	BTI #7160012
<i>Individual Materials</i>	
Sodium Fluorescein Powder, 1-mg vial	BTI #98155
Methylumbellifерone, 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
<b>Injection System Tests</b>	
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 [143](#))

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 [23](#).

### 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 HTX and, if applicable, the external dispense module. Instructions are also included for preparing the reader and dispense module for shipment.

Product Registration .....	8
Important Information .....	8
1: Unpack and Inspect the Synergy HTX .....	9
2: Select an Appropriate Location .....	12
3: Remove the Shipping Hardware .....	13
4: Install the Fluorescence Lamp Assembly .....	14
5: Install the Power Supply .....	15
6: Unpack and Inspect the Dispense Module .....	15
7: Install the Dispense Module .....	16
8: Connect the Host Computer .....	17
9: Install Gen5 Software .....	18
10: Turn on the Reader .....	18
11: Establish Communication .....	18
12: Set Dispenser Calibration Values .....	19
13: Run a System Test .....	20
14: Test the Injection System .....	21
Operational/Performance Qualification .....	22
Repackaging and Shipping Instructions .....	23

## 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.bioteck.com](http://www.bioteck.com) or by contacting BioTek Customer Care.

## Important Information



This chapter contains installation and setup tasks for a Synergy HTX 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 HTX



The Synergy HTX with all available modules weighs up to 38 pounds (17 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.

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

1. Open the outer shipping box. Remove the foam blocks to access the inner shipping box.
2. Open the inner shipping box. Remove the accessories box and set it aside. Remove the vertical supports.
3. The reader is attached to a shipping panel that has two handles for lifting. Lift the reader out of the box and place it on a level surface. Remove its protective storage bag.
4. Carefully tip the reader onto its back. Using a screwdriver, remove the screws and washers that attach the panel to the reader. Carefully set the reader upright.
5. Locate the supplied plastic tool storage pocket. Place the screws and washers inside the bag. Attach the pocket to the back of the reader for storage. Do not block any air vents.
6. Place the panel into the inner shipping box for storage. Place this box and all packaging materials into the outer shipping box for reuse if the reader needs to be shipped again.

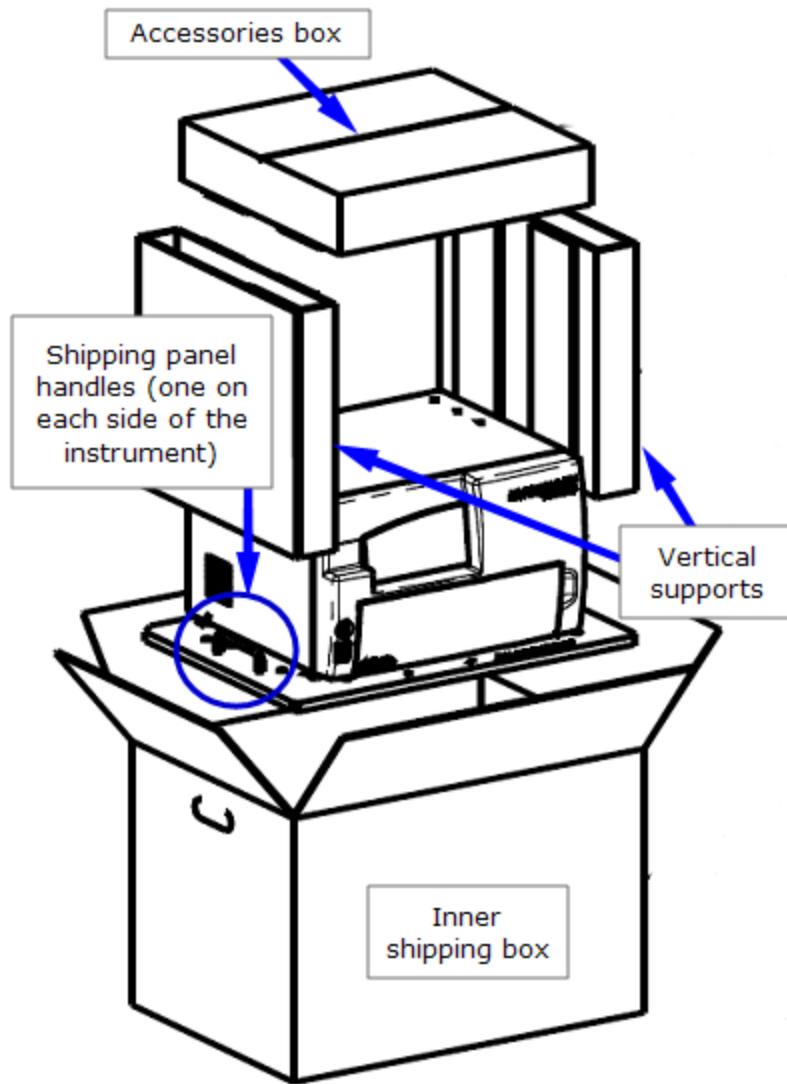


Figure 2-1: Unpacking the reader

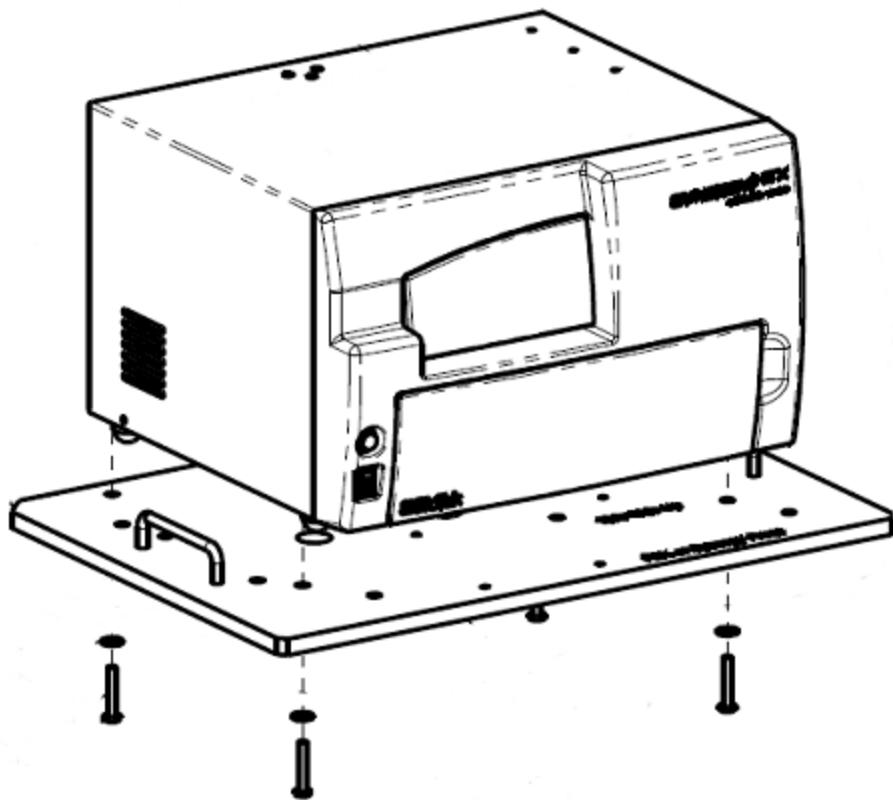


Figure 2-2: Removing the shipping panel



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 HTX, 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 the microplate carrier shipping bolt before turning on the reader.

Replace the bolt before repackaging the reader for shipment. If necessary, contact BioTek to order a replacement bolt (PN 1342008) or o-ring (PN 49259).

1. Pull down the microplate loading door on the front of the reader.
2. Using the supplied wrench, remove the carrier shipping bolt with its o-ring and warning tag.
3. Store the wrench, bolt, o-ring, and tag in the supplied plastic tool storage bag.

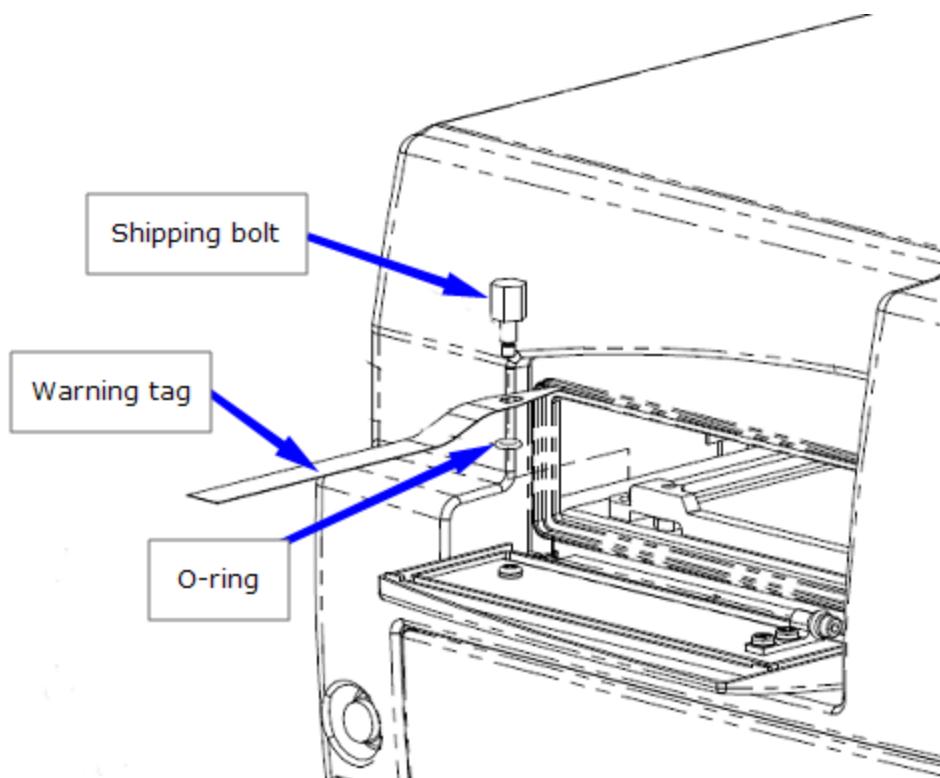


Figure 2-3: Removing the microplate carrier shipping bolt

## 4: Install the Fluorescence Lamp Assembly

*Applies only to models with fluorescence capability*



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



**Warning!** When replacing an existing lamp assembly: The fluorescence 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.

1. Locate the lamp assembly in the accessories box. The lamp is attached to a bracket that also holds a condenser lens and a heat absorber. Two cables are attached to the back of the lamp.
2. Open the reader's hinged door. The lamp compartment is on the left.
3. Orient the lamp assembly as shown below and slide it all the way into the compartment.
4. Plug the lamp cables into the power source located to the right of the lamp. Either cable can be plugged into either socket.

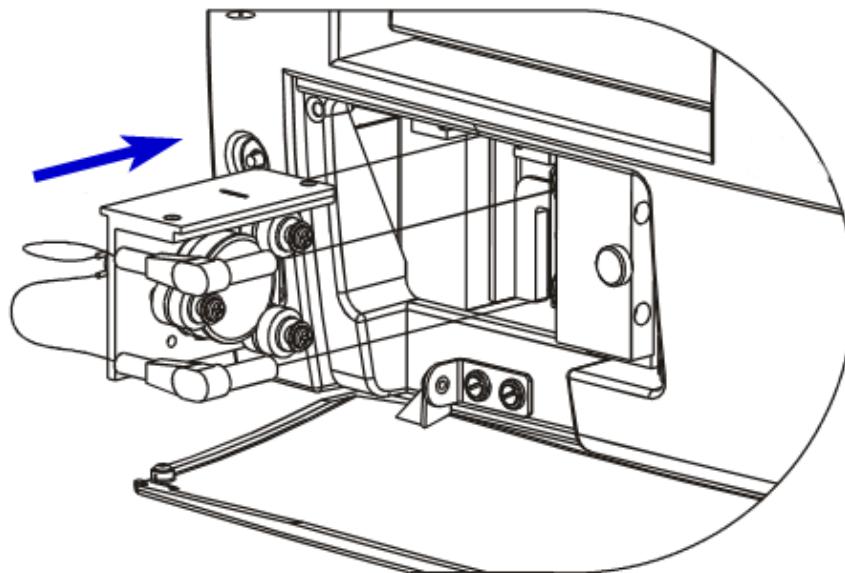


Figure 2-4: Installing the fluorescence lamp assembly (Note: the replacement lamp part number is 7080500)

## 5: Install the Power Supply



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

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

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.

## 6: 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 2-7](#) and [Figure 2-8](#) starting on page 26.

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.

## 7: Install the Dispense Module

*Applies only to models equipped with injectors*

1. Place the dispense module to the left side or 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. Each is labeled with a left-pointing arrow.

**① 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.



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.

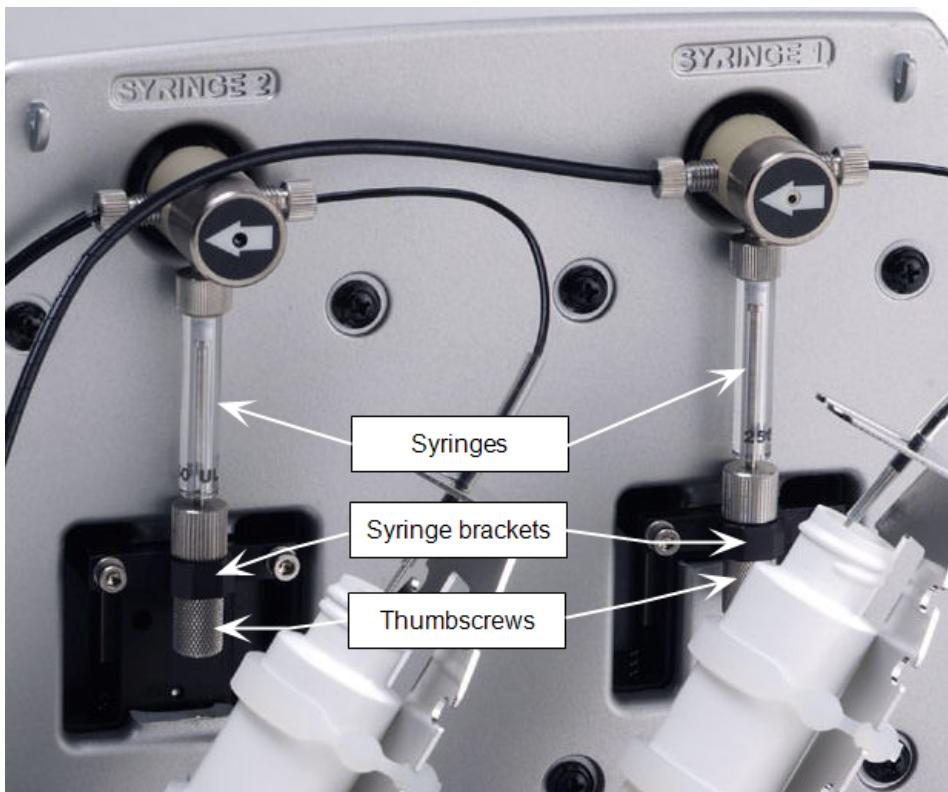


Figure 2-5: Dispense module, close-up view of syringes

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.

---

## 8: Connect the Host Computer

The Synergy HTX 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.

## 9: Install Gen5 Software



The Synergy HTX 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.

## 10: 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 HTX. 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. See *Figure 3-1* on page 30.

## 11: 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 HTX**. Click **OK**.
4. Perform one of the following steps, as applicable:
  - Select **Plug & Play**. (A reader must be connected to the computer and turned on to appear in the Available Plug & Play Readers list.)
  - Select **Com Port** and select 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.

---

## 12: Set Dispenser Calibration Values

*Applies only to models equipped with injectors*

Before using the external dispense module with the Synergy HTX, you must set its calibration values in Gen5.

① 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). You will enter the **measured** calibration values into Gen5.

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

## 13: 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 HTX**.
  - 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 143. 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 22.

## 14: 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.

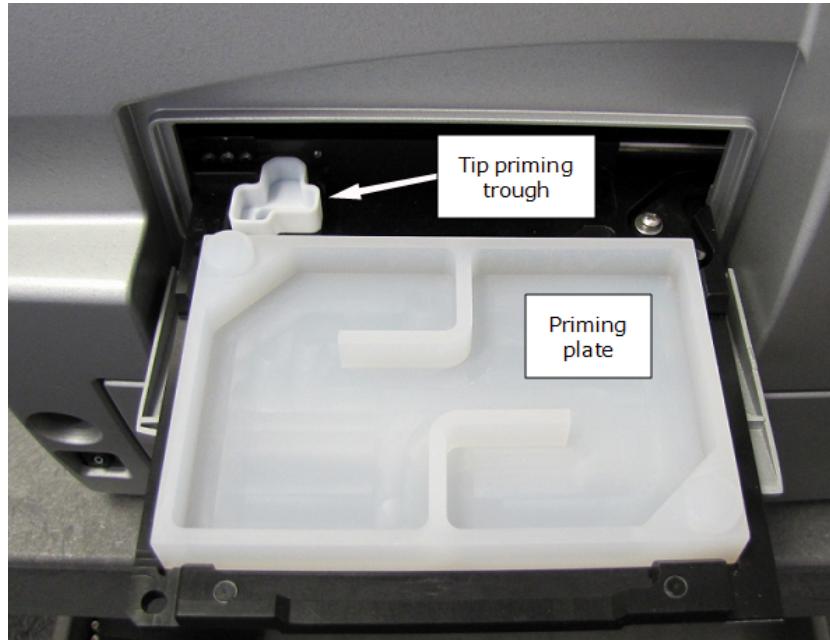


Figure 2-6: Priming trough and plate installed on the carrier

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 HTX** 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 HTX 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 107 to learn about BioTek's recommended OQ/PQ procedures for the Synergy HTX.

A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Synergy HTX is available for purchase (BTI #1340508). Contact your local BioTek dealer for more information.

## Rewrap 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 [74](#) 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 HTX with all available modules weighs up to 38 pounds (17 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 panel and carrier shipping bolt before repackaging the reader. If necessary, please contact BioTek to order a replacement panel or bolt.

When preparing to ship the Synergy HTX 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 (BTI #7093001 for the reader, #7083001 for the dispense module).

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 [74](#).
3. If you will also be shipping the dispense module, perform the steps described on page [24](#).

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 lamp assembly and pack it in bubble wrap (see p. 14).
7. Replace the microplate carrier shipping bolt (see p. 13).
8. Tip the reader onto its back. Attach the shipping panel to the bottom of the reader using the four screws and washers. See *Figure 2-2* on page 11.
9. Wrap the plastic bag around the reader and shipping panel.
10. 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.
11. Grasp the handles on the shipping panel and carefully lower the reader into the inner shipping box. See *Figure 2-1* on page 10.
12. Slide the foam vertical supports into place around the reader. Place the accessories box on top.
13. Close and seal the inner box with tape.
14. Place four foam corner blocks around the inner shipping box. Close and seal the outer box with tape.
15. 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 74. 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 HTX**.
4. Perform this step twice, once per dispenser: Click the **Prime** tab 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.

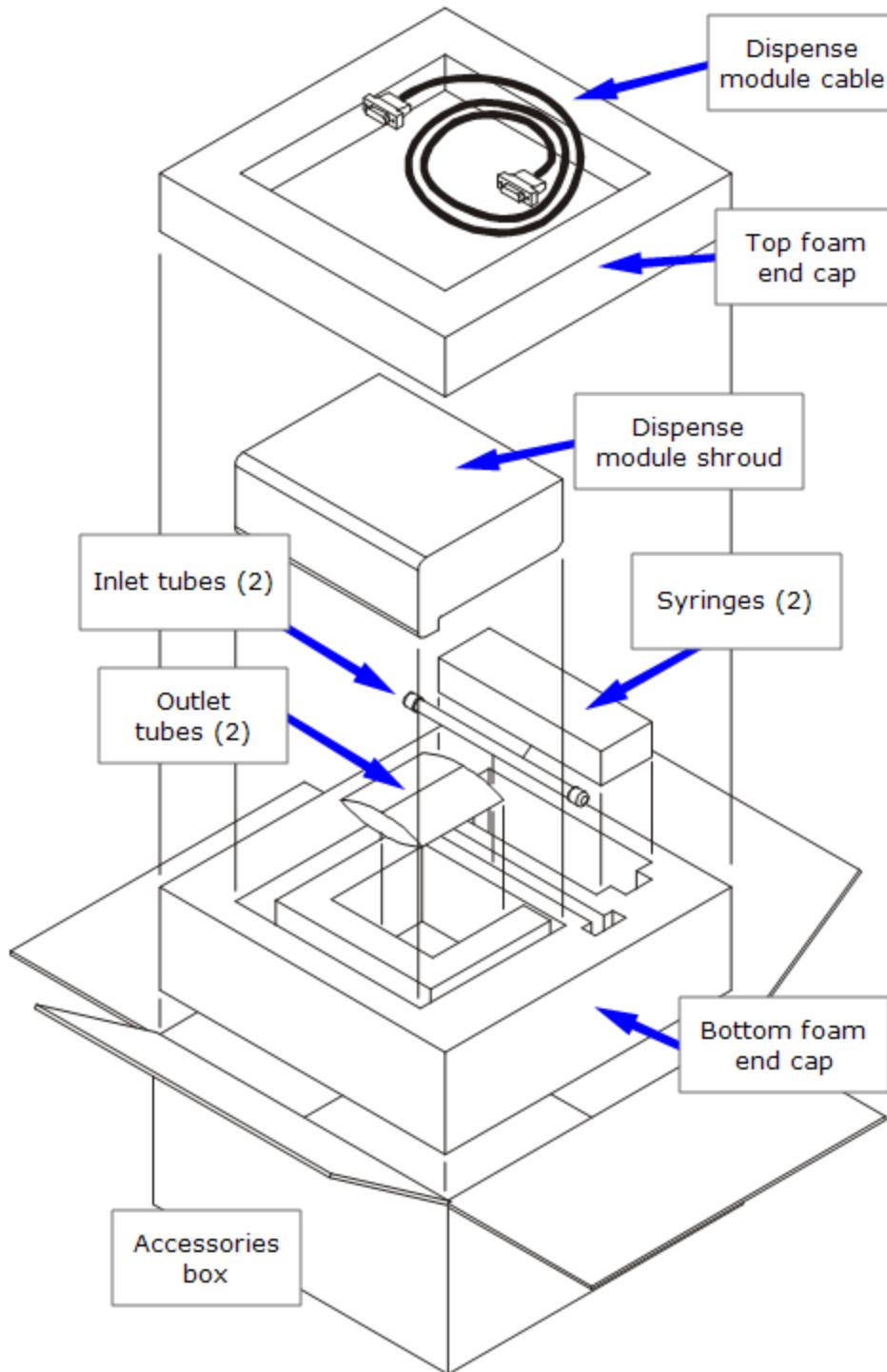


Figure 2-7: Packing the dispense module accessories

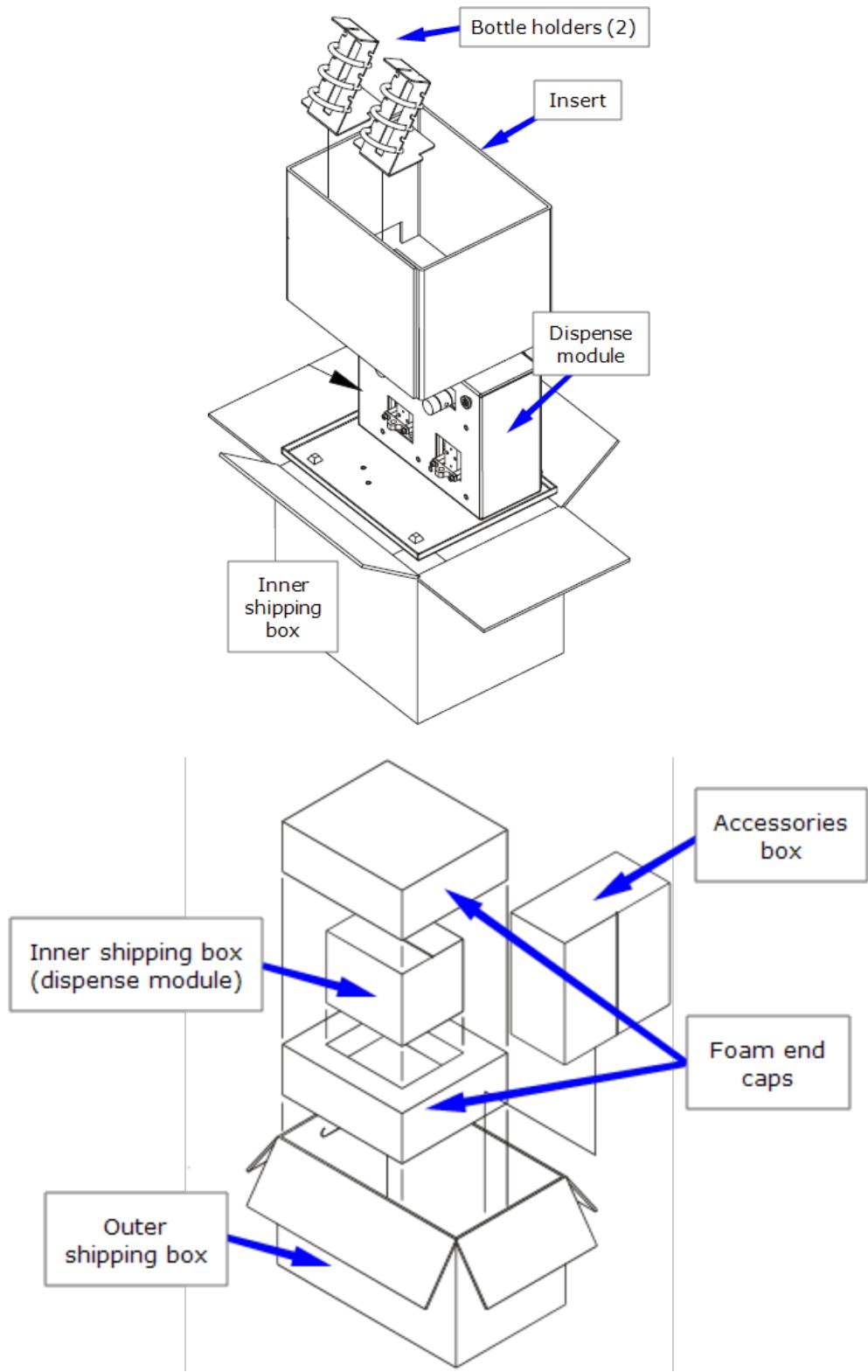


Figure 2-8: The inner (top) and outer (bottom) shipping boxes



## *Chapter 3*

# Getting Started

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

Key Components .....	30
Lamp Assembly and Filter Wheel Access .....	31
Excitation and Emission Filter Wheels .....	32
Installing the Time-Resolved Cartridge .....	34
Configuring the System for Luminescence Measurements .....	35
Injection System .....	35
External Dispense Module .....	35
Internal Tubing .....	37
Priming the Injection System .....	37
Gen5 Software .....	38
Viewing and Modifying Filter Wheel Information .....	38
Protocols and Experiments .....	39
Dispense Module Control .....	40
Plate Shaking Options .....	41
Recommendations for Optimum Performance .....	42
General .....	42
Luminescence Measurements .....	43
Models with Injectors .....	43
Incubation and Partial Plates .....	43

## Key Components

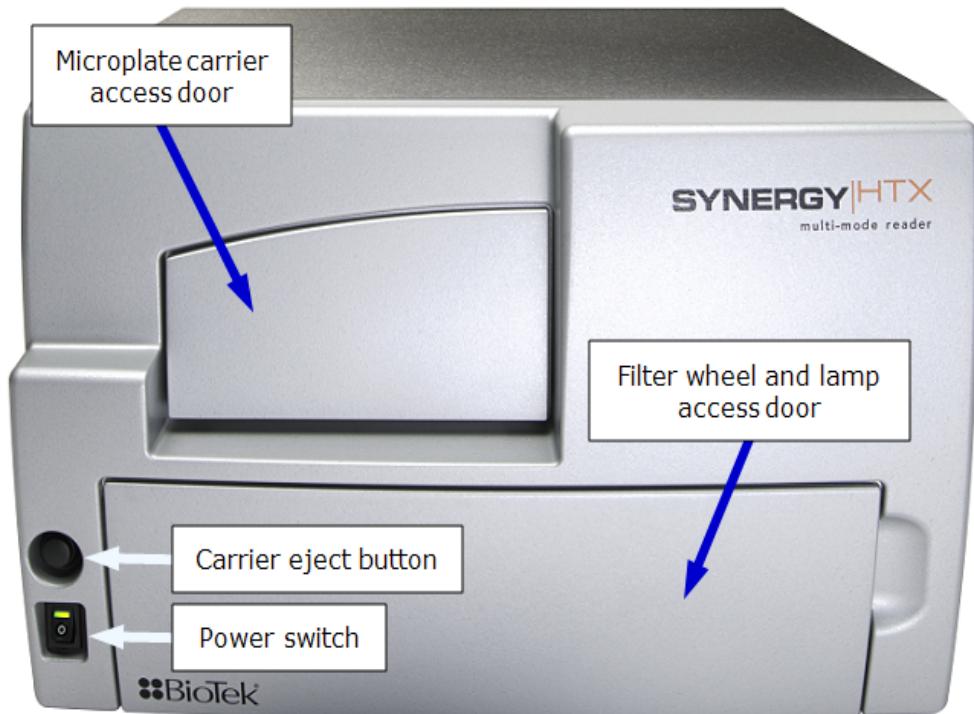


Figure 3-1: Synergy HTX, front view

- The power switch contains an LED, which is illuminated green when the power is on.
- The carrier eject button can be used to move the microplate carrier into or out of the measurement chamber and also to stop the instrument from “beeping” when it encounters an error.
- The microplate carrier supports microplates and adapter plates as described in the **Specifications** appendix. The plate is positioned so that well A1 is in the left rear corner of the carrier. A spring clip holds the plate securely in place. The microplate loading door helps to ensure a light-impermeable measurement chamber. When a plate read is initiated, the carrier slides into the measurement chamber and then moves on the X and Y axes to align each microwell with the top or bottom fluorescence probe, or bottom absorbance probe, as specified in the Gen5 procedure. When the read is complete, the plate carrier slides to its full-out position.

For fluorescence and luminescence reading modes, the height of the top optic probe can be adjusted. Use the Read Height option to define how far the top probe shall be offset from the top surface of the plate during the read. In Gen5, this option is found in a Read step within a Procedure. Refer to the Gen5 Help for further instructions.

## Lamp Assembly and Filter Wheel Access

*Applies only to models with fluorescence and/or luminescence capability*

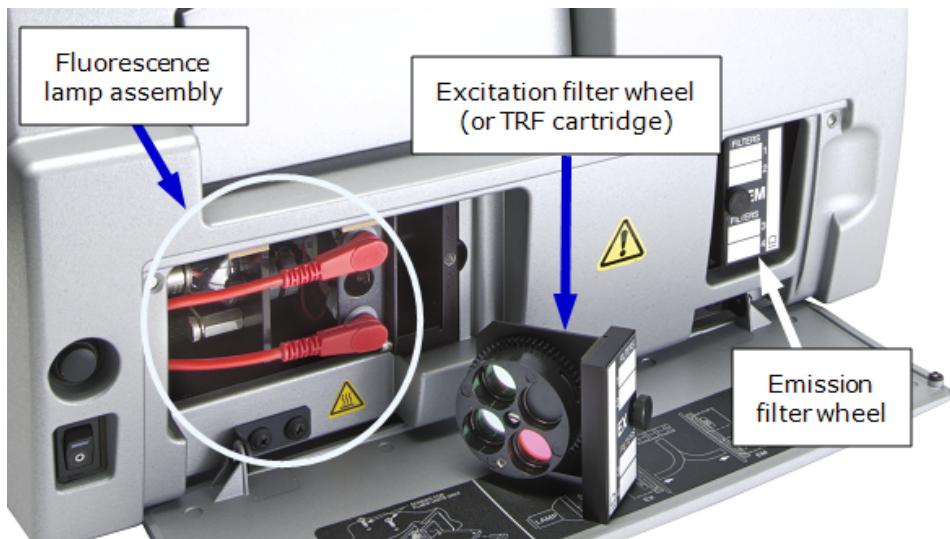


Figure 3-2: Fluorescence lamp assembly and filter wheels

- The fluorescence lamp assembly and the excitation and emission filter wheels are accessible via a hinged door on the front of the instrument. To open the door, insert your finger into the notch on the right side and pull the door downward. A diagram showing the location of the lamp assembly and the orientation of the excitation and emission filter wheels is printed on the inside of the hinged door.
- For models with the Time-Resolved Fluorescence feature, remove the excitation filter wheel and replace it with the "TR" cartridge before running a time-resolved fluorescence assay. See page 34 for more information on the TR cartridge.

<span style="font-size: 2em; font-weight: bold;">i</span>	<p>The Synergy HTX has two lamps:</p> <p><b>Standard Fluorescence.</b> The 20-watt tungsten halogen lamp's life is rated at an average of 1000 hours, and it is user-replaceable. The intensity of the bulb will slowly drop over time until the instrument's run-time self-check detects a low lamp current signal and Gen5 displays an error message. The lamp (PN 7080500) should be replaced at this time. Keeping a spare lamp on hand is recommended.</p> <p><b>Absorbance and Time-Resolved Fluorescence.</b> This bulb should outlive the useful life of the reader. If there is a problem with the lamp, however, the intensity may drop and the run-time self-check will detect a low signal level and generate an error message. If this happens, the instrument will require service. Contact BioTek for assistance (this lamp is not user-replaceable).</p>
-----------------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

## Excitation and Emission Filter Wheels

Synergy HTX models with fluorescence capability are equipped with one excitation filter wheel and one emission filter wheel; readers with luminescence capability use an emission filter wheel only. (A monochromator is used for absorbance measurements.)

A filter in the excitation wheel selects the narrow band of light to which the sample will be exposed. A filter in the emission wheel selects the band of light with the maximum fluorescence signal, to be measured by the photomultiplier (PMT).

Each filter wheel is labeled EX or EM, and can contain up to four filters and/or black “plugs.” 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.

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 4E18 (saturation) error.

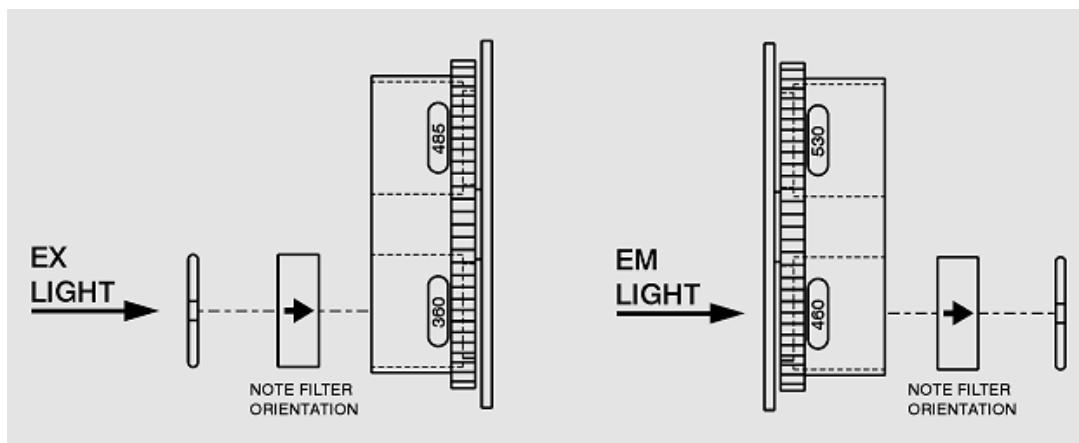


Figure 3-3: Diagram showing the proper orientation of the filters in their wheels



The Synergy HTX 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 HTX does not automatically detect which filters are installed.

See page [38](#) for information on updating Gen5’s filter table.

## Removing the Filter Wheels, Changing Filters

It is important to note that:

- The excitation and emission filter wheels are not interchangeable and are labeled as follows: EX = Excitation, EM = Emission. (TR = Time-Resolved Cartridge; see page 34)
- Filter direction within a filter wheel is important, and the direction differs depending on the filter wheel. There is a diagram on the inside of the front panel door indicating this.
- Each filter is marked with an arrow indicating the proper direction of light. Refer to the figure on the previous page for proper filter orientation.

### To remove a filter wheel:

1. **Important!** Turn off the instrument.
2. Open the filter wheel access door using the depression on the right side of the door.
3. Observe the two thumbscrews within the compartment. The left thumbscrew holds the excitation filter wheel in place; the right secures the emission filter wheel.
4. Remove the thumbscrew and slide the filter wheel's supporting metal bracket straight out of the compartment.

The emission filter wheel will "spring" out when removed. This is because a shutter behind the wheel closes quickly to protect the PMT.



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

### To remove a filter or plug:

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

### To replace a filter or plug:

1. Hold the metal bracket with the filter wheel facing up.
2. Properly orient the filter or plug (see *Figure 3-3* on page 32), and then drop it into the desired filter wheel location.
3. Using your fingers, squeeze the sides of the C-clip filter 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 C-clip until it sits flush against the filter.

4. Clean both sides of the filter with lens paper.

#### To reinstall a filter wheel:

1. Ensure that all filters and/or plugs are properly inserted.
2. Slide the filter wheel back into its chamber.
3. Replace the thumbscrew.
4. Close the front door.
5. Turn on the instrument.

## Installing the Time-Resolved Cartridge

*Applies only to models that support Time-Resolved Fluorescence*

The "TR" cartridge (see [Figure 3-4](#)) must be installed in place of the excitation filter wheel before a TRF assay can be run. The TR cartridge allows light from the xenon flash bulb to be input to the fluorescence optical system within the Synergy HTX. Excitation wavelengths are selected by adjusting the monochromator from 200 to 999 nm in 1-nm increments, with a fixed bandwidth of 10 nm.

The Synergy HTX automatically detects the presence of the TR cartridge. At the start of a time-resolved fluorescence assay, Gen5 will prompt you to install the TR cartridge if it is missing.

1. **Important!** Turn off the instrument.
2. Open the filter wheel access door using the depression on the right side of the door. Observe the two thumbscrews within the compartment. The left thumbscrew holds the Excitation filter wheel in place.
3. Remove the left thumbscrew and slide the filter wheel's supporting metal bracket straight out of the compartment.
4. Slide the TR cartridge into the compartment and replace the thumbscrew. Close the front door and turn on the instrument.



Figure 3-4: The "TR" cartridge, for time-resolved fluorescence

## Configuring the System for Luminescence Measurements

- For best results when taking luminescence measurements, the excitation filter wheel should have no empty locations, and it should have at least one “plug” (also referred to as a “dummy filter”) installed to prevent light from reaching the samples. Remove the excitation filter wheel and examine its contents; ensure that there are no empty locations and there is at least one plug installed.
- If your tests require that the light emitted from the samples remain unfiltered, the emission filter wheel should have an empty location in it. Remove the emission filter wheel and examine its contents; ensure that there is an empty location.



If you made any changes to either filter wheel, you must update Gen5’s filter table. Select “PLUG” to indicate the presence of a plug and “HOLE” to indicate an empty location. Click **Send Values** to download the information to the reader. See the example in *Figure 3-7* on page 38.

# Injection System

## External Dispense Module

If a syringe is leaking, it may need to be replaced. See **As-Needed Maintenance** starting on page 73 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.

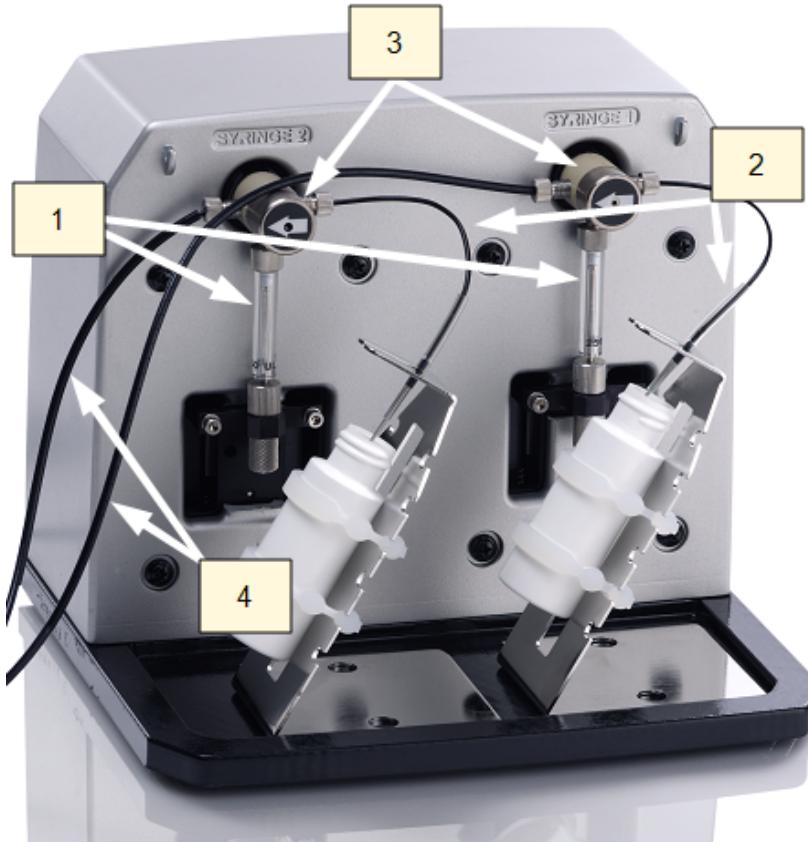


Figure 3-5: Dispense module components

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 HTX.

## 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.

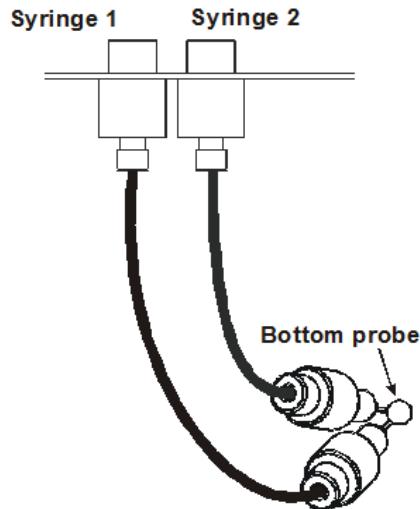


Figure 3-6: Injectors inside the reader

---

The tubing and injectors should be cleaned at least quarterly. See **Preventive Maintenance** starting on page 45 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 2-6* on page 21):

- 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.

## Gen5 Software

Gen5 supports all Synergy HTX 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.

### Viewing and Modifying Filter Wheel Information

If configured with fluorescence or luminescence capability, the Synergy HTX ships with a set of excitation and emission filters installed, and the reader's onboard software is preconfigured with the filter values and their locations. When Gen5 establishes communication with the reader, it uploads and stores this information. To view or modify the information, select **System > Instrument Configuration**, highlight the **Synergy HTX**, and click **View/Modify**. Click **Setup** and the **Fluorescence/Luminescence** tab.

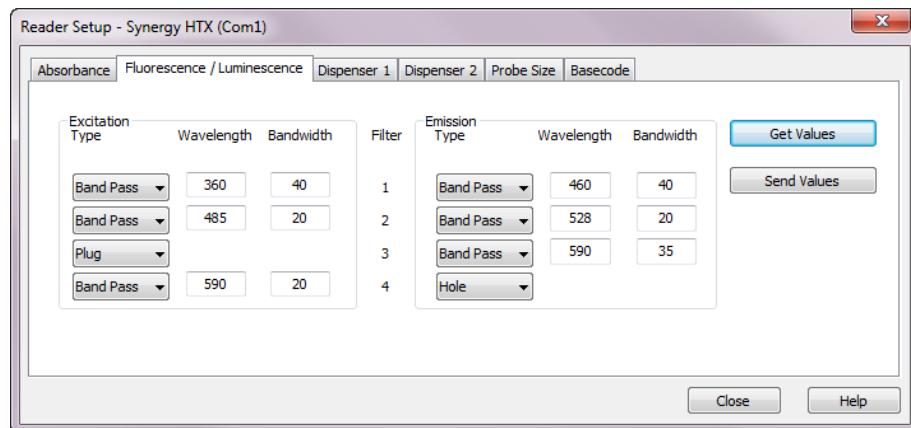


Figure 3-7: Configuring EX/EM filter wheel information in Gen5



**Important!** It is critical that the filter wheel information in Gen5 and the reader's software exactly matches the configuration of the installed filter wheels.

To change the settings and download them to the instrument:

1. Select **Band Pass**, **Plug**, or **Hole** for the excitation and emission filter wheels.
2. For each filter type, enter the wavelength value and its accompanying bandwidth. The bandwidth is printed on the side of each filter.
3. **Important!** When finished, click **Send Values** to download the information to the reader. (Clicking Get Values uploads information from the reader.)

4. Click **OK** to save the settings and close this dialog. The settings become available for selection in the Read step dialog in a Procedure.

## 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 HTX** 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 HTX** 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.

## Plate Shaking Options

The Synergy HTX supports multiple plate shaking options, as described below. Shaking is controlled using Gen5 by adding a Shake step to a protocol's procedure.

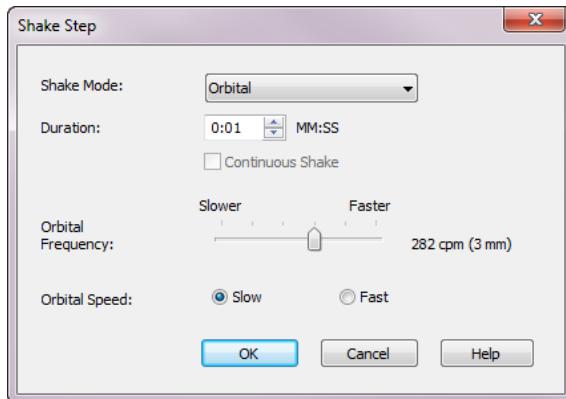


Figure 3-8: Gen5 Shake Step options

Mode	Speed	Amplitude (in 1-mm steps)	Frequency
Linear	-	1 mm to 6 mm	18 Hz to 6 Hz
Orbital	Slow	1 mm to 6 mm	10 Hz to 3 Hz
Orbital	Fast	1 mm to 6 mm	14 Hz to 5 Hz

Note: Frequency is based on the Amplitude selected

## 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 HTX supports standard flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with flat-bottomed wells. See **Specifications** starting on page [137](#) 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](mailto: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*

# Preventive Maintenance

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

Overview .....	46
Recommended Maintenance Schedule .....	47
Warnings and Precautions .....	48
Clean Exposed Surfaces .....	49
Inspect/Clean Excitation and Emission Filters .....	50
Flush/Purge Fluid Path .....	51
Run a Dispense Protocol (Optional) .....	52
Empty/Clean the Tip Priming Trough .....	53
Clean the Priming Plate .....	53
Clean the Internal Components .....	53

## Overview

A general Preventive Maintenance (PM) regimen for the Synergy HTX 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 HTX 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
<b>All models:</b>			
Clean exposed surfaces			✓
Inspect/clean excitation and emission filters (if equipped)		✓	
Decontaminate the instrument	<i>before shipment or storage</i>		
<b>Models with injectors and an external dispense module:</b>			
Flush/purge the fluid path	✓		
(Optional) Run a Dispense protocol			✓
Empty/clean tip prime trough	✓		
Clean priming plate			✓
Clean internal components		✓	✓
Tubing and injector heads			
Optical probes			
Internal surfaces			

## Warnings and Precautions

	<b>Warning! Internal Voltage.</b> Turn off and unplug the instrument for all maintenance and repair operations.
	<b>Warning!</b> Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears.
	<b>Warning!</b> Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.
	<b>Caution!</b> The buildup of deposits left by the evaporation of spilled fluids within the read chamber can impact measurements. Be sure to keep System Test records before and after maintenance so that changes can be noted.
	<b>Caution! Models with injectors.</b> Before removing the reader's cover to expose internal parts, purge the dispense module, turn off the instrument, and disconnect the fluid line, power cable, and PC cable.
	<b>Warning!</b> The fluorescence 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.
	<b>Warning!</b> The instrument with all available modules weighs up to 38 pounds (17 kg) depending on the model. Use two people when lifting and carrying the instrument.

	<b>Important!</b> Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. <b>Do not allow water or other cleaning solution to run into the interior of the instrument.</b> If this happens, contact BioTek's Technical Assistance Center.
	<b>Important!</b> Do not apply lubricants to the microplate carrier or carrier track. Lubricant attracts dust and other particles, which may obstruct the carrier path and cause errors.

---

## 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 to better access the interior; see instructions starting on page [53](#).

---

## 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

① 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 **Getting Started** chapter contains illustrations for identifying the filter wheels and their unique characteristics. It also contains instructions for replacing filters, if necessary.

3. Inspect the glass filters for speckled surfaces or a "halo" effect. This may indicate deterioration due to moisture exposure over a long period of time. If you have any concerns about the quality of the filters, contact your BioTek representative.
4. Using cotton balls or lens-cleaning tissue moistened with a small amount of high-quality alcohol, clean each filter by lightly stroking its surface in one direction. Ensure that the filters remain in their current locations.
5. Use a magnifying glass to inspect the surface; remove any loose threads left by the cotton ball.
6. Replace the filter wheels in their respective positions and replace the thumbscrews. Close the hinged door.

## 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.

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 HTX**.
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 HTX**.
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 132).

## Run a Dispense Protocol (Optional)

*Applies only to models equipped with injectors*

After flushing/purging the system (described on page 51) and before running an assay that requires dispense, take a moment to visually inspect the dispense accuracy.



Use a DI H<sub>2</sub>O-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<sub>2</sub>O-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 53 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*

The internal components that require routine cleaning include the optical probes, the surface beneath the microplate carrier, and the internal dispense tubes and injector heads.

The components should be cleaned at least *quarterly*. In addition, if fluid has spilled inside the instrument and/or if an unusually high background signal has been flagged by the assay controls (typically blanks or negative controls), the optical probes and the surface beneath the microplate carrier should be cleaned.

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

For all tasks:

- Protective gloves
- Safety glasses

For removing the shroud and some of the internal components:

- Screwdriver
- 1/8" hex key
- 3/32" hex key

For cleaning the internal dispense tubes and injector heads, as well as for wiping the surface under the plate carrier:

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

For cleaning the optical probes:

- Clean cotton swabs
- Isopropyl alcohol
- Lens-cleaning tissue

## 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. Remove four mounting screws: one at the bottom rear corner on each side, and two at the top center of the rear panel.
4. Stand facing the front of the instrument. Grasp both sides of the shroud, slide it toward you, and pull it straight off the instrument. Set the shroud aside.

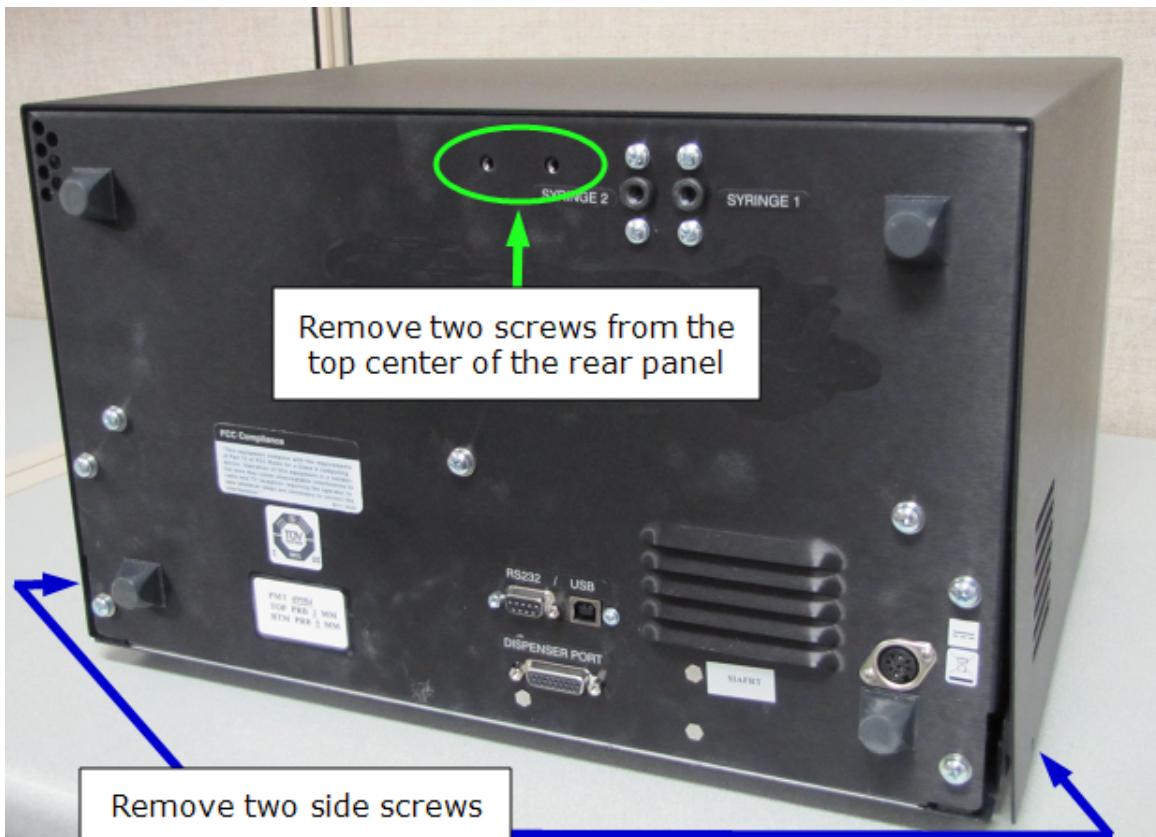


Figure 4-1: Removing the screws that secure the shroud

When reinstalling the shroud, press down firmly on the top to maintain a good seal while tightening the top screws.

## Remove the Internal Dispense Tubes and Injector Heads

Review the photo below to identify the components described in this section:

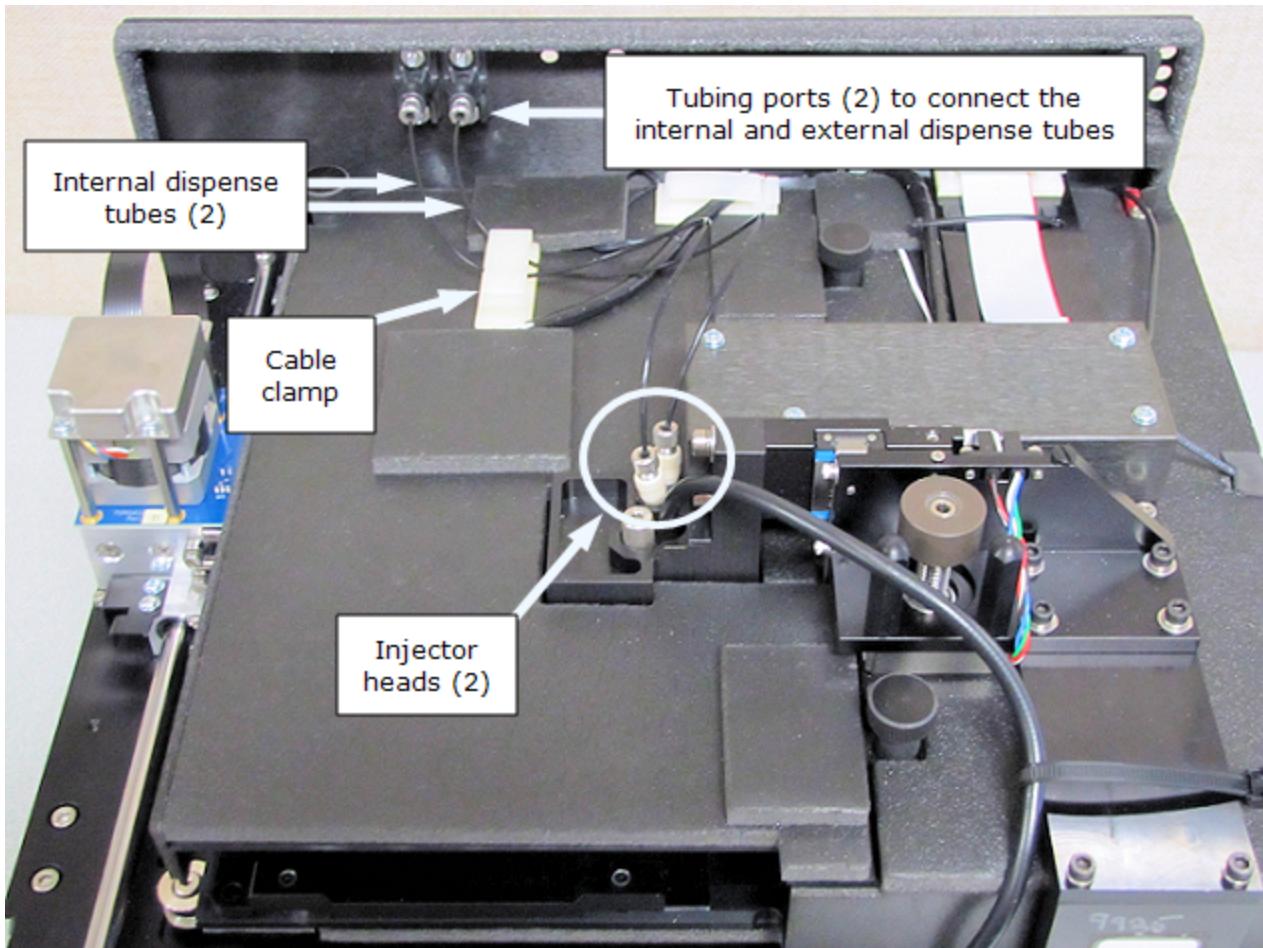


Figure 4-2: Internal components for the injection system



**Important!** When reinstalling the internal dispense tubes, be sure to align the tubing ports with the injector heads as shown in the next diagram. Look for the SYRINGE number labels on the instrument's rear panel.

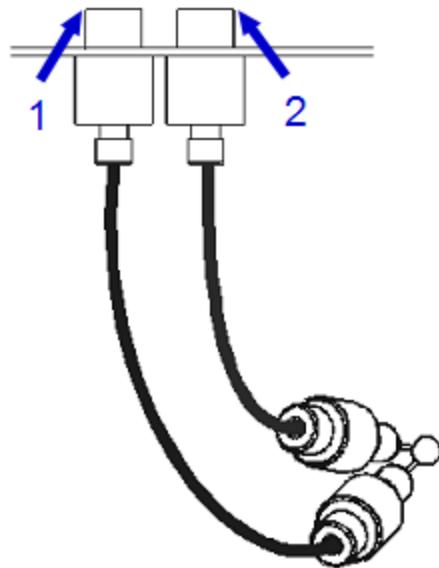


Figure 4-3: Identifying the numbered syringe ports on the back of the reader

Perform these steps to remove both sets of internal dispense tubes and injector heads (refer to [Figure 4-4](#) and [Figure 4-5](#)):

1. Open the cable clamp to release the tubes.
2. Locate the tubing ports on the reader's rear wall. Turn each tube's thumbscrew counterclockwise and gently pull the tube from the port.
3. Locate the injector heads. Turn each tube's thumbscrew counterclockwise to disconnect the tube from the injector head.

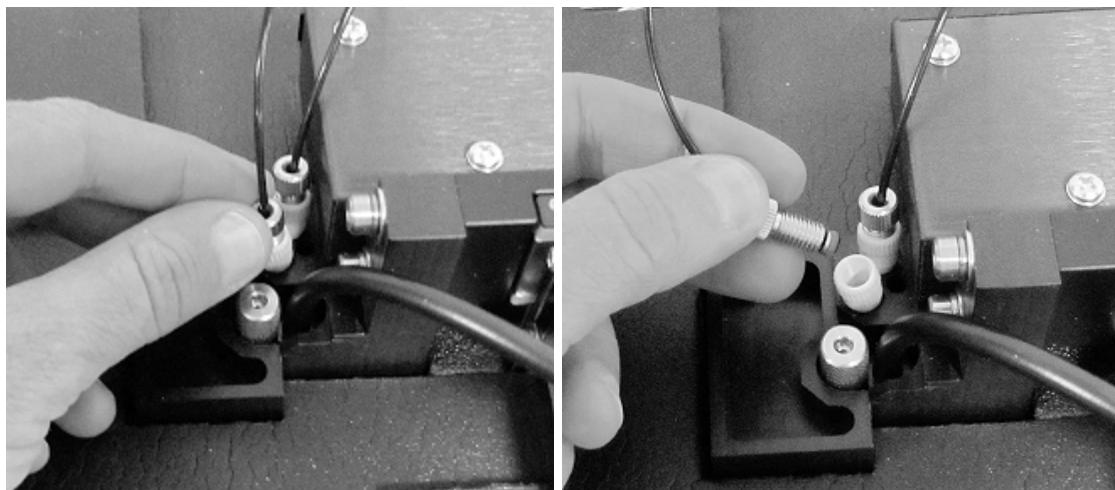
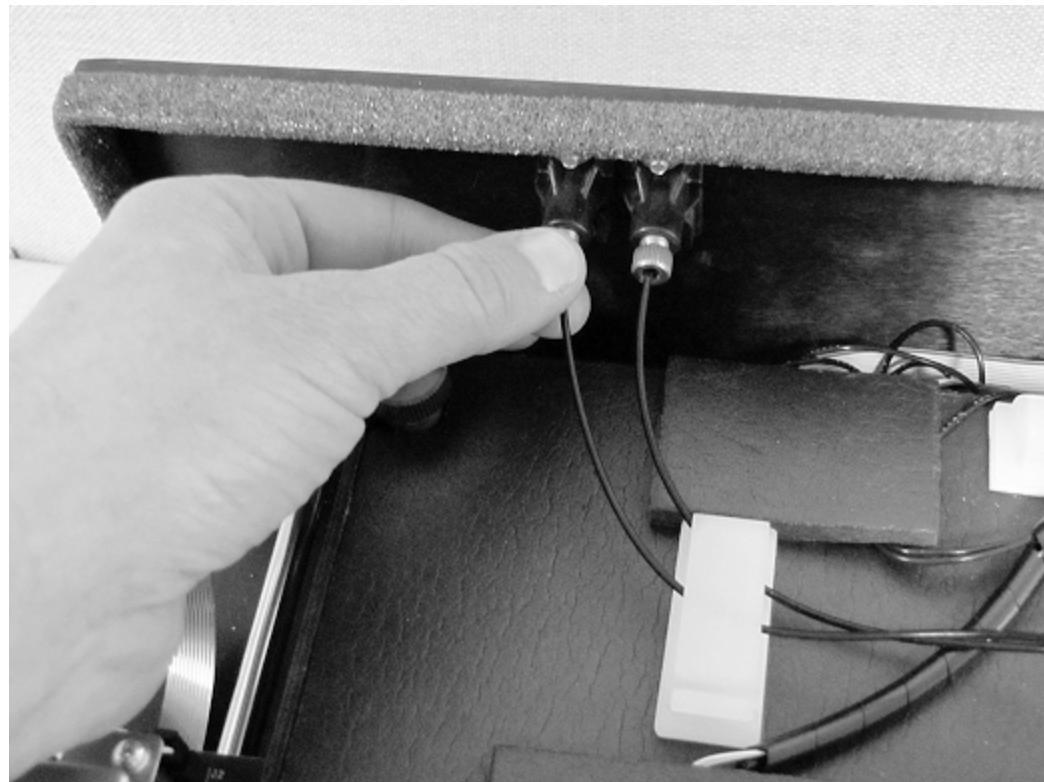


Figure 4-4: Removing the dispense tubes

4. Turn the injector heads counterclockwise and gently pull them out of their sockets.

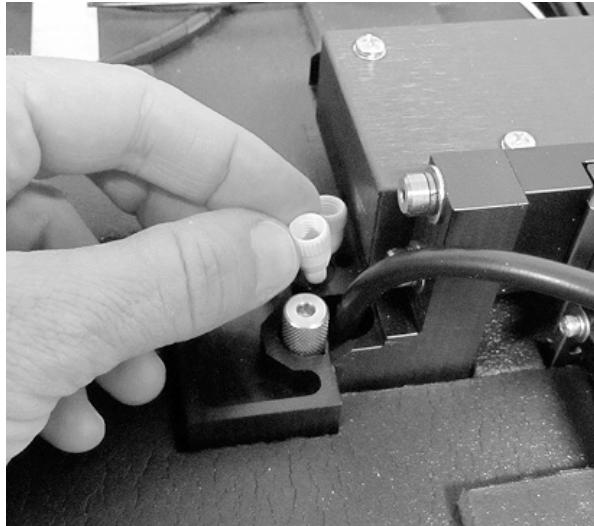


Figure 4-5: Removing the injector heads

① Be sure to seat the injector tips securely when reinstalling. See [Figure 4-22](#) on page [71](#).

## Clean the Dispense Tubes and Injector Heads

As discussed on page 46, 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 4-6: 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).

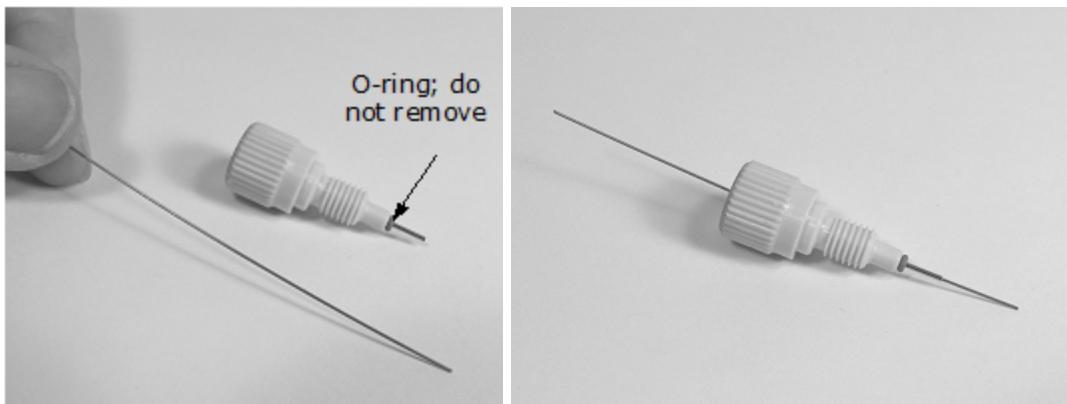


Figure 4-7: Using the stylus to clean an injector head

## Clean the Optical Probes

The optical probes should be cleaned at least *quarterly*. They should also be cleaned if reagent has spilled and/or if an unusually high background signal has been flagged by the assay controls (typically blanks or negative controls).

Contaminated probes can lead to a loss of sensitivity (e.g., instead of being able to meet a 10 pg/mL concentration detection limit, the instrument may only be able to meet 20 pg/mL). Another indicator is the %CV in the Corners liquid test—it may increase due to “noise” in the chamber from any spilled fluorescing compounds.

- To access the optical probes, the first step is to unplug the reader and remove its shroud (cover). If you haven’t already done this, turn to page 53 now for instructions.
- We recommend cleaning the internal tubes and injector heads along with the optical probes. Instructions for removing and cleaning these components are provided on pages 64 through 67.

Before starting this procedure:

- Gather some supplies:
  - Small container of isopropyl alcohol
  - Small container of deionized or distilled water
  - Lens-cleaning tissue
  - Cotton swabs
- Review [Figure 4-8](#) to identify the components described in this section.

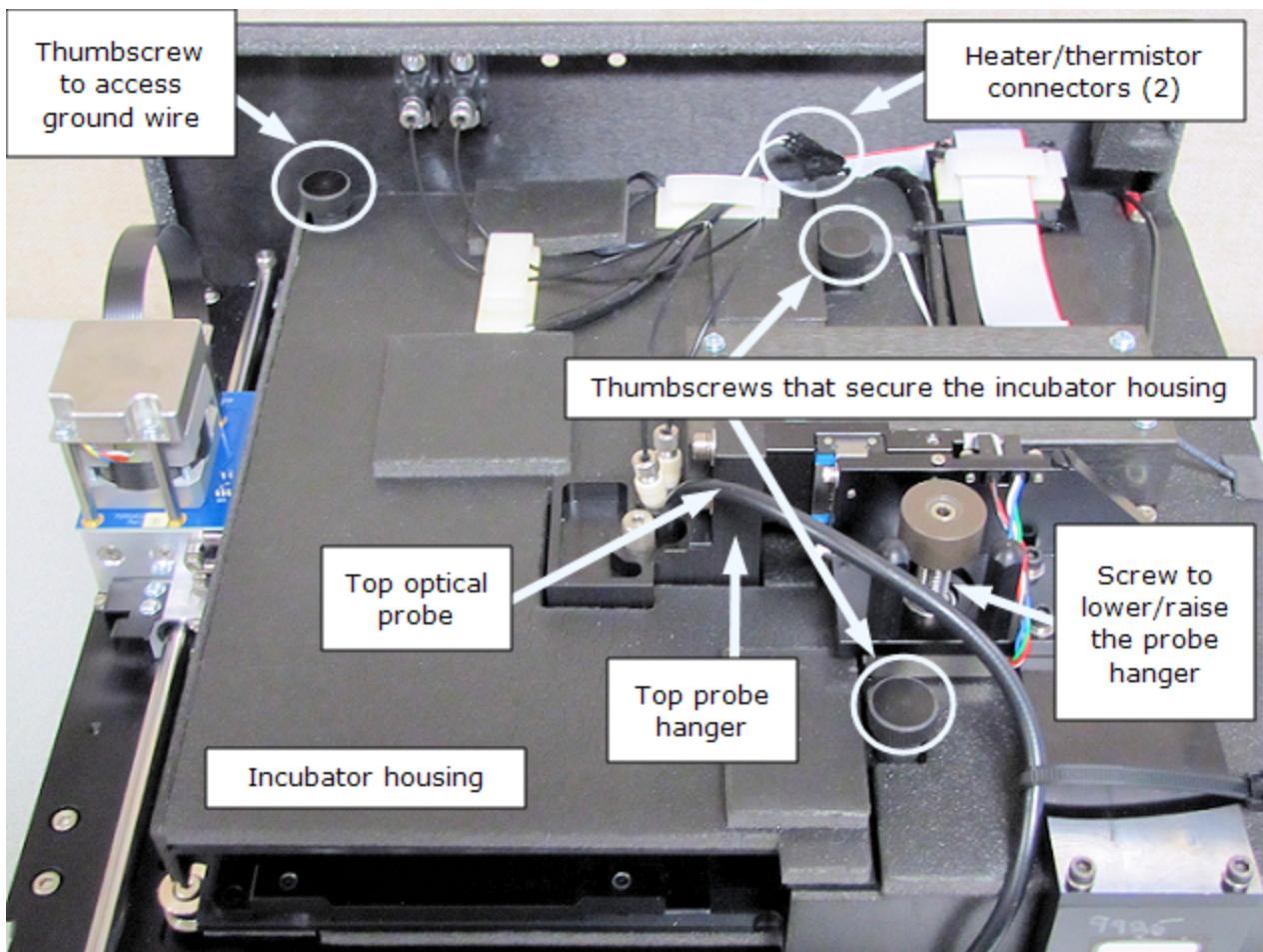


Figure 4-8: Internal components discussed in this section

Once the shroud has been removed and the internal tubes and injector heads have been removed and cleaned, perform the steps on the next few pages to remove a few more components and then clean the optical probes.

1. Disconnect the heater and thermistor wires. To do this, depress the small tab (see arrow below) and separate the connectors.



Figure 4-9: Disconnecting the heater and thermistor wires

2. Remove the thumbscrew located in the left rear of the instrument and set it aside. This exposes the ground wire.

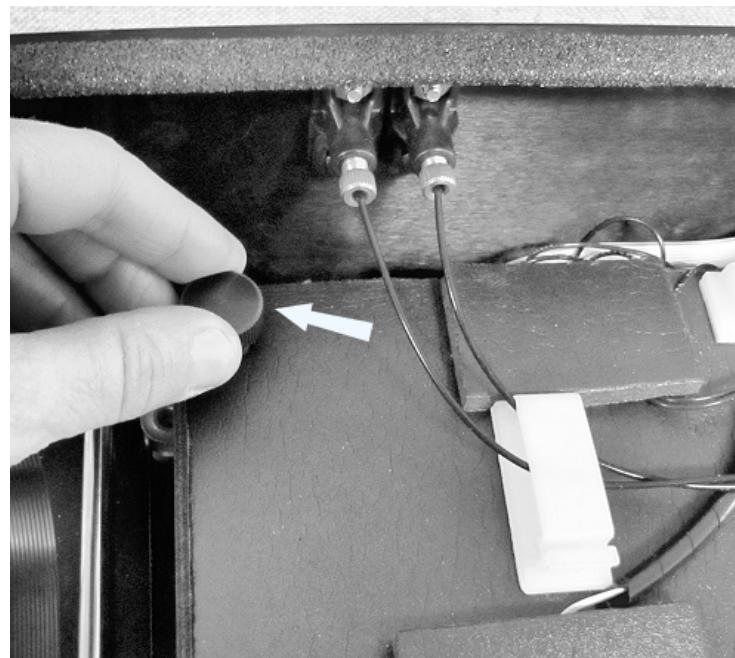


Figure 4-10: Removing a thumbscrew to expose the ground wire

3. Lift the ground wire and move it off to the side.



Figure 4-11: Moving the ground wire

4. Remove the two thumbscrews that secure the incubator housing and set them aside.
5. Turn the top probe screw counterclockwise to lower the probe hanger all the way to the bottom. (Rotate the screw, not the ring around it.)

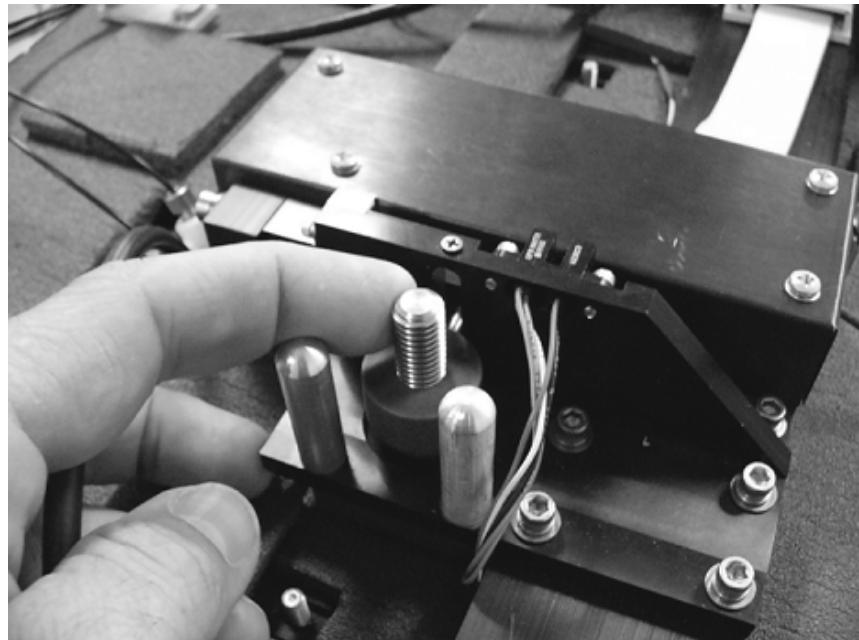


Figure 4-12: Lowering the probe hanger

6. Lift the left side of the incubator housing and carefully slide it out.

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.

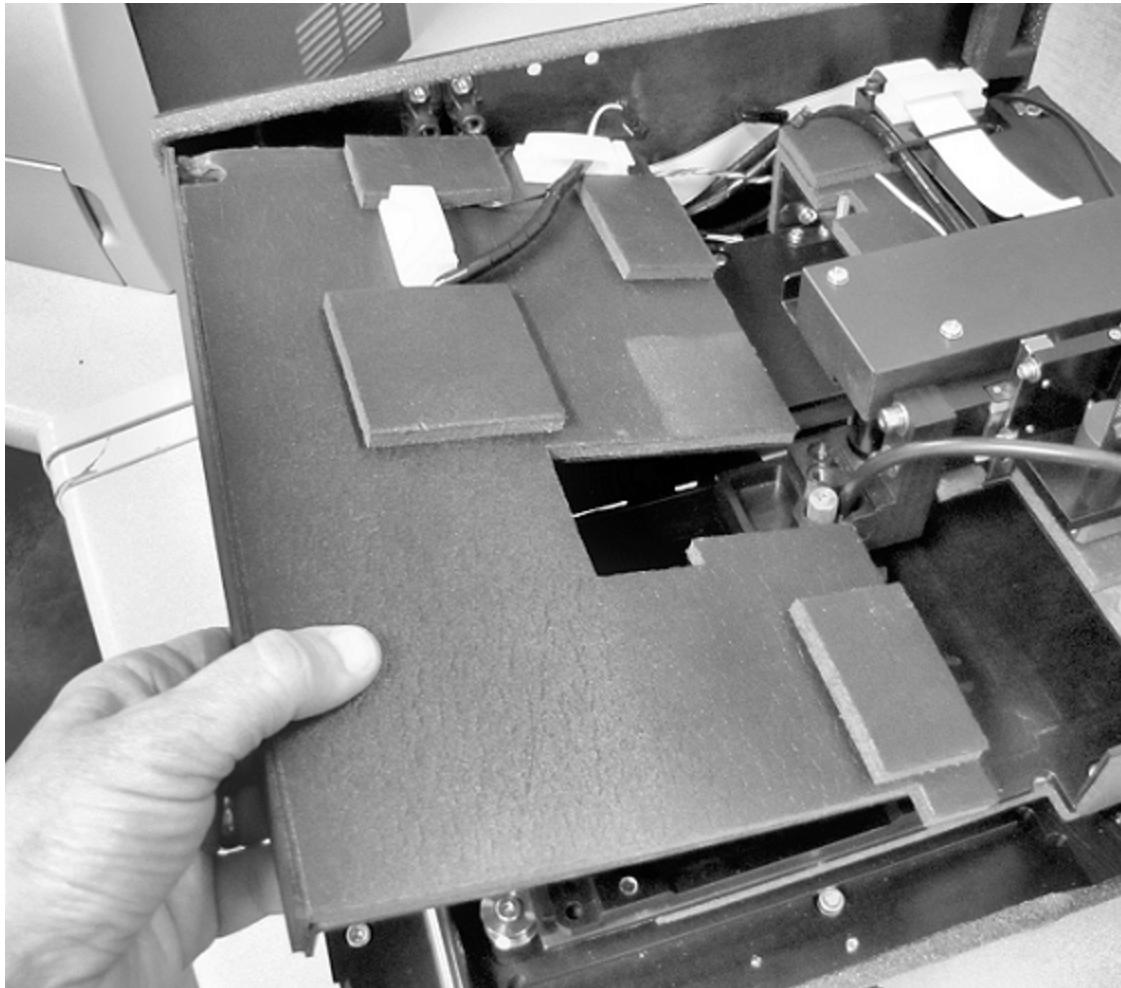


Figure 4-13: Removing the incubator housing

7. Use a 1/8" hex key to remove the top optical probe's holding screw.

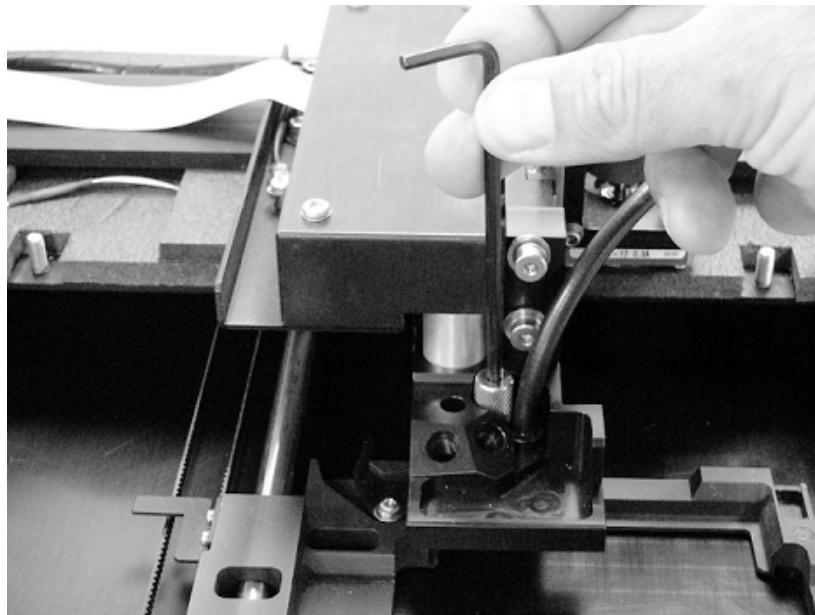


Figure 4-14: Removing the top probe's holding screw

8. Gently pull the optical probe up and out of its socket to expose it for cleaning. Soak the end of the probe in alcohol for **one minute maximum**. Wipe with lens-cleaning tissue and set aside.

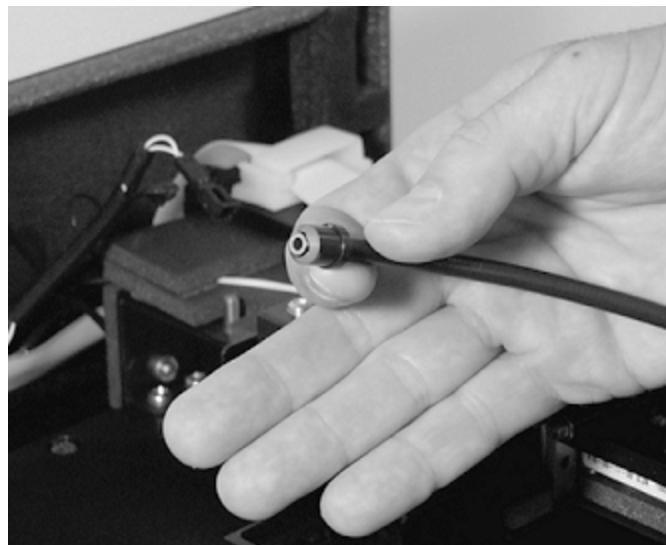


Figure 4-15: Optical probe, ready for cleaning

9. Use a 3/32" hex key to remove the two shoulder screws securing the top probe hanger. Remove the screws and set them aside.

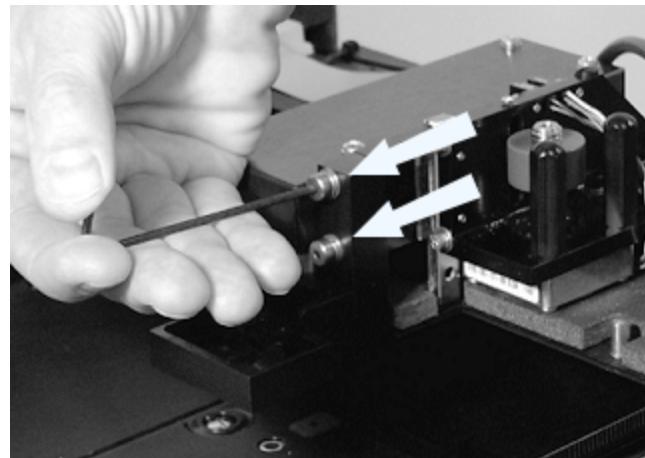


Figure 4-16: Removing two screws on the top probe hanger

10. Lower the top probe hanger and slide to the left to remove it. Turn the hanger upside down to clean the absorbance lens (see instructions on the next page). **Do not touch the lens with your fingers!** Inspect the block for spills or other contamination. Carefully clean with mild detergent if necessary.

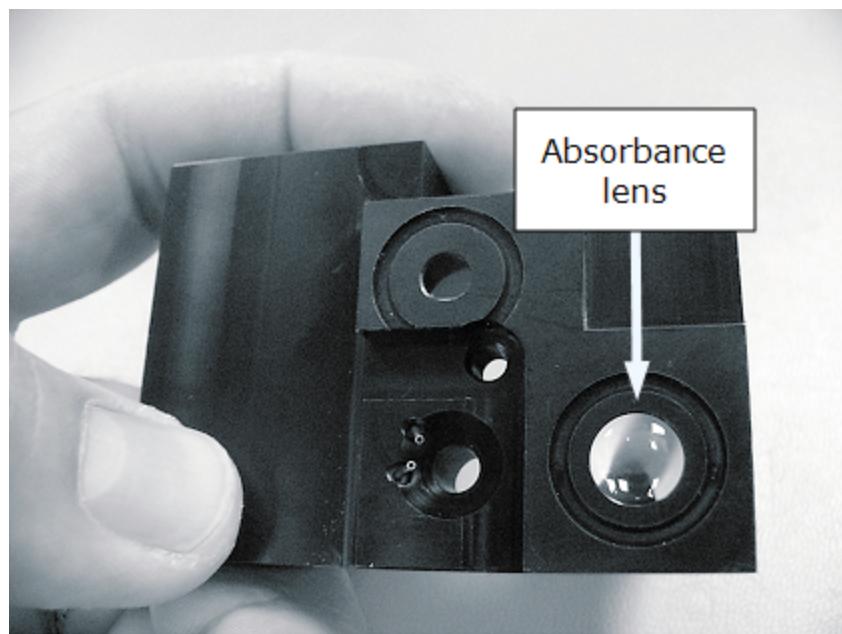


Figure 4-17: Viewing the underside of the probe hanger



**Caution!** When cleaning the absorbance lens with the swab, apply very little pressure to the lens! Applying too much pressure can push the lens out of its holder; reinstallation must be performed by BioTek service personnel. If the lens does fall out, contact BioTek TAC.

11. Use a cotton swab moistened with alcohol to *gently* clean the lens on the top probe hanger.



Figure 4-18: Cleaning the absorbance lens

12. Slide the microplate carrier out of the way. Use a cotton swab moistened with alcohol to clean the lens on the instrument surface.

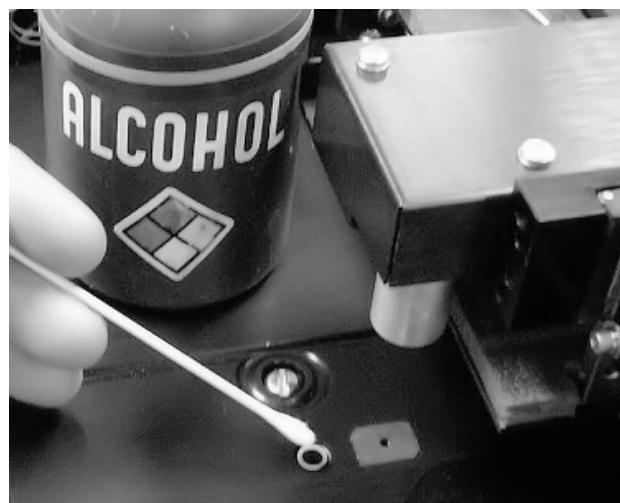


Figure 4-19: Cleaning the lens on the instrument surface

## Clean the Reader's Internal Surface

1. If you have not already done so, unplug the instrument and remove its shroud. Follow the instructions under [Clean the Optical Probes](#) to (at a minimum) disconnect the incubator wires, detach the ground wire, lower the top optic probe hanger, and remove the incubator housing.
2. Manually slide the microplate carrier to the left to engage the support pin, and then away from the center surface.

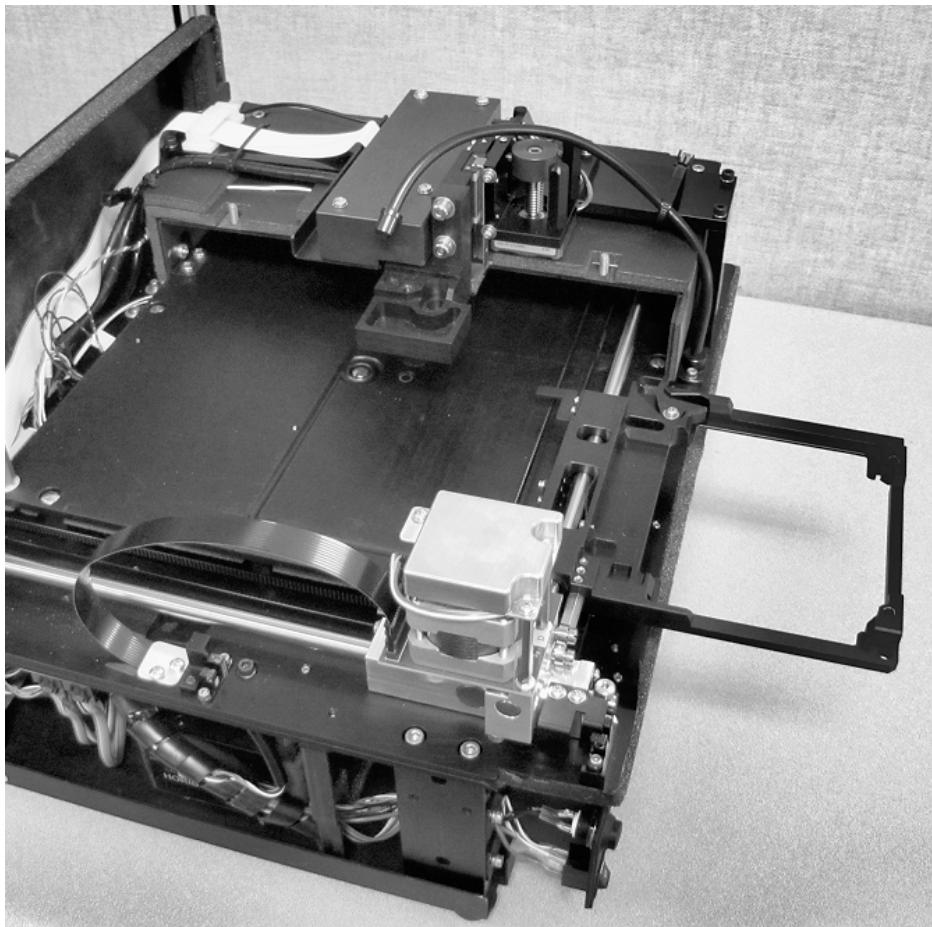


Figure 4-20: Microplate carrier fully extended

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

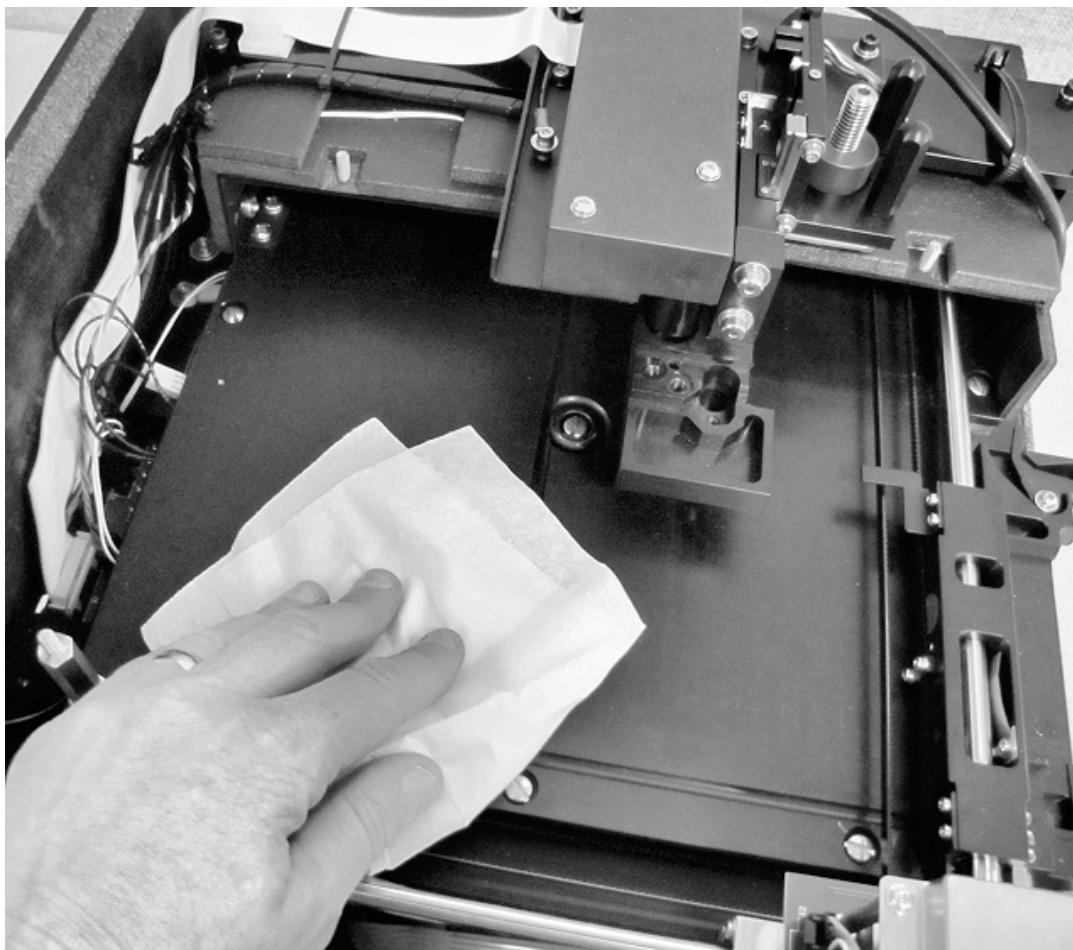


Figure 4-21: Wiping the reader's internal surface

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

## Reassemble the Components

Perform these steps in the order listed to reassemble the components. Refer to the page numbers shown for further instructions and photos demonstrating the steps.

1. Slide the microplate carrier back into the instrument.
2. Insert the two injector heads into their sockets in the top probe hanger. **Do not touch the absorbance lens with your fingers!** Ensure that the injector heads are properly seated in the hanger. The knurled plastic should sit flush against the hanger surface, as shown below (left).
3. Attach the two internal dispense tubes to the injector heads, as shown below (right). **Do not overtighten the thumbscrews!**

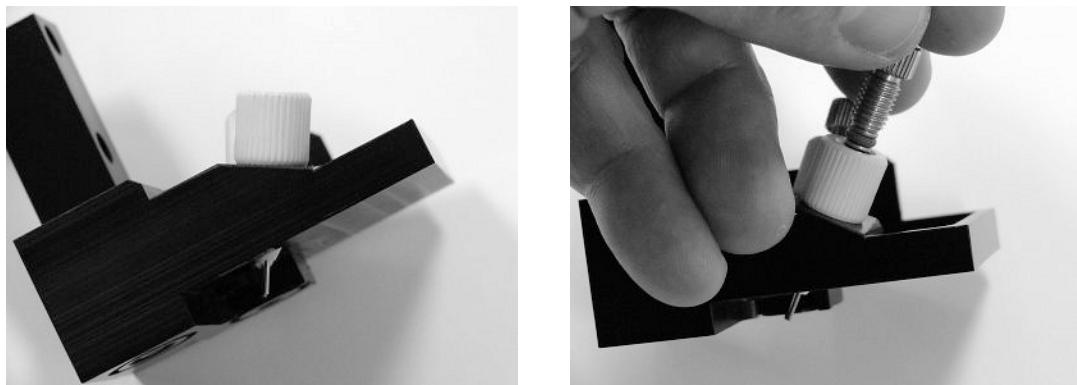


Figure 4-22: Reinstalling the injector heads and tubing

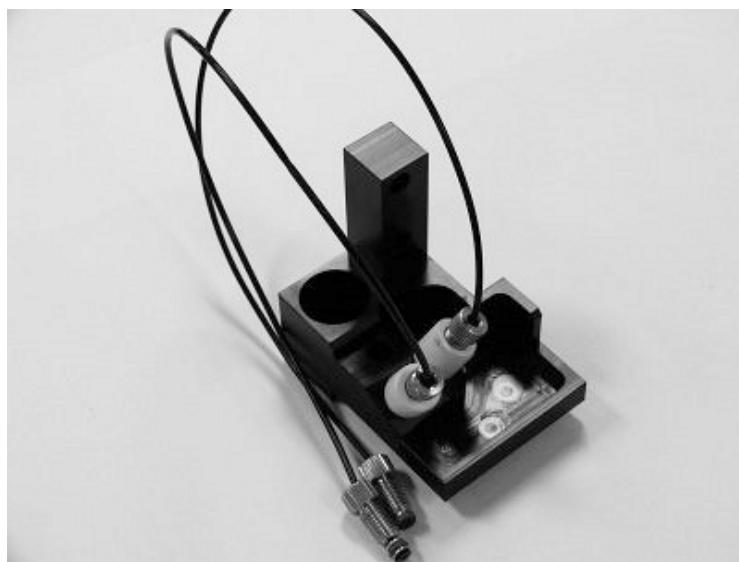


Figure 4-23: Top probe hanger, ready for reinstallation

4. Replace the top probe hanger and shoulder screws (using the 3/32" hex key). See page [67](#).
5. Insert the top optic probe into its socket and replace its holding screw (using the 1/8" hex key). See page [66](#).
6. Replace the incubator housing and two thumbscrews. Do not slide the two "forks" on the housing's right side under the fixed foam housing. See page [65](#).
7. Replace the groundwire and its thumbscrew. See page [64](#).
8. Reconnect the heater and thermistor wires. Be sure to connect wires of the same color. See page [63](#).
9. Attach the two internal dispense tubes to the tubing ports, taking care to align the correct port with the correct injector head. See page [58](#).
10. Slide the two internal dispense tubes into the cable clamp and close the clamp. See page [58](#).
11. Review the steps you just performed to make sure the components have been properly reassembled.
12. Slide the shroud onto the instrument.
13. Replace the four screws to securely attach the shroud to the base. See page [55](#).

## Performance Check

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

- Plug the instrument in and turn it on; allow its run-time system test to complete. Run a System Test through Gen5.
- Run any required OQ/PQ tests.

## *Chapter 5*

# **As-Needed Maintenance**

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

Decontamination .....	74
Required Materials .....	74
Procedure for Models without the Dispense Module .....	75
Procedure for Models with the Dispense Module .....	76
Dispense Module, Syringe Replacement .....	79
Syringe Maintenance Position .....	79
Replace the Syringe .....	80

## 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 HTX 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 78.

### 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 77.

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 51) 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  $\mu\text{L}$ , 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  $\mu\text{L}$ .
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  $\mu\text{L}$ .
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  $\mu\text{L}$ , and keep the default dispense Rate.
5. Run five prime cycles, for a total of 25,000  $\mu\text{L}$ .
6. Pause for 10 minutes and then run one prime cycle with 5000  $\mu\text{L}$ . This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
7. Empty the beaker containing the outlet tubes.
8. Wipe all surfaces with deionized water.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

## Clean the Tubing and Injectors

Turn to [Clean the Internal Components](#) on page 53 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 53 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 HTX**. 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.

## 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 HTX** and click the **Prime** tab.
2. Select the appropriate Dispenser number (1 or 2) associated with the syringe.
3. Click **Maintenance**. The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve.

## Replace the Syringe

Refer to [Figure 2-5 on page 17](#).

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 HTX**.
8. Click the **Prime** tab and click **Initialize**.

## *Chapter 6*

# Instrument Qualification Process

This chapter describes the tests that BioTek Instruments, Inc. has developed for complete qualification of all models of the Synergy HTX. 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 107 contains the actual step-by-step test procedures.

Instrument System Test .....	82
Plate Shaker Test .....	82
Absorbance Testing .....	83
Luminescence Testing .....	91
Fluorescence Testing .....	94
Injection System Testing .....	102

---

## Instrument System Test

Each time the Synergy HTX 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 [143](#) for information on error codes and troubleshooting tips.

Refer to **Sample Reports** on page [155](#) to see a sample System Test Report for Synergy HTX.

---

## 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 HTX, 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  $\pm$  3 nm ( $\pm$ 2 nm instrument,  $\pm$ 1 nm filter allowance).

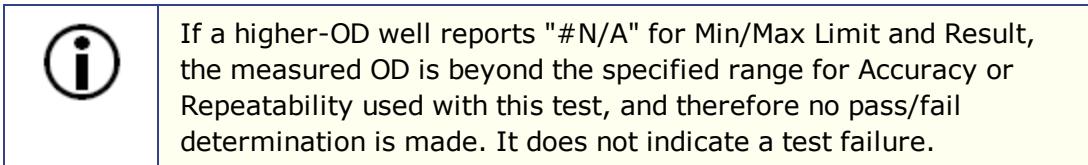
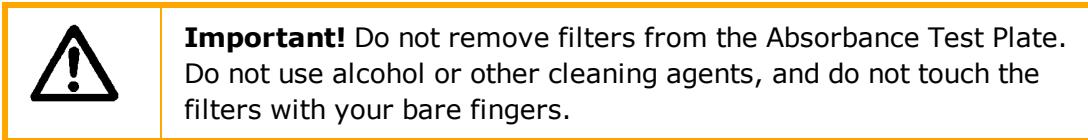
- **Alignment:** The test plate has precisely machined holes to confirm mechanical alignment. The amount of light that shines through these holes is an indication of whether the microplate carrier is properly aligned with the absorbance optical path. A reading of more than 0.015 OD for any of the designated 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<sup>2</sup> 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 155 to see a sample Absorbance Plate Test Report for Synergy HTX.

## Troubleshooting

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



## 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 [116](#).

**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 [83](#).) This test is optional since the Synergy HTX 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.

① The Plate Type setting in each Gen5 protocol should match the actual plate in use.

### Synergy HTX Abs Test 1.prt

Parameter	Setting
Plate Type	96 WELL PLATE
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	Monochromators
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 HTX Abs Test 2.prt**

<b>Parameter</b>	<b>Setting</b>
Plate Type	96 WELL PLATE
Shake Step	Linear, 4 minutes, default frequency
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	Monochromators
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
<i>Data Reduction</i>	Define two Delta OD transformations (450–630 nm), one per Read data set

**Synergy HTX Abs Test 3.prt**

<b>Parameter</b>	<b>Setting</b>
Plate Type	96 WELL PLATE
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	Monochromators
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 [115](#).

Absorbance specifications used with the liquid tests:

Accuracy:

$\pm 1.0\% \pm 0.010$  OD from 0.000 to 2.000 OD  
 $\pm 3.0\% \pm 0.010$  OD from 2.000 OD to 3.000 OD

Repeatability:

$\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD  
 $\pm 3.0\% \pm 0.005$  OD from 2.000 OD to 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  $\times 0.010 + 0.005$ ). For each well, its Standard Deviation should be less than its Allowed Deviation.  
Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a Mean of 0.8004 and a Standard Deviation of 0.0018. The Mean multiplied by 1.0% ( $0.8004 \times 0.010$ ) equals 0.008, and when added to 0.005 equals 0.013; this is the Allowed Deviation for well A1. Since the Standard Deviation for well A1 is less than 0.013, the well meets the test criteria.
3. 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  $\pm 1.0\% \pm 0.010$  OD, then the expected range for the Mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD.  $1.902 \times 0.010 + 0.010 = 0.029$ ;  $1.902 - 0.029 = 1.873$ ;  $1.902 + 0.029 = 1.931$ .

### Absorbance Liquid Test 2

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:
  - o Calculate the mean absorbance for each well, and average the means for each concentration.
  - o 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  $\pm 1.0\% \pm 0.005$  OD. (If above 2.000 OD, apply the  $\pm 3.0\% \pm 0.005$  specification.)
- The Standard Deviation for each set of readings should be less than the Allowed Deviation.

Example: Readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a Mean of 1.951, and a Standard Deviation of 0.0026. The Mean (1.951) multiplied by 1.0% ( $1.951 \times 0.010$ ) = 0.0195, which, when added to the 0.005 ( $0.0195 + 0.005$ ) = 0.0245 OD, which is the Allowed Deviation. Since the Standard Deviation is less than this value, the reader meets the test criteria.

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  $\pm 1.0\% \pm 0.010$  OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. ( $1.902 \times 1.0\% = 0.019 + 0.010 = 0.029$ , which is added to and subtracted from 1.902 for the range.)

### 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  $\times 0.010 + 0.005$ ). For each well, its Standard Deviation should be less than its Allowed Deviation.

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

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

3. Calculate 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](http://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 HTX LumTest\_Harta.prt

Parameter	Setting
Plate Type	"8030015 Harta - with 8032028 adapter"
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
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	150
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	100 msec
Dynamic Range	Standard
Read Height	1.00 mm

<b>Parameter</b>	<b>Setting</b>
READ STEP 2	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Background
Read wells	F1-G12
Filter Set	1
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	150
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	100 msec
Dynamic Range	Standard
Read Height	1.00 mm
READ STEP 3	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Battery check
Read wells	A7-A8
Filter Set	1
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	50
Integration Time	0:01.00 MM:SS.ss
Delay After Plate Movement	100 msec
Dynamic Range	Extended
Read Height	1.00 mm

## Results Analysis

The Luminescence Test procedure is described on page [120](#).

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**:  $(A2 - \text{Mean of the buffer cells}) / (3 * \text{Standard deviation of buffer cells})$
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  $\leq 60$  amol to pass.
- If the reader is equipped with the red-shifted PMT, the detection limit must be  $\leq 500$  amol to pass.

If you do not know which PMT is installed (#49984=low-noise PMT; #49721=red-shifted PMT), please contact BioTek TAC.

## 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 provides two options for testing the fluorescence system. One uses a solid state Fluorescence Test Plate (package BTI# 14000006\*; contact BioTek Customer Care regarding availability). The other uses liquid plates, the materials for which are available for purchase from BioTek (see [Materials for Conducting Liquid Tests](#) on page 5).

\*Fluorescence Test Plate BTI# 7092092 cannot be used for these tests.

### BioTek Fluorescence Test Plate

The Fluorescence Test Plate simplifies the process for conducting fluorescence qualification tests on the Synergy HTX. The test plate is solid and therefore immune to the pipetting errors, evaporation issues, and costs experienced with conventional Liquid Tests.

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

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

### Results Analysis

Refer to the *Fluorescence Test Plate User Guide* for descriptions of the data reduction calculations for each test. The tests must meet the following criteria to pass:

<b>Corners Test</b>	%CV < 3.0
<b>Sensitivity Tests</b>	
<i>Sodium Fluorescein analogue</i>	
Top optics	Detection Limit <= 53 pM
Bottom optics	Detection Limit <= 30 pM
<i>Methylumbellifерone analogue</i>	
Top optics	Detection Limit <= 160 pg/mL
Bottom optics	Detection Limit <= 160 pg/mL
<b>Linearity Test</b>	R <sup>2</sup> >= 0.9500

## 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 top and bottom fluorescence probes.
- Fluorescence Intensity (Sensitivity):** The Sensitivity Test measures a fluorescent compound (Sodium Fluorescein or Methylumbellif erone) and buffer solution to test the fluorescence reading capability of the instrument (top and bottom optics). 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.
- Linearity:** The Linearity Test verifies that the system is linear, that is, signal changes proportionally with changes in concentration.

### Gen5 Protocol Parameters

The information in this section represents the recommended reading parameters for the Gen5 protocols used with liquid testing. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

① The Plate Type setting in each Gen5 protocol should match the actual plate in use.

#### Synergy HTX FI\_T\_SF.prt and Synergy HTX FI\_B\_SF.prt

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915) (FI_T) "Greiner SensoPlate" (#655892) (FI_B)
Read Step 1	
Kinetic loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read"
Read well	D7
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Scale to High Wells, D7, 50000

<b>Parameter</b>	<b>Setting</b>
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)
Read Step 2	
Kinetic loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read Buffer"
Read wells	C9-E9
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Use first filter set gain from FIRST read step
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)
Read Step 3	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Corners Read"
Read wells	A1-A3, A10-A12, H1-H3, H10-H12
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm

<b>Parameter</b>	<b>Setting</b>
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Scale to high wells, A3, 50000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)
Read Step 4	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Linearity Read"
Read wells	C1-F5
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Scale to high wells, C1, 50000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)

### Synergy HTX FI\_T\_MUB.prt

<b>Parameter</b>	<b>Setting</b>
Plate Type	"Costar 96 black opaque" (#3915)
Read Step 1	
Kinetic loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters

<b>Parameter</b>	<b>Setting</b>
Step Label	"Sensitivity Read"
Read well	D7
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to High Wells, D7, 80000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm
Read Step 2	
Kinetic loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read Buffer"
Read wells	C9–E9
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Use first filter set gain from FIRST step
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm
Read Step 3	
Detection Method	Fluorescence intensity
Read Type	Endpoint

Parameter	Setting
Optics Type	Filters
Step Label	"Linearity Read"
Read wells	C1–F5
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to high wells, C1, 80000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm

## Results Analysis

The Fluorescence Liquid Test procedures begin on page [122](#).

### Corners Test

1. Calculate the Mean of the 12 "corner" wells (A1–A3, A10–A12, H1–H3, and H10–H12).
2. Calculate the Standard Deviation of the same 12 wells.
3. Calculate the %CV: (Standard Deviation/Mean)\*100

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

### Sensitivity Test

1. Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (C9, D9, E9).
2. Among the three buffer wells, find the Median Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the SF (or MUB) Concentration well (D7).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean SF (or MUB) Concentration, Buffer Median STD with its corresponding Buffer Mean:  

$$(<\text{SF or MUB}>\text{Mean}-\text{BufferMean})/(3*\text{BufferSTD})$$
5. Calculate the Detection Limit:

**Sodium Fluorescein:** Using the known concentration value of SF and the calculated SNR: 1000/SNR

- *Bottom 5 mm probe:* The Detection Limit must be  $\leq 30 \text{ pM}$  ( $10 \text{ pg/mL}$ ) to pass
- *Top 3 mm probe:* The Detection Limit must be  $\leq 53 \text{ pM}$  ( $20 \text{ pg/mL}$ ) to pass

**Methylumbellif erone:** Using the known concentration value of MUB and the calculated SNR: 17.6/SNR

- The Detection Limit must be  $\leq 0.16 \text{ ng/mL}$  ( $0.91 \text{ nM}$ ) 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:

<i>Using Sodium Fluorescein</i>	
<b>x</b>	<b>y</b>
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
<i>Using Methylumbellif erone</i>	
<b>x</b>	<b>y</b>
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  **$\geq 0.9500$**  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.
- 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.

- ① The Plate Type setting in each Gen5 protocol should match the actual plate in use.

**Synergy HTX Disp 1 Test.prt** and **Synergy HTX 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

<b>Parameter</b>	<b>Setting</b>
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
<i>Data Reduction</i>	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 HTX Disp 1 Test No Read.prt and Synergy HTX 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	<i>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.</i>

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

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 [132](#).

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  $\mu$ L, 20  $\mu$ L, 5  $\mu$ L), 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:  $(\text{Standard Deviation} / \text{Mean}) \times 100$
4. Calculate the Accuracy % Error:

$$((\text{ActualWeight}-\text{ExpectedWeight})/\text{ExpectedWeight}) * 100$$

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

Dispense Volume	To pass, %CV must be:	To pass, Accuracy % Error must be:
80 $\mu$ L	$\leq 2.0\%$	$\leq 2.0\%$
20 $\mu$ L	$\leq 7.0\%$	$\leq 5.0\%$
5 $\mu$ L	$\leq 10.0\%$	$\leq 20.0\%$

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 [53](#). If tests continue to fail, contact BioTek TAC.



## *Chapter 7*

# Instrument Qualification Procedures

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

**Instrument Qualification Process** starting on page 81 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 .....	108
IQ/OQ/PQ Description .....	109
Recommended Qualification Schedule .....	110
System Test .....	111
Plate Shaker Test .....	112
Absorbance Plate Tests .....	113
Absorbance Liquid Tests .....	115
Luminescence Test .....	120
Fluorescence Plate Tests .....	121
Fluorescence Liquid Tests .....	122
Injection System Tests .....	132

## Overview

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

Every Synergy HTX 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 110 to determine which qualification tests shall be conducted for your Synergy HTX model and to meet your site's regulatory requirements.

A Product Qualification Package (BTI #1340508) for the Synergy HTX 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 HTX 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.

	<b>IQ</b>	<b>OQ</b>	<b>PQ</b>	
<b>Tasks/Tests</b>	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 <u>or</u> 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		✓	✓	
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 82 describes this test and explains where to find information on error codes and troubleshooting tips, as well as sample test reports for Synergy HTX.

### 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.
- If applicable, adjust the Gen5 Absorbance table for Synergy HTX to wavelength values that will confirm operation of the reader at its limits. For example, set 200 and 999 nm (lower and upper limits of the monochromator) and then any four wavelengths in between that best represent your assays. To access this feature, select **System > Instrument Configuration > Synergy HTX > View/Modify > Setup > Absorbance**.

### 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; it will show either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL \*\*\* ERROR (error code) DETECTED." If the test failed, go to page 143 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 Mode to Linear and the Duration to 30 seconds. You can select any frequency. 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 83 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 HTX.

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 [84](#).
  - Test descriptions are provided on page [83](#).

## Absorbance Liquid Tests

*Absorbance Liquid Tests* starting on page 85 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

	<p>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:</p> <ul style="list-style-type: none"><li>▪ Perform the tests exactly as described here.</li><li>▪ Rerun the tests using your particular plates, solutions, and so on.</li><li>▪ 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.</li></ul>
-----------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

### 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 HTX Abs Test 1.prt** described on page 86

### 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  $\mu$ L in a flat-bottom microwell.

## Test Procedure

① 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  $\mu$ L/well of the stock solution into column 1.
3. Pipette 200  $\mu$ L/well of the diluted solution into column 2.
4. Create a Gen5 experiment based on the **Synergy HTX 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 [88](#) to perform calculations and determine pass/fail.
  - Troubleshooting tips are provided on page [90](#).
  - Test descriptions are provided on page [85](#).

## 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 [113](#)). 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 HTX Abs Test 2.prt** described on page [87](#)

## 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 HTX 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 [88](#) to perform calculations and determine pass/fail.
  - Troubleshooting tips are provided on page [90](#).
  - Test descriptions are provided on page [83](#).

## 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 HTX 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 HTX Abs Test 3.prt** described on page [87](#)

## 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 HTX Abs Test 3** protocol and read the plate.
  - Save the experiment. Refer to the instructions on page [89](#) to perform calculations and determine pass/fail.
  - Troubleshooting tips are provided on page [90](#).
  - Test descriptions are provided on page [85](#).

## Luminescence Test

*Luminescence Testing* starting on page 91 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 91:
  - **Synergy HTX 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 HTX 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 93.
6. When finished, turn off the Harta reference plate to preserve battery life.

## Fluorescence Plate Tests

[BioTek Fluorescence Test Plate](#) on page 94 introduces the test plate and references the User Guide for the test methods. Use of the test plate is offered as an alternative to conducting the fluorescence liquid tests described in the next section.

### Requirements

Refer to the **Getting Started** section of the *Fluorescence Test Plate User Guide* for information on the required materials and prerequisite tasks.

### Test Procedure

The **Qualification Tests** section of the *Fluorescence Test Plate User Guide* contains a procedure for cleaning the plate and then creating and running experiments based on supplied Gen5 protocols.

As described in the User Guide, when each experiment is finished, Gen5 exports the measurement data to a prepared Microsoft Excel .xls file. The worksheet(s) within that file calculate results and determine pass or fail.

① For use with the Synergy HTX, identify the reader-specific Gen5 protocols on the USB flash drive that came with the test plate. Use only those protocols that apply to your reader model and your organization's qualification requirements.

## Fluorescence Liquid Tests

*Fluorescence Liquid Tests* starting on page 95 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 perfectly clean and free from dust and bottom scratches. Use new microplates from sealed packages.

Manufacturer part numbers are subject to change.

Methylumbellifereone can be used as an alternative or supplemental method for performing these tests for the top probe. See instructions starting on page 128.

- 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
- 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 95:

Synergy HTX FI_B_SF.prt	Corners, Sensitivity, Linearity tests, Bottom optics, Sodium Fluorescein (SF)
Synergy HTX FI_T_SF.prt	Corners, Sensitivity, Linearity tests, Top optics, Sodium Fluorescein (SF)

## Test Solutions



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:

<b>Mix this SF solution:</b>	<b>with buffer:</b>	<b>to make:</b>	
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	<b>3.3 nM</b>	<i>Corners Test</i>
4.24 mL of 3.3 nM SF	9.76 mL	<b>1 nM</b>	<i>Sensitivity/Linearity Tests</i>

## 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 [124](#).

Refer to the pipette map below 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 HTX FI\_B\_SF.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 HTX FI\_T\_SF.prt** and read the plate.
4. Save the experiments. Refer to the instructions starting on page [99](#) to perform calculations and determine pass/fail.
  - Troubleshooting tips are provided on page [101](#).
  - Test descriptions are provided on page [95](#).

## Pipette Map

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:

*Refer to the illustration on the next page.*

Using a single-channel pipette:

- Pipette **200 µL** of the **3.3 nM SF** solution into the “corner” wells.
- Pipette 200 µL of the buffer in the wells surrounding the SF. (Omit if using a solid black plate or Greiner SensoPlate.)
- Pipette 200 µL of the **1 nM SF** solution into well D7.
- Pipette 200 µL of the buffer solution into wells C9, D9, and E9.

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

- Pipette **150 µL** of the buffer into wells C2-F5. 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	BUF
	C	<b>150 µL:</b> 1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM			BUF				
Sensitivity /Linearity	D	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM	<b>200 µL:</b> 1 nM		BUF				
	E	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM			BUF				
	F	1 nM	0.5 nM	0.25 nM	0.125 nM	0.0625 nM							
	G	BUF	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	

## 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 <i>Materials for Conducting Liquid Tests</i> 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
- 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 HTX FI\_T\_MUB.prt**, described in detail under *Gen5 Protocol Parameters* starting on page 95

## Test Solutions

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

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

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

## Test Procedure

1. If you have not already done so, prepare the test solutions; see page [129](#).

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 HTX FI\_T\_MUB.prt** and read the plate.
3. Save the experiment. Refer to the instructions starting on page [99](#) to perform calculations and determine pass/fail.
  - Troubleshooting tips are provided on page [101](#).
  - Test descriptions are provided on page [95](#).

## Pipette Map

Using a single-channel pipette:

- Pipette **200 µL** of the **17.6 ng/mL (100 nM) MUB** solution into well D7.
- Pipette 200 µL of the buffer solution into wells C9, D9, and E9.

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

- Pipette **150 µL** of the buffer into wells C2-F5. 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			
D	100 nM	50 nM	25 nM	12.5 nM	6.25 nM		MUB 100 nM		BUF			
E	100 nM	50 nM	25 nM	12.5 nM	6.25 nM				BUF			
F	100 nM	50 nM	25 nM	12.5 nM	6.25 nM							
G												
H												

## Injection System Tests

*Injection System Testing* starting on page 102 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  $\pm 1.0\% \pm 0.010$  OD or better and a repeatability specification of  $\pm 1.0\% \pm 0.005$  OD or better.

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

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200  $\mu$ L hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250-mL beaker
- New 96-well, clear, flat-bottom microplates
- 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 102:

For models with Absorbance capabilities:

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

For models without Absorbance capabilities:

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

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

Synergy HTX **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 <sub>3</sub> Na	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 HTX 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 HTX 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 [105](#) to perform calculations and determine pass/fail.
  - Test descriptions are provided on page [102](#).

## 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 HTX 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 determine pass/fail according to the instructions on page [105](#).

---

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 HTX 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 [105](#) to perform calculations and determine pass/fail.
  - Test descriptions are provided on page [102](#).

## 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				

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

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

80 µL weight: \_\_\_\_\_ g

Expected weight: 2.5600 g

Accuracy % Error: \_\_\_\_\_ %

Must be < = 2.0%  P  F

Standard Deviation: \_\_\_\_\_  
Mean: \_\_\_\_\_

%CV: \_\_\_\_\_ %

Must be < = 7.0%  P  F

Reader Model: \_\_\_\_\_  
Reader S/N: \_\_\_\_\_

Reading Date: \_\_\_\_\_  
Comments: \_\_\_\_\_

20 µL weight: \_\_\_\_\_ g

Expected weight: 0.6400 g

Accuracy % Error: \_\_\_\_\_ %

Must be < = 5.0%  P  F

Standard Deviation: \_\_\_\_\_  
Mean: \_\_\_\_\_

%CV: \_\_\_\_\_ %

Must be < = 10.0%  P  F

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

5 µL weight: \_\_\_\_\_ g  
Expected weight: 0.1600 g

Accuracy % Error: \_\_\_\_\_ %

Must be < = 20.0%  P  F

Standard Deviation: \_\_\_\_\_  
Mean: \_\_\_\_\_

%CV: \_\_\_\_\_ %

Must be < = 10.0%  P  F

## 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				

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

5 µL Dispense Delta ODs @630/750 nm				
9	10	11	12	

80 µL weight: \_\_\_\_\_ g

Expected weight: 2.5600 g

**Accuracy % Error:** \_\_\_\_\_ %

Must be < = 2.0%  P  F

Standard Deviation: \_\_\_\_\_

Mean: \_\_\_\_\_

**%CV:** \_\_\_\_\_ %

Must be < = 7.0%  P  F

Reader Model: \_\_\_\_\_

Reader S/N: \_\_\_\_\_

Reading Date: \_\_\_\_\_

Comments: \_\_\_\_\_

20 µL weight: \_\_\_\_\_ g

Expected weight: 0.6400 g

**Accuracy % Error:** \_\_\_\_\_ %

Must be < = 5.0%  P  F

Standard Deviation: \_\_\_\_\_

Mean: \_\_\_\_\_

**%CV:** \_\_\_\_\_ %

Must be < = 10.0%  P  F

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

Signature: \_\_\_\_\_

5 µL weight: \_\_\_\_\_ g

Expected weight: 0.1600 g

**Accuracy % Error:** \_\_\_\_\_ %

Must be < = 20.0%  P  F

Standard Deviation: \_\_\_\_\_

Mean: \_\_\_\_\_

**%CV:** \_\_\_\_\_ %

Must be < = 10.0%  P  F

## *Appendix A*

# Specifications

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

General Specifications .....	138
Absorbance Specifications .....	139
Fluorescence Specifications .....	141
Luminescence Specifications .....	142
Dispense/Read Specifications .....	142

## General Specifications

### Microplates

The Synergy HTX accommodates standard 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry up to 1.125" (28.575 mm) high, and the BioTek Take3 and Take3 Trio Micro-Volume Plates.

### Hardware and Environmental

Light Source	Absorbance: Xenon flash light source, 10W maximum average power  Fluorescence: Tungsten halogen, 20W power
Dimensions	Approximately 16" D x 16" W x 10" H (40.6 cm x 40.6 cm x 25.4 cm)  Note: For dimensions that include installation with a BioStack, refer to the <i>BioStack Operator's Manual</i>
Weight	Approximately 38 lbs. (17 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	100 VA max, 130 VA max with injectors
Incubation	Temperature control range from 4° over ambient to 50°C.  Temperature variation ±0.5°C @37°C, tested with Innovative Instruments, Inc. temperature test plate.  Top and bottom incubation controlled via software-adjustable gradient.

## Absorbance Specifications

### Optics

Wavelength Range	200 to 999 nm
Wavelength Accuracy	$\pm 2$ nm
Wavelength Precision	$\pm 0.2$ nm (standard deviation)
Wavelength Bandpass	2.4 nm
Resolution	0.0001 OD
Increment	1 nm
Measurement Range	0.000 to 4.000 OD

### 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

*Tested with certified neutral density glass*

96-well plate, normal read speed:

0.000 to 2.000 OD  $\pm 1.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms  
 2.000 to 3.000 OD  $\pm 3.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms

384-well plate, normal read speed:

0.000 to 2.000 OD  $\pm 2.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms  
 2.000 to 2.500 OD  $\pm 3.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms

96-well and 384-well plate, sweep read speed:

0.000 to 1.000 OD  $\pm 1.0\%$   $\pm 0.010$  OD

### Linearity

*By liquid dilution*

96-well plate, normal read speed:

0.000 to 2.000 OD  $\pm 1.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms  
 2.000 to 3.000 OD  $\pm 3.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms

384-well plate, normal read speed:

0.000 to 2.000 OD  $\pm 2.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms  
 2.000 to 2.500 OD  $\pm 3.0\%$   $\pm 0.010$  OD, Delay after plate movement: 100 ms

96-well and 384-well plate, sweep read speed:

0.000 to 1.000 OD  $\pm 1.0\% \pm 0.010$  OD

### Repeatability

*Tested with certified neutral density glass measured by one standard deviation (8 measurements per data point)*

96-well and 384-well plate, normal read speed:

0.000 to 2.000 OD  $\pm 1.0\% \pm 0.005$  OD, Delay after plate movement: 100 ms  
2.000 to 3.000 OD  $\pm 3.0\% \pm 0.005$  OD, Delay after plate movement: 100 ms

96-well and 384-well plate, sweep read speed:

0.000 to 1.000 OD  $\pm 2.0\% \pm 0.010$  OD

### Read Timing

Minimum kinetic interval (450 nm): Sweep mode, < 20 seconds, 96-well plate

Time elapse from plate in to plate out (450 nm): Sweep mode, <35 seconds, 96-well plate

## Fluorescence Specifications

### Optics

Optic Probes	<i>Configuration is model-dependent</i> Top, 3 mm probe Bottom, 5 mm probe
Detection	PMT, low-noise standard; red-shifted (850 nm) option available

### Sensitivity

*The following specifications apply to the Normal mode of reading.*

5 mm optical probe, bottom reading

DL Sodium Fluorescein in PBS, Excitation 485/20, Emission 528/20  
 <= 30 pM

DL Propidium Iodide in PBS, Excitation 485/20, Emission 645/40  
 <= 62.5 ng/mL

3 mm optical probe, top reading

DL Sodium Fluorescein in PBS, Excitation 485/20, Emission 528/20  
 <= 53 pM

DL Methylumbelliflone in CBB, Excitation 360/40, Emission 460/40  
 <= 0.16 ng/mL

### 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 measurements per data point; 100 ms delay after plate movement.*

96-well read, minimum kinetic interval: <55 seconds

### Time-Resolved Fluorescence

*For "T" models*

Delay: 0, or 20 to 2,000  $\mu$ s

Integration Time: 20 to 2,000  $\mu$ s

Granularity: 10- $\mu$ s step

## Luminescence Specifications

$\leq$  60 amol/well DL ATP in a 96-well plate (low-noise PMT) , 20 amol typical

$\leq$  500 amol/well in a 96-well plate (red-shifted PMT)

10-second integration, PMT sensitivity 150, 16 blank wells

## Dispense/Read Specifications

*Applies only to models equipped with injectors*

Plate Type	Dispenses to standard 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry
Detection Method	Absorbance, Fluorescence (including TRF), Luminescence
Volume Range	5–1000 $\mu$ L with a 5–20 $\mu$ L tip prime
Accuracy	$\pm 1 \mu$ L or 2.0%, whichever is greater
Precision	Dispensing a 200 $\mu$ L solution of deionized water, 0.1% Tween 20, and dye at room temperature:  $\leq 2.0\%$ for volumes of 50–200 $\mu$ L $\leq 4.0\%$ for volumes of 25–49 $\mu$ L $\leq 7.0\%$ for volumes of 10–24 $\mu$ L $\leq 10.0\%$ for volumes of 5–9 $\mu$ L
Injection Speeds	225, 250, 275, and 300 $\mu$ L/sec

Maximum delay between the end-of-dispense and start-of-read processes (96-/384-well plates, default probe heights only)

Absorbance: T  $\leq$  3 sec

Top Filter Fluorescence: T  $\leq$  1 sec

Bottom Filter Fluorescence: T  $\leq$  1 sec

Luminescence: T  $\leq$  0.5 sec

## *Appendix B*

### Error Codes

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

Overview .....	144
Error Codes .....	145

## Overview

When a problem occurs during operation with the Synergy HTX, 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](mailto:tac@biotek.com)

For errors that are displayed during operation of the Synergy HTX 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
0200	<p><b>24VDC dropped below safe level</b></p> <ul style="list-style-type: none"> <li>External power supply has failed.</li> <li>Verify connection to AC mains.</li> <li>Power supply connection to instrument is loose or broken.</li> </ul> <p>Contact BioTek TAC.</p>
150x	<p><b>Temperature is out of range, or External 24-volt power supply is low</b></p> <ul style="list-style-type: none"> <li>x=1: Zone 1</li> <li>x=2: Zone 2</li> <li>x=3: Zones 1 and 2</li> <li>x=4: Zone 3</li> <li>x=5: Zones 1 and 3</li> <li>x=6: Zones 2 and 3</li> <li>x=7: Zones 1, 2, and 3</li> <li>x=8: Zone 4</li> <li>x=9: Zones 1 and 4</li> <li>x=A: Zones 2 and 4</li> <li>x=B: Zones 1, 2, and 4</li> <li>x=C: Zones 3 and 4</li> <li>x=D: Zones 1, 3, and 4</li> <li>x=E: Zones 2, 3, and 4</li> <li>x=F: Zones 1, 2, 3, and 4</li> </ul> <p>Contact BioTek TAC.</p>

Code	Description and possible remedy
152x	<p><b>One or more incubator zones are defective</b></p> <ul style="list-style-type: none"> <li>• x=1: Zone 1</li> <li>• x=2: Zone 2</li> <li>• x=3: Zones 1 and 2</li> <li>• x=4: Zone 3</li> <li>• x=5: Zones 1 and 3</li> <li>• x=6: Zones 2 and 3</li> <li>• x=7: Zones 1, 2, and 3</li> <li>• x=8: Zone 4</li> <li>• x=9: Zones 1 and 4</li> <li>• x=A: Zones 2 and 4</li> <li>• x=B: Zones 1, 2, and 4</li> <li>• x=C: Zones 3 and 4</li> <li>• x=D: Zones 1, 3, and 4</li> <li>• x=E: Zones 2, 3, and 4</li> <li>• x=F: Zones 1, 2, 3, and 4</li> </ul> <p>Turn the incubator on and wait at least 10 minutes for it to stabilize. Contact BioTek TAC.</p>
2101	<p><b>Plate dimensions incorrect. Row count &lt; 1 or &gt; 99.</b></p> <p>Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.</p>
2102	<p><b>Plate dimensions incorrect. Column count &lt; 1 or &gt; 99.</b></p> <p>Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.</p>
2109	<p><b>Plate dimensions incorrect. Plate height &gt; 28.575 mm.</b></p> <p>Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.</p>
230x	<p><b>Plug not found in filter wheel</b></p> <ul style="list-style-type: none"> <li>• x=2: excitation filter wheel</li> <li>• x=3: emission filter wheel</li> </ul> <p>Protocol contains a plug in a filter wheel, but it was not found. Verify that the plug is physically installed and that this is accurately reflected in the filter table.</p>

<b>Code</b>	<b>Description and possible remedy</b>
2313	<p><b>Empty hole not found in emission filter wheel</b></p> <p>The protocol requires a "hole" in the emission filter wheel, but no empty locations were detected. Verify that the emission filter wheel contains an empty location and that this is accurately reflected (as a hole) in the filter table.</p>
2326	<p><b>TRF cartridge not installed</b></p> <p>The protocol is defined for time-resolved fluorescence (TRF) but the TRF cartridge is not installed in the excitation filter wheel slot. Verify that the TRF cartridge is installed in the reader and that this is accurately reflected in the filter table.</p>
2327	<p><b>Excitation filter wheel not installed</b></p> <p>Verify that the excitation filter wheel is installed in the reader.</p>
240x	<p><b>Read area of the plate will not fit in the inside open area of the carrier</b></p> <ul style="list-style-type: none"> <li>• x=3: First row position + Y offset &lt; 2.54 mm or &gt; 83.57 mm</li> <li>• x=4: Low row position + Y offset &lt; 2.54 mm or &gt; 83.57 mm</li> <li>• x=5: First column position + X offset &lt; 4.57 mm or &gt; 120.65 mm</li> <li>• x=6: Last column position + X offset &lt; 4.57 mm or &gt; 120.65 mm</li> <li>• x=7: Plate width &lt; 84.15 mm or &gt; 86.11 mm</li> <li>• x=8: Plate length &lt; 125.73 mm or &gt; 128.40 mm</li> </ul> <p>Review definition for plate defined in Plate Type database. Ensure counts or dimensions do not exceed limits. See the Gen5 Help for a description of measuring plates.</p>
2B0x	<p><b>Syringe failure</b></p> <ul style="list-style-type: none"> <li>• x=1: Syringe failed to reach home sensor (optical sensor should be on)</li> <li>• x=2: Syringe moved off home sensor, but sensor didn't change state (optical sensor should be off)</li> <li>• x=3: Syringe clean position too far from home sensor</li> <li>• x=4: Steps to clear sensor at runtime deviated from value saved when homing (verify error).</li> </ul> <p>Protocol definition is incorrect or reader is being controlled by incorrectly programmed third-party software.</p> <p>Syringe was not installed correctly or was not cleaned, preventing a move to home sensor.</p> <p>Contact BioTek TAC.</p>
2B0A	<p><b>Plate not in carrier for system prime operation</b></p> <p>Place priming plate in carrier.</p>

<b>Code</b>	<b>Description and possible remedy</b>
2C01	<b>Dispenser configuration incorrect</b>
2C05	<b>Volume calibration data is invalid</b>
2C07	<b>Volume calibration is needed to override defaults</b>
	Verify that the dispenser calibration values have been loaded into Gen5. See the <b>Installation</b> chapter.
	Contact BioTek TAC.
2D09	<b>Tip prime volumes specified could overflow the tip priming trough</b>
	Empty the tip priming trough.
	Contact BioTek TAC.
2D0A	<b>Tip prime trough or plate is full or may overflow</b>
	Empty the tip priming plate.
2D15	<b>Invalid kinetic interval selected for plate mode/plate synchronous mode</b>
	Enter a valid kinetic interval.
	Contact BioTek TAC.
2D16	<b>Assay missed scheduled start of read (well synchronous mode)</b>
	Verify computer setup. Hibernate or sleep mode should not be enabled.
	Contact BioTek TAC.
2D22	<b>Invalid volume selected for tip prime</b>
	Select a valid volume
	Contact BioTek TAC.
2D23	<b>Invalid volume selected for dispense</b>
	Select a valid volume.
	Contact BioTek TAC.
2D24	<b>Invalid rate selected for dispense</b>
	Select a valid rate.
	Contact BioTek TAC.
2D28	<b>Dispenser module not attached</b>
	Verify that the [supplied] cable between the external dispense module and the reader is connected.
	Contact BioTek TAC.
2D2A	<b>Dispense not primed successfully</b>
	Reinitialize the dispenser, then repeat the prime operation.
	Contact BioTek TAC.

<b>Code</b>	<b>Description and possible remedy</b>
2D46	<b>Invalid wavelength specified</b> Each wavelength value must be between 230 and 999 nm.
3700	<b>Absorbance reference channel failed noise test</b>
3710	<b>Absorbance measurement channel failed noise test</b>
3800	<b>Absorbance reference channel failed offset test</b>
3810	<b>Absorbance measurement channel failed offset test</b>  Humidity is outside the environmental specification of instrument.  Note the specification in <b>Appendix A</b> and move to an area with lower humidity.  Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
390Y	<b>Absorbance reference channel dark range outside of limits (measurement &lt; 100 counts), where Y= readset</b>
391Y	<b>Absorbance measurement channel dark range outside of limits, where Y = readset</b>  Humidity is outside the environmental specification of instrument. Note the specification in <b>Appendix A</b> and move to an area with lower humidity.  Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
3900	<b>Absorbance reference channel dark range outside of limits</b>
3910	<b>Absorbance measurement channel dark range outside of limits</b>  Humidity is outside the environmental specification of instrument. Note the specification in <b>Appendix A</b> and move to an area with lower humidity.  Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
3E0y	<b>Absorbance reference channel saturated during one of the following steps:</b> <ul style="list-style-type: none"> <li>• Absorbance Blank Data Collection, y=readset #</li> <li>• Absorbance Gain Calibration</li> <li>• Absorbance Blank Data Collection</li> <li>• Absorbance Spectral Scan</li> </ul> Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spilled fluid in chamber or dirty absorbance optics.

<b>Code</b>	<b>Description and possible remedy</b>
3E1y	<p><b>Absorbance measurement channel saturated during one of the following steps:</b></p> <ul style="list-style-type: none"> <li>• Absorbance Blank Data Collection, y=readset #</li> <li>• Absorbance Gain Calibration</li> <li>• Absorbance Blank Data Collection</li> <li>• Absorbance Spectral Scan</li> </ul> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spilled fluid in chamber or dirty absorbance optics.</p>
3F0y	<p><b>Absorbance reference signal out of range:</b></p> <ul style="list-style-type: none"> <li>• Absorbance Read Process, y = readset#</li> <li>• Absorbance Optics Test</li> </ul> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spilled fluid in chamber or dirty absorbance optics.</p>
3F1y	<p><b>Absorbance measurement out of range:</b></p> <ul style="list-style-type: none"> <li>• Absorbance Read Process, y = readset#</li> <li>• Absorbance Optics Test</li> </ul> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spilled fluid in chamber or dirty absorbance optics.</p>
3F00	<p><b>Absorbance reference correction value out of range:</b></p> <ul style="list-style-type: none"> <li>• Absorbance Spectral Scan</li> </ul> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.</p> <p>Check for spilled fluid in chamber or dirty absorbance optics.</p>
4xxx	<p><b>PMT well overload</b></p> <p>Gain/Sensitivity too high. Chemistry too concentrated.</p> <p>Verify that the physical filter configuration matches the Gen5 Filter Table.</p>
4810	<p><b>PMT measurement offset test failure (offset is &lt; 700 or &gt; 2450 counts)</b></p> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.</p>
4A0X	<p><b>PMT gain out of range</b></p> <p>x = readset</p> <p>Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.</p>

<b>Code</b>	<b>Description and possible remedy</b>
4B10	<b>PMT measurement value is too low</b>
4B11	<b>Failed high-voltage PMT test</b>
4B12	<b>Failed low-voltage PMT test</b>
4B15	<b>Failed well overload test for absorbance or fluorescence</b>
4B18	<b>Failed background overload test</b>
	Ensure that the Gen5 Filter Table accurately reflects the physical configuration of the excitation and emissions filter wheels.
	Ensure that the door is fully closed and there is no light leakage.
4E0X	<b>Flash-on reference value at full scale during the flash fluorescence read (x=readset #)</b>
4E11	<b>PMT test failed at 750 volts</b>
4E12	<b>PMT test failed at 500 volts</b>
	Too much light in chamber. Door not closed completely. Ensure that instrument case is completely installed and secured.
	Ensure that the Gen5 Filter Table accurately reflects the physical configuration of the excitation or emission filter wheels.
	Gain set too high; try adjusting setting in protocol.
4E18	<b>PMT saturation detected</b>
	Too much light in chamber. Door not closed completely. Ensure the instrument case is completely installed and secured.
	Ensure the Gen5 Filter Table accurately reflects the physical configuration of the excitation and emission filter wheels.
	Sensitivity set too high. Try adjusting sensitivity (gain) setting.
	Fluorescence standards dispensed to plate exceed value established by initial standard values recorded.
	Contamination within read chamber. Clean read chamber.
	Verify there is no filter wavelength overlap between excitation and emission positions 2 and 3.

<b>Code</b>	<b>Description and possible remedy</b>
4F0X	<p><b>Fluorescence signal out of range (too low)</b></p> <p>Too much light in chamber. Door not closed completely. Ensure the instrument case is completely installed and secured.</p> <p>Ensure the Gen5 Filter Table accurately reflects the physical configuration of the excitation and emission filter wheels.</p> <p>Sensitivity set too high. Try adjusting sensitivity (gain) setting.</p> <p>Fluorescence standards dispensed to plate exceed value established by initial standard values recorded.</p> <p>Contamination within read chamber. Clean read chamber.</p> <p>Verify there is no filter wavelength overlaps between excitation and emission positions 2 and 3.</p>
5000 5200	<p><b>Carrier x-axis failed to home</b></p> <p>Shipping screw may be installed. See the <b>Installation</b> chapter for removal instructions.</p> <p>An object may be obstructing the path.</p>
5001 5201	<p><b>Carrier y-axis failed to home</b></p> <p>Y-axis rails are dusty or rusty. Dirt in the roller bearings is causing them to jam.</p> <p>An object may be obstructing the path.</p>
5002 5202	<p><b>Excitation filter wheel axis failed to home</b></p> <p>Filter wheel not inserted correctly.</p> <p>Filter wheel obstructed.</p> <p>Filter not clipped in.</p> <p>Gear teeth of filter wheel binding with gear teeth of the motor. Remove filter wheel, spin wheel by hand, and reinsert.</p>
5003 5203	<p><b>Emission filter wheel axis failed to home</b></p> <p>Filter wheel not inserted correctly.</p> <p>Filter wheel obstructed.</p> <p>Filter not clipped in.</p> <p>Gear teeth of filter wheel binding with gear teeth of the motor. Remove filter wheel, spin wheel by hand, and reinsert.</p>
5006 5206	<p><b>Probe z-axis failed to home</b></p> <p>Shipping bracket not removed. See the <b>Installation</b> chapter for removal instructions.</p>

<b>Code</b>	<b>Description and possible remedy</b>
5402	<b>Excitation filter wheel failed positional verify</b>
5403	<b>Emission filter wheel failed positional verify</b>
	Ensure filter cartridge is fully inserted.
5700	<b>Carrier x-axis obstructed</b>
5701	<b>Carrier y-axis obstructed</b>
	Axis may have hit probe z-axis.
	Tip priming trough is not correctly inserted.
5702	<b>Excitation filter wheel obstructed</b>
5703	<b>Emission filter wheel obstructed</b>
	Ensure filter cartridge is fully inserted.
5706	<b>Probe z-axis obstructed</b>
	Shipping bracket not removed.
	Verify plate height matches selected plate type.
	Manually turn the z-axis up, remove any microplates from the carrier, and attempt a successful system test.
5708	<b>Dispenser syringe 1 obstructed</b>
5709	<b>Dispenser syringe 2 obstructed</b>
	Verify nothing is blocking the syringe drive.
5800	<b>Carrier x-axis obstructed</b>
5801	<b>Carrier y-axis obstructed</b>
	Shipping screw still installed.
	An object may be obstructing the carrier's path.
5A00	<b>Carrier x-axis obstructed</b>
5A01	<b>Carrier y-axis obstructed</b>
	Plate has hit something.
	Plate cover not accounted for when creating plate dimension file.
	Tip prime trough dislodged.
5B00	<b>Plate height violation</b>
	Plate is inside chamber when it should be outside.
	<ul style="list-style-type: none"> <li>• The read was aborted and "home all axes" not performed.</li> </ul>
	<ul style="list-style-type: none"> <li>• The carrier is inside the reader and the newly defined plate height is different from the most recently specified plate height. To resolve this error, eject the carrier before running the experiment.</li> </ul>



## *Appendix C*

### **Sample Reports**

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

## Gen5 System Test Report

Reader: Synergy HTX (Serial Number: 1506132)  
Basecode: P/N 1340200 (v1.02)  
Gen5 Version: 2.06.10  
Date and Time: 6/17/2015 9:23:27 AM  
User: 9925  
Company:  
Comments:

### Test Results

SYSTEM TEST PASS

Operator ID: \_\_\_\_\_

Notes: \_\_\_\_\_  
\_\_\_\_\_

### SYSTEM SELF TEST

1340200 Version 1.02	1506132	SW1	S1	
		1100	1000	
		AF	T	
Voltage Reference Test	Min	Low	High	Max
24V Power	1953			
Xenon Flash	1430	1740	2166	2476
Motor Power	2050			
Tungsten Lamp	11			1719

### ABSORBANCE

Optics Test	Ref	Meas	Gain	Resets
#1:200			1.61	2
Light	13517	39328		
Dark	9875	9886		
Delta	3642	29442		
#2:352			1.72	4
Light	12718	39321		
Dark	9873	9882		
Delta	2845	29439		
#3:620			1.55	1
Light	12733	39591		
Dark	9878	9893		
Delta	2855	29698		
#4:790			2.59	2
Light	12905	39620		
Dark	9872	9889		
Delta	3033	29731		
#5:860			1.80	1
Light	12967	39661		
Dark	9878	9895		

	Delta	3089	29766		
#6:962				2.15	1
	Light	13065	39721		
	Dark	9877	9897		
	Delta	3188	29824		
Noise Test		Ref	Meas		
	Max	9835	9881		
	Min	9832	9879		
	Delta	3	2		

#### FLUORESCENCE/LUMINESCENCE

##### Filter PCB

Bias current offset	-0.7	counts	PASS
Offset voltage	1476	counts	PASS
750V measurement	34.1	counts	PASS
750V noise	6	counts	
750V offset	1482	counts	
500V measurement	2.2	counts	
500V noise	2	counts	
500V offset	1480	counts	
Reset offset	1516	counts	

##### Excitation Wheel

- #1:360/40
- #2:485/20
- #3:PLUG
- #4:PLUG

##### Emission Wheel

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

#### CALIBRATION

##### Carrier - Bottom Fluorescence Probe

Upper Left	x= 9696	y= 1804	
Lower Left	x= 9692	y= 7324	
Lower Right	x= 1004	y= 7328	
Upper Right	x= 1008	y= 1804	
Delta 1	9696 - 9692=	+4	
Delta 2	1008 - 1004=	+4	
Delta 3	1804 - 1804=	+0	
Delta 4	7328 - 7324=	+4	

##### Carrier - Top Fluorescence Probe

Upper Left	x= 9724	y= 232	
Lower Left	x= 9712	y= 5756	
Lower Right	x= 1024	y= 5756	
Upper Right	x= 1040	y= 232	
Delta 1	9724 - 9712=	+12	
Delta 2	1040 - 1024=	+16	
Delta 3	232 - 232=	+0	

Delta 4                5756 - 5756= +0

Carrier - Absorbance Probe

Upper Left	x=11256	y= 1788
Lower Left	x=11240	y= 7308
Lower Right	x= 2552	y= 7308
Upper Right	x= 2560	y= 1788
Delta 1	11256 - 11240=	+16
Delta 2	2560 - 2552=	+8
Delta 3	1788 - 1788=	+0
Delta 4	7308 - 7308=	+0

Carrier - Injectors

Upper Left	x= 9724	y= 1388
Lower Left	x= 9712	y= 6912
Lower Right	x= 1024	y= 6912
Upper Right	x= 1040	y= 1388
Delta 1	9724 - 9712=	+12
Delta 2	1040 - 1024=	+16
Delta 3	1388 - 1388=	+0
Delta 4	6912 - 6912=	+0

Carrier - Test Sensors

Middle Sensor	y=11940	
Tested	11944	
Delta	+4	
Back Sensor	x=11632	y= 7916
Tested	11632	7916
Delta	+0	+0

Probe Height                34.25 mm

Monochromator                B=+0.002274 C=-0.306332

INCUBATION

Temperature Setpoint: 0.0                Current Average: 23.9                A/D Test: PASS

Zone 1: 24.0	Min: 23.9	Max: 24.0	Range: PASS	Thermistor: PASS
Zone 2: 23.8	Min: 23.7	Max: 23.8	Range: PASS	Thermistor: PASS
Zone 3: 23.9	Min: 23.9	Max: 23.9	Range: PASS	Thermistor: PASS
Zone 4: 24.0	Min: 23.9	Max: 24.0	Range: PASS	Thermistor: PASS

0000

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

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

Date: \_\_\_\_\_

### Absorbance Test Plate Results

Reader: Synergy HTX (Serial Number: 1506132)  
Basecode: P/N 1340200 (v1.02)  
Date and Time: 6/16/2015 3:40:38 PM  
Absorbance Plate: 7 Filter Test Plate (P/N 7260522) - S/N 283882  
Last Plate Certification: July 2014  
Next Plate Certification Due: July 2015  
User: 7358  
Comments:

#### Peak Absorbance Results

Well	C6	C6	C6	C6
Reference	256	365	407	652
Tolerance	3	3	3	3
Read	256	365	407	652
Result	PASS	PASS	PASS	PASS

#### Alignment Results

Wells	A1	A12	H1	H12
Read	0.001	0.001	0.001	0.001
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	0.141	0.553	1.077	1.614	1.698	2.243
Min Limit	0.118	0.522	1.035	1.562	1.644	2.133
Max Limit	0.164	0.584	1.119	1.666	1.752	2.353
Read 1	0.154	0.558	1.085	1.622	1.703	2.250
Result	PASS	PASS	PASS	PASS	PASS	PASS

#### Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.154	0.558	1.085	1.622	1.703	2.250
Min Limit	0.147	0.548	1.069	1.601	1.681	2.177
Max Limit	0.160	0.569	1.101	1.643	1.725	2.322
Read 2	0.153	0.558	1.084	1.621	1.702	2.245
Result	PASS	PASS	PASS	PASS	PASS	PASS

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

Date: \_\_\_\_\_