Synergy™ HTX

Operator's Manual

BioTek®

Contents

Chapter 1

Introduction

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

Synergy HTX Multi-Mode Microplate Reader

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 (see **Chapter 3, Getting Started**).

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.

Both Synergy HTX models support 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. Absorbance mode reads plates up to 0.8" (20.3 mm) in height; fluorescence mode reads plates up to 1.25" (31.75 mm). Polymerase Chain Reaction (PCR) tubes up to 1.25" (31.75 mm) are also readable with the use of existing adapter 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

❖ Part numbers and package contents are subject to change. Contact BioTek Customer Care with any questions.

Item	Part #
Synergy HTX Operator's Manual	1341000
Power supply	76061
Power cord set (specific to installation environment):	
Europe (Schuko)	75010
USA/International	75011
United Kingdom	75012
Australia/New Zealand	75013
RS-232 serial cable	75034
USB cable	75108
with USB Driver Software	7090204
Wrench	7772028
Fluorescence lamp assembly* (Note: The replacement lamp assembly is PN 7080500)	7080501
Filter "plugs" (2) (also referred to as "dummy filters" or "blanks")*	7082073
Plastic storage bag and fastener strips	—
Time-Resolved Fluorescence cartridge assembly ("T" models only)	7090523
Models with injectors, an external dispense module (packed separately), with the following accessories:	
Outlet tubes (2, plus 2 spare) from dispense module to instrument	7082120
Inlet tubes (2) from supply bottles to syringe drives	7082121
250 µl syringes (2)	7083000
Syringe thumbscrews (2)	19511
Priming plate	7132158
Injector tip priming trough	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 plastic storage bag	2872304

** If applicable to your reader model.*

Optional Accessories

❖ Accessory availability and part numbers are subject to change. Contact BioTek Customer Care with questions or visit www.biotek.com and use the Accessories search tool.

Item	Part #
7-filter Absorbance Test Plate	7260522
Fluorescence Test Plate	7092092
Product Qualification (IQ-OQ-PQ) package	1340508
PCR Tube Adapter Plates	6002072 and 6002076
Terasaki Adapter Plate	7330531
Take3 Micro-Volume Plate	TAKE3
Take3 Trio Micro-Volume Plate	Take3Trio
BioCell Quartz Vessel and Adapter Plate	7272051/7270512
Additional Fluorescence Filters; contact BioTek for part numbers and availability	
The Synergy HTX is compatible with the BioStack Microplate Stacker. Contact BioTek or visit our website to learn more.	

For Use with Liquid Tests (see Chapter 5)	Part #
Absorbance Liquid Test Solutions:	
BioTek Wetting Agent Solution	7773002
BioTek QC Check Solution #1	
25 mL	7120779
125 mL	7120782
Dispense Module Liquid Test Solution:	
BioTek Green Test Dye	7773003
BioTek Blue Test Dye	7773001
BioTek QC (Yellow) Test Dye	7120782
Individual Fluorescence Liquid Test Solutions:	
Sodium Fluorescein Powder	98155
Liquid Test Kit using Sodium Fluorescein	7160013
Liquid Test Kit using Methylumbelliferone (“MUB”)	7160012

Product Support & Service

Technical Assistance Center (TAC)

If your instrument(s) or software 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 service or repair, please contact our Technical Assistance Center. BioTek's "TAC" is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays. You can send a fax or an e-mail any time. You can also request technical assistance via our website: **www.biotek.com**.

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

Please be prepared to provide the following information:

- Your name and company information, along with a daytime phone or fax number, and/or an e-mail address
- The product name, model, and serial number
- The onboard software part number and version (available through Gen5 at **System > Instrument Configuration > Get Basecode Information**)
- Gen5 software version information (**Help > About Gen5**)
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed in Gen5 (see also **Appendix C, Error Codes**)
- A text file of the diagnostic history of the instrument (available via Gen5 by selecting **System > Diagnostics > History**, then selecting the appropriate file and clicking **Export**)

Returning Instruments for Service/Repair

If you need to return an instrument to BioTek for service or repair, please contact the TAC for a service authorization number *before* shipping the instrument. Repackage the instrument properly (see **Chapter 2, Installation**), write the number on the shipping box, and ship to BioTek.

Applications Support

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

Phone: (888) 451-5171 **E-Mail:** applications@biotek.com

Chapter 3

Getting Started

This chapter describes some of the Synergy HTX's key components and provides an introduction to using Gen5 to control the instrument.

Key Components

Power Switch, Carrier Eject Button, Microplate Carrier

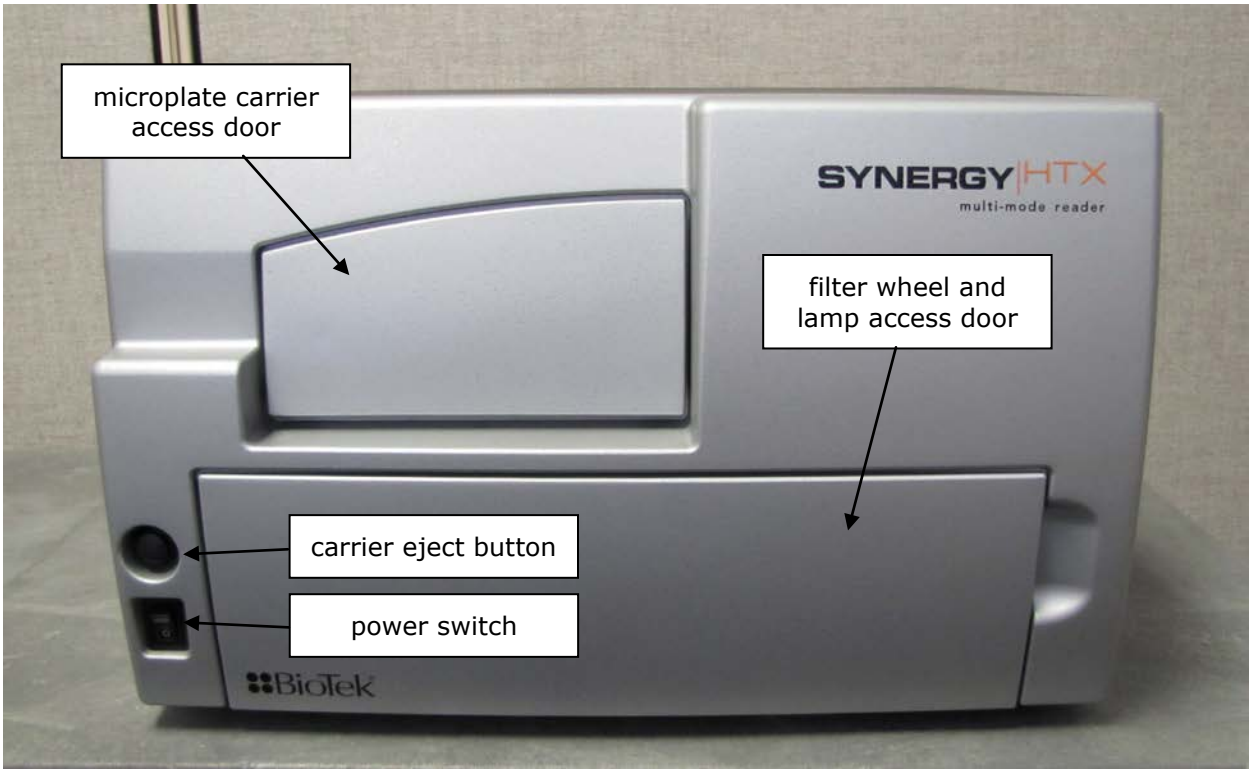


Figure 12: Power switch, carrier eject button, microplate carrier

- The power switch contains an LED, which is illuminated green when the power is on.
- The microplate 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 **Appendix A, Specifications**. 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 optical 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 Synergy HTX models with fluorescence and luminescence capability.

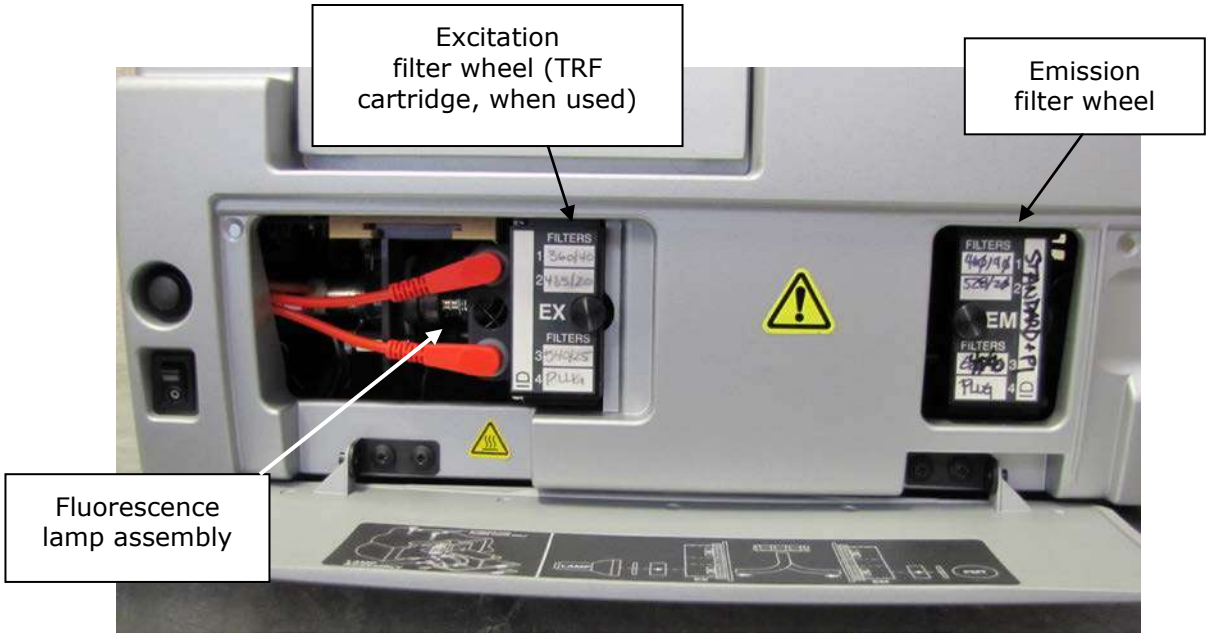


Figure 13: Accessing the fluorescence lamp assembly and filter wheels

- 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, slip 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 37 for more information on the TR cartridge.

- The Synergy HTX has two lamps: one for standard fluorescence, one for absorbance and time-resolved fluorescence:

  Standard Fluorescence: 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.

  Absorbance and Time-Resolved Fluorescence: 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).

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.

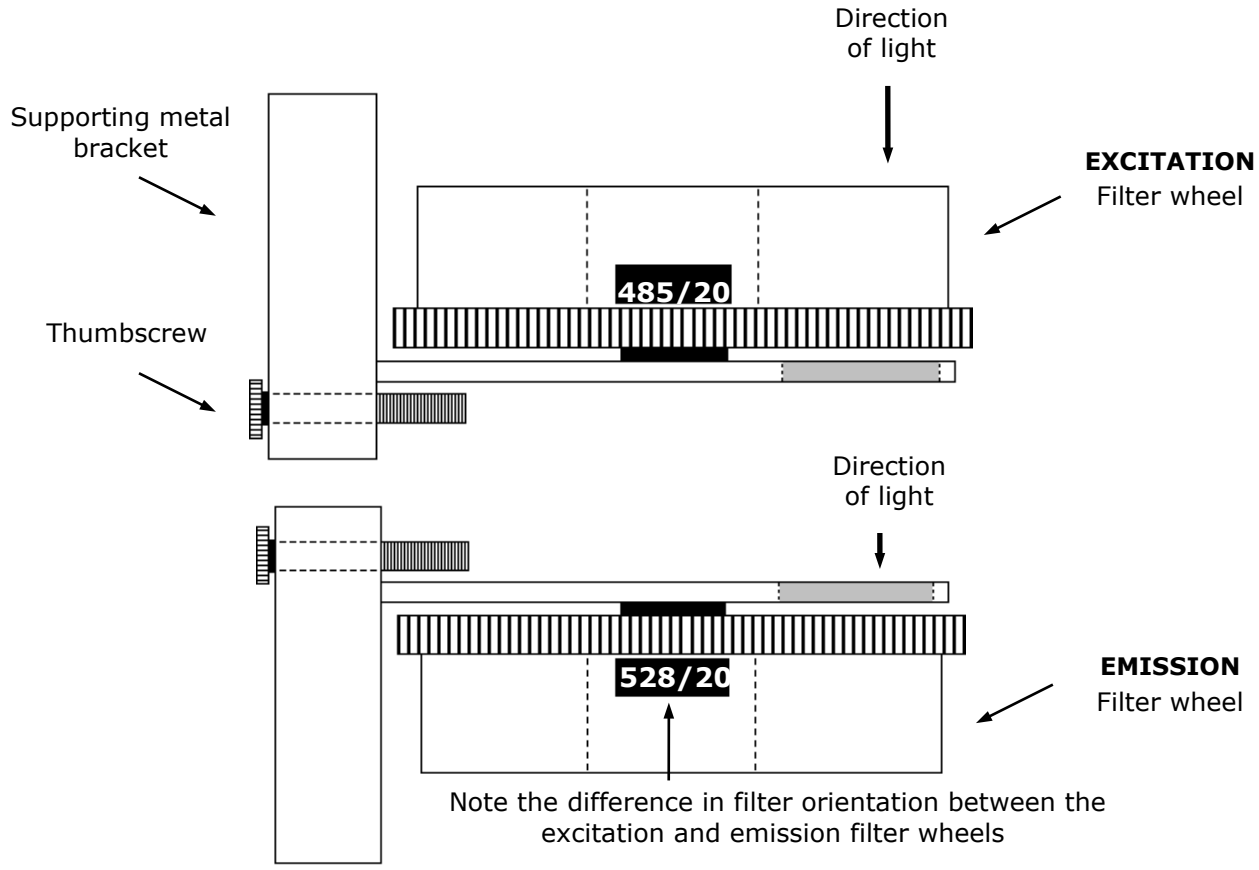


Figure 14: Profiles of the excitation and emission filter wheels, showing proper filter orientation

Important! The Synergy HTX is shipped with a set of excitation and emission filters installed, and the Synergy HTX'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 42 for information on updating Gen5's filter table.

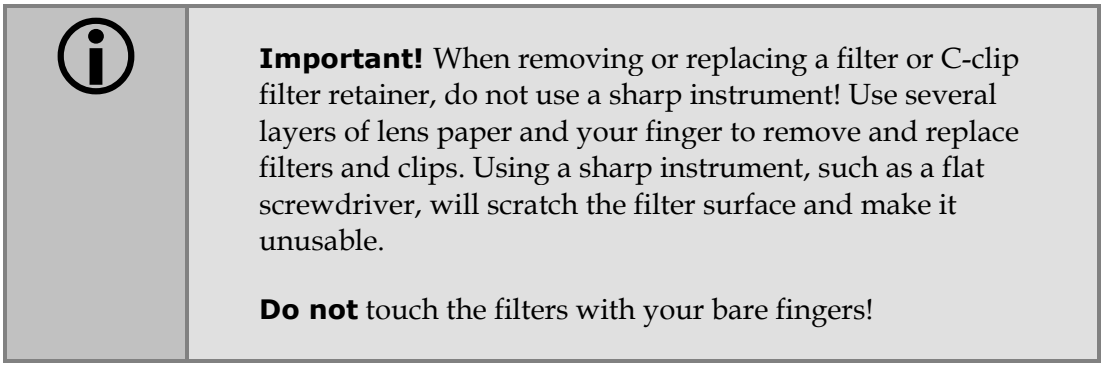
Removing the Filter Wheels

The filter wheels can be removed if different filter wheels need to be installed. 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 37.)
- 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 figures 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. **Note:** 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 its 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 page 34), 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 inserted properly (see above).
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 Fluorescence Cartridge

For Synergy HTX models that support time-resolved fluorescence, the "TR" cartridge 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, the operator will be prompted to install the TR cartridge if it is missing.

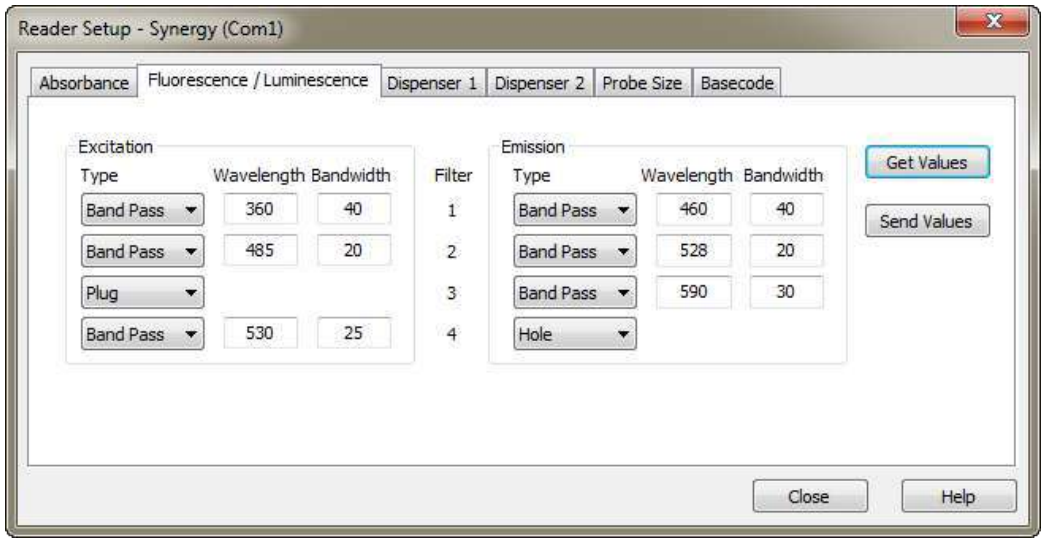
1. **Important!** Turn off the instrument.
2. Open the filter wheel access door using the depression on the right side of the door. Observe the two thumbscrews within the compartment. The left thumbscrew holds the Excitation filter wheel in place. See the figure on page 33.
3. Remove the left thumbscrew 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.

❖ See page 43 for more information on creating Gen5 protocols.

Figure 15: The "TR" cartridge, for time-resolved fluorescence assays

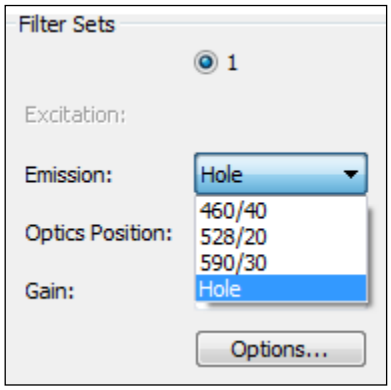
Configuring the System for Luminescence Measurements

- 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 (see page 35) 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.



Updating Gen5's filter table; for complete instructions, see page 42.

- When defining a filter set in a Read step in a Gen5 procedure, selecting "Hole" indicates the empty location in the emission filter wheel. See page 43 for information on Read steps and procedures.



The External Dispense Module

Applies only to Synergy HTX models with injectors.

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.

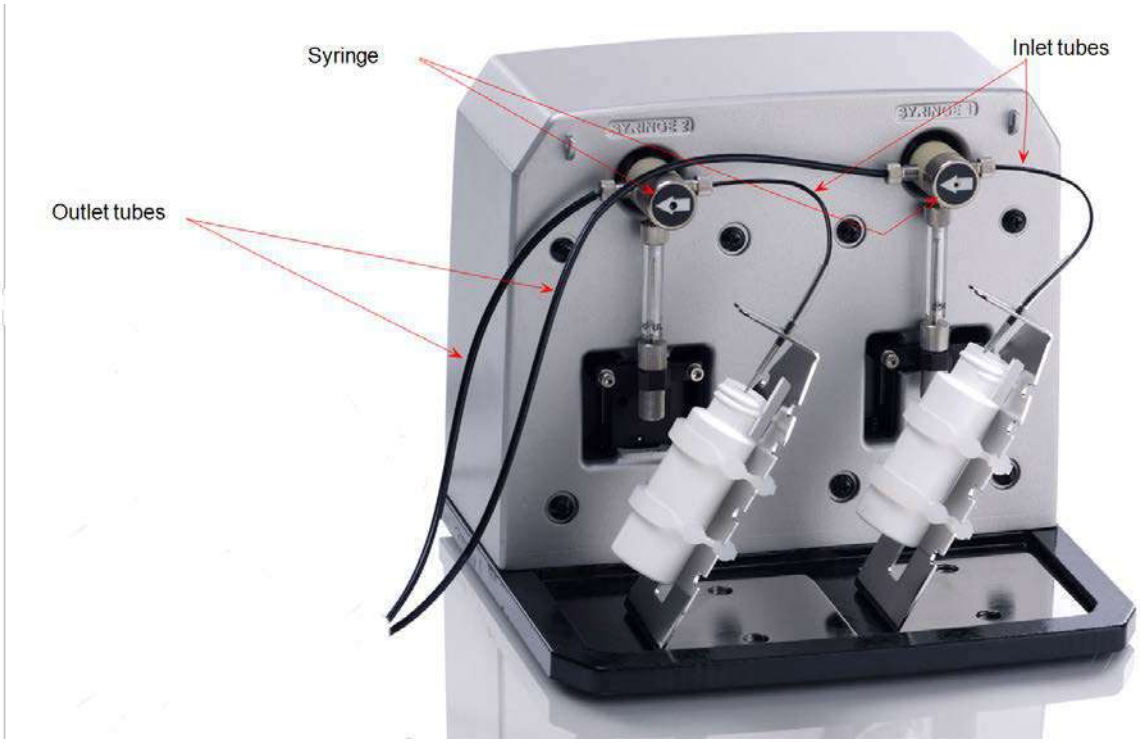


Figure 16: Dispense module components

- Two 250-µL syringes draw fluid from the supply bottles.
- Inlet tubes transport fluid from the supply vessels 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.

- Three-way valves switch the syringe flow from the inlet tubes to the outlet tubes.
- Outlet tubes transport fluid from the syringes into the instrument, through the tubing ports on the Synergy HTX's rear panel. The outlet tubes are opaque PTFE tubes with threaded fittings on each end that are used to deliver fluid from the syringes to the instrument.

Inside the Synergy HTX, 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.

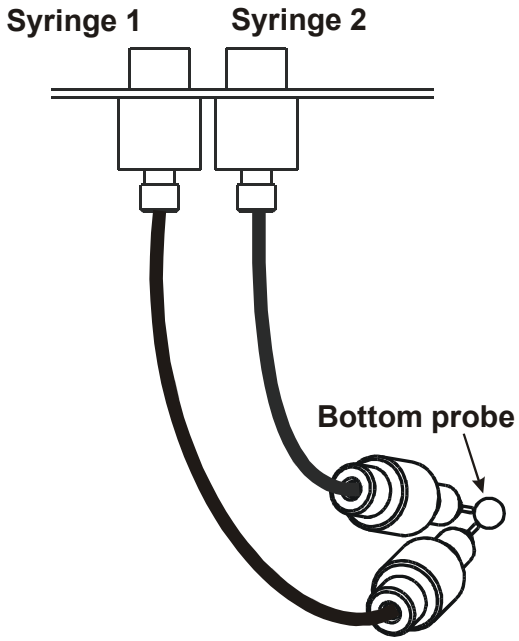


Figure 17: Close-up view of the injectors inside the instrument

❖ The tubing and injectors should be cleaned at least quarterly. See **Chapter 4, Preventive Maintenance** for more information.

Priming the System

Before an assay requiring fluid dispense is run, the system should be fully primed with the reagent or other fluid used by the assay. At the start of the assay (and optionally at the start of each dispense to a well), an additional injector tip prime can be performed. The tip prime compensates for any fluid loss at the injector tip due to evaporation since the last dispense. All priming activities are controlled via Gen5 (see page 46).

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

- The priming plate is about the same size as a standard microplate and 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 priming 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.

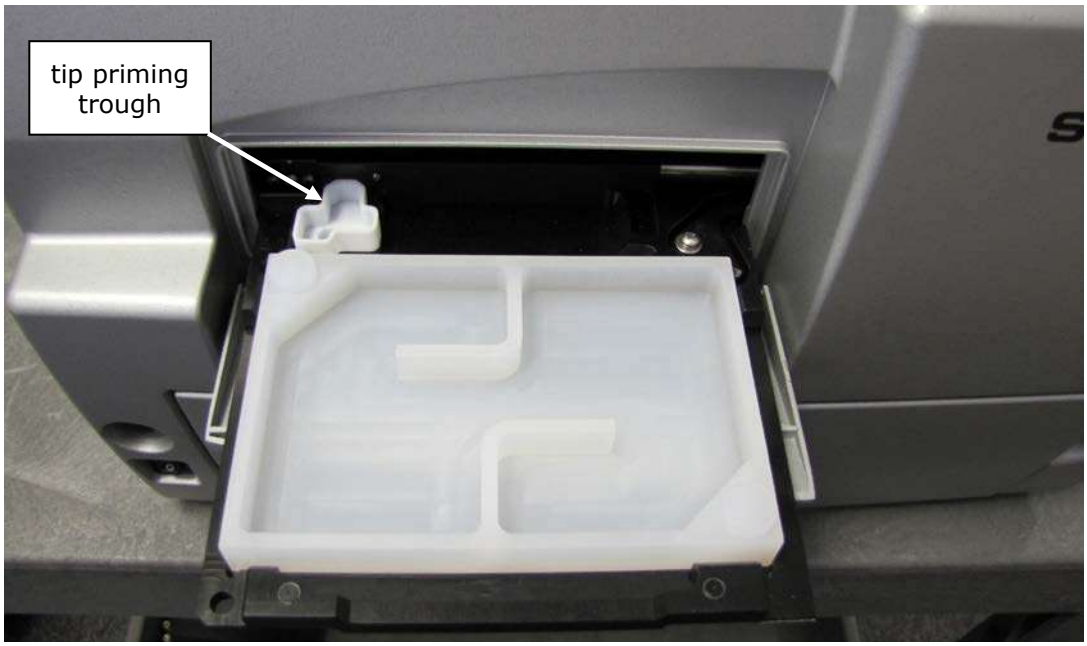


Figure 18: Priming plate and tip priming trough

Gen5 Software

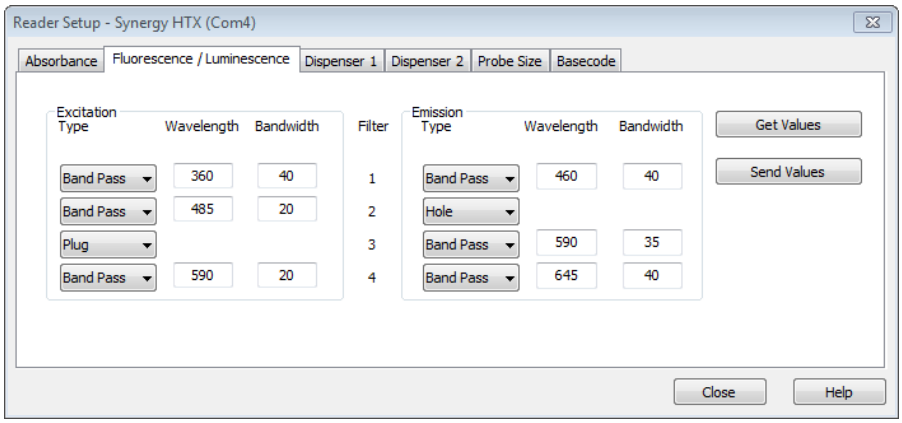
BioTek's Gen5 software supports all Synergy HTX reader models. Use Gen5 to control the reader and the dispense module, perform data reduction and analysis on the measurement

values, print /export results, and more. This section provides brief instructions for creating experiments and reading plates. It also explains how to use Gen5 to perform some functions that are specific to the dispense module.

Viewing/Updating the Filter and Wavelengths Tables

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 "asks" for this information and then stores it in a filter table on the computer.

To view this table in Gen5, select **System > Instrument Configuration**, highlight the Synergy HTX reader, and click **View/Modify.** Click **Setup** and then click the **Fluorescence/Luminescence** tab.



Regarding the Absorbance Wavelengths table:

The Synergy HTX performs absorbance reads in the range of 200 to 999 nm.

Click the Absorbance tab to specify and calibrate 6 wavelengths to be made available as default selections within a protocol's Reading Parameters dialog.

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. 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.

Creating Protocols and Experiments

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

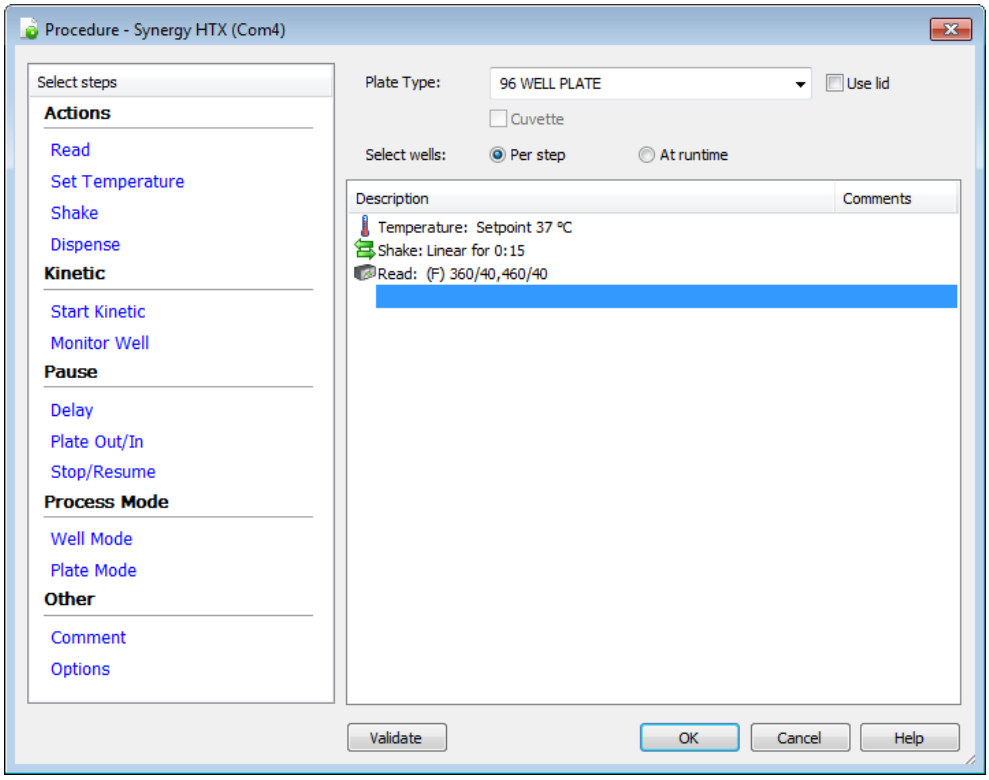


Figure 19: Defining the procedure within a Gen5 protocol

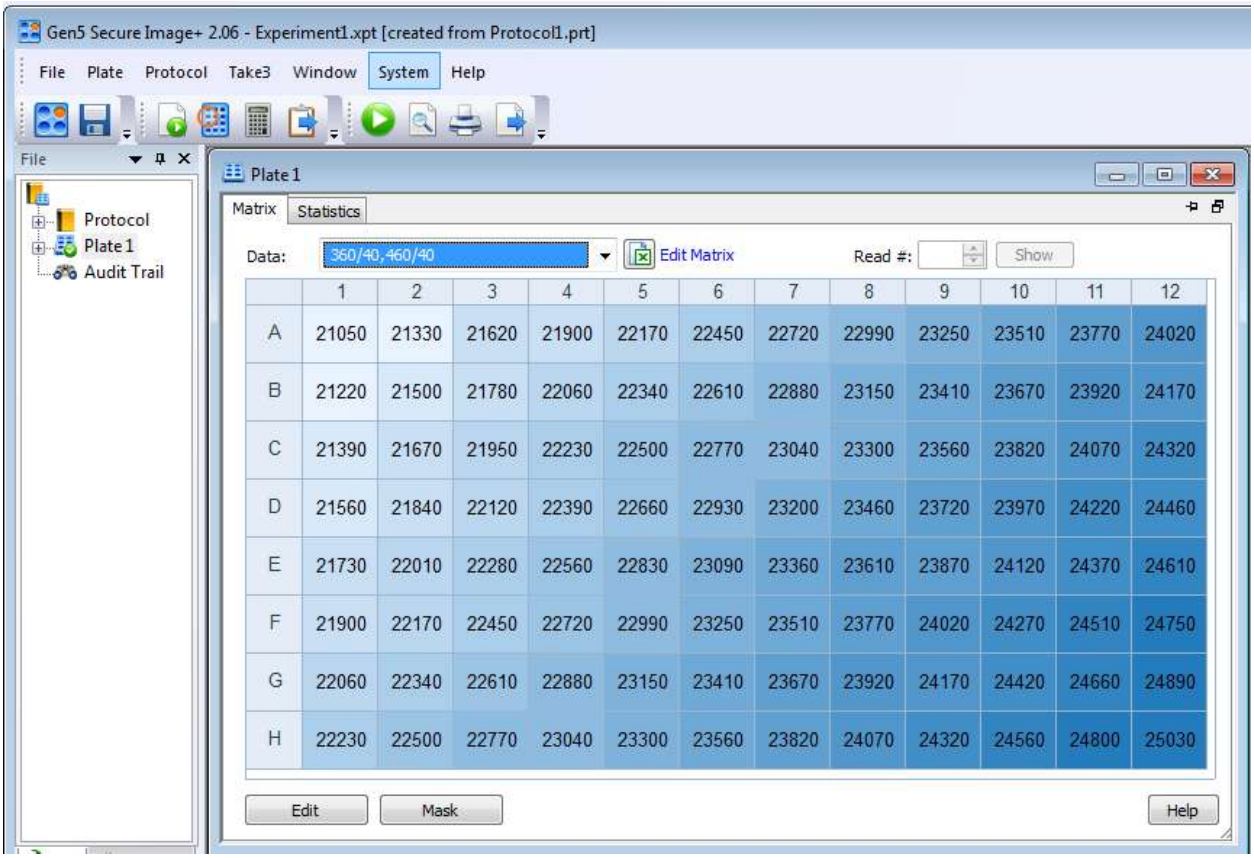


Figure 20: An experiment (containing measurement data), based on a predefined protocol

The instructions below briefly describe how to create a simple protocol in Gen5. For more information, or if the instructions below do not match what you see in Gen5, refer to the *Gen5 Getting Started Guide* or Help system.

1. To create a new protocol, from the Task Manager, select **Protocols > Create New**.
2. Select **Protocol > Procedure**. If prompted to select a reader, select the **Synergy HTX** and click **OK**.
3. Select a plate type.

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

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

   Tips:

   - Add a Dispense step to define the volume and rate at which fluid will be dispensed, and from which dispenser.

- Add a Read step to specify the detection method and filter sets or wavelength values, enable time-resolved fluorescence, and set the Top Probe Vertical Offset value.
- To define a Kinetic read, place an Endpoint Read step inside a Kinetic Start/End loop.

5. Open the Plate Layout dialog and assign blanks, samples, controls, and/or standards to the plate.
6. Open the Data Reduction dialog to add data reduction steps. Categories include Transformation, Well Analysis, Curve Analysis, and more.
7. Create a report or export template, via one of the Report/Export Builder options.
8. Save the file with an identifying name.

The instructions below briefly describe how to create an Experiment based on an existing protocol and then read a plate. See Gen5's Help system for complete instructions.

1. To create a new experiment, from the Task Manager click, **Experiments > Create using an existing protocol**.
2. Select the desired protocol and click **OK**.
3. Select **Plate > Read** or click the Read Plate icon.
4. Click **OK** when the Load Plate dialog appears. The plate will be read.
5. When the read is complete, measurement values will appear in Gen5. To view them, select the desired data set (e.g., "528/20,645/40") from the Data drop-down list.
6. If you have not already done so, save the file with an identifying name.

Controlling the Dispense Module

Applies only to Synergy HTX models with injectors.

Gen5 is used to perform several dispense module-specific functions, including initializing, priming, and purging. Gen5 also contains certain configuration items that must be set before using the dispense module. Read the following sections to become familiar with these functions and configuration items.

Initialization

If the dispense module was connected to the reader before the reader was turned on, or if a System Test was run via Gen5, the dispense module should initialize automatically. If for any reason the module does not initialize automatically, you can initialize it from Gen5:

1. In Gen5, select **System > Instrument Control > Synergy HTX** and click the **Prime** tab.

2. Select the desired Dispenser number (1 or 2) and click **Initialize**. The syringe drive will move to its home position and its sensors will be verified. Upon successful completion, the Initialized field should show "Yes".

Prime Utility

Before running an experiment with a Dispense step, the dispense module and its associated tubing must be primed with the fluid to be used. Gen5 provides a special utility for this task. To prime the dispense module:

1. 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.
2. **Important!** Place the priming plate on the carrier.
3. In Gen5, 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, from 5 to 5000 µL.
   The minimum recommended prime volume is 1100 µL.
6. Select a prime Rate, in µL/second.
7. Click **Prime** to start the process.
8. When the process is complete, carefully remove the priming plate from the carrier and empty its contents. If the priming plate is empty, the prime volume was too low.

Purge Utility

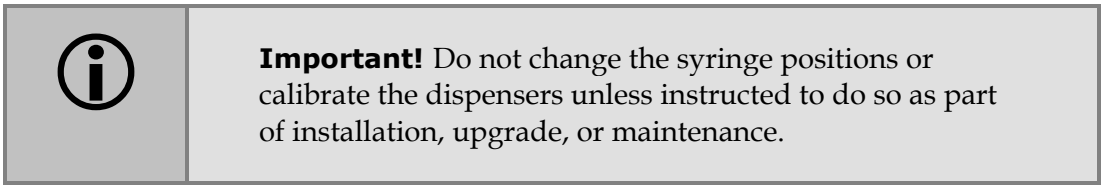
Gen5 provides a special utility to purge fluid from the dispense tubing and syringe by pumping the fluid in reverse, back into the supply bottle. To purge the dispense module:

1. In Gen5, 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.
4. Select a prime Rate in µL/second.
5. Click **Purge** to start the process.

Syringe Maintenance Position

Gen5 provides access to special syringe setup functions for maintenance and calibration purposes. If a syringe needs to be installed or replaced, it must first be moved to its "Maintenance Position." To do this using Gen5:

1. 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.
4. See "Install Dispense Module Components" in **Chapter 2, Installation** for information on installing/removing the syringes.



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

Recommendations for Optimum Performance

- Microplates should be perfectly 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 instrument is on and successfully communicating with the controlling software. You may want to run a System Test if the instrument 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 optically clear, flat-bottomed wells. See **Appendix A, Specifications** for more information on the supported plates.
- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µL per well in a 96-well plate and 25 µL in a 384-well plate.
- Dispensing solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies; however, for best results, remove the air bubbles by degassing the plate in a vacuum chamber before reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Agitate the microplate before reading to help bring this problem 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.

- 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 them overnight, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. See **Chapter 4, Preventive Maintenance** for more information.
- **For models with injectors:** 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.

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 fluid.
- 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 step-by-step instructions for maintaining the Synergy HTX and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

Recommended Maintenance Schedule

Overview

A general **Preventive Maintenance (PM)** regimen for all Synergy HTX models 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 keep the dispense module and injectors 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 them overnight, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. Perform a visual inspection of the dispensing accuracy before conducting an assay that requires a dispense step to verify instrument performance.

It is important to keep the dispensing lines scrupulously clean at all times. Take special care when using molecules active at very low concentrations (e.g., enzymes, inhibitors). Remove any residual reagent in the dispensing lines using a suitable cleaning solution (review the reagent's package insert for specific recommendations).

A daily cleaning regimen is the best way to ensure accurate performance and a long life for your instrument and dispense module. BioTek also recommends flushing the module with DI water before conducting the decontamination procedure described in **Chapter 5, As-Needed Maintenance**.

Recommended Maintenance Schedule

The following charts recommend Preventive Maintenance tasks and the frequency with which each task should be performed.

- ❖ It is important to note that the risk and performance factors associated with your assays may require that some or all of the procedures be performed more frequently than presented in the schedule.

Task	Page	Daily	Quarterly	As Needed
All models:				
Clean exposed surfaces	55			✓
Inspect/clean excitation and emission filters (if equipped)	56		✓	
Decontamination	*see Chapter 5*	*before shipment or storage*		
Models with injectors only:				
Flush/purge the fluid path	57	✓		
(Optional) Run Dispense protocol	58			✓
Empty/clean tip prime trough	59	✓		
Clean priming plate	59			✓
Clean internal components tubing and injector heads	67		✓	
Clean optical probes	68		✓	
Clean internal surfaces	76		✓	

Warnings and Precautions

	**Warning! Internal Voltage.** Turn off and unplug the instrument for all maintenance and repair operations.
	**Warning!** Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears.
	**Warning!** Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.
	**Important!** Do not immerse the instrument, spray it with liquid, or use a "wet" cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact BioTek's Technical Assistance Center.
	**Important!** Do not apply lubricants to the microplate carrier or carrier track. Lubrication 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 reader to produce an error.
	**Caution!** The buildup of deposits left by the evaporation of spilled fluids within the read chamber can impact measurements. Be sure to keep System Test records before and after maintenance so that changes can be noted.
	**Caution! Models with injectors.** 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.
	**Warning!** 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.

Cleaning Exposed Surfaces

Important! Turn off and unplug the instrument for all cleaning operations.

Important! Do not immerse the instrument, spray it with liquid, or use a "wet" cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact BioTek's Service Department.

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
- Mild detergent (optional)

To clean the exposed surfaces:

1. Turn off and unplug the instrument.
2. Moisten a clean cotton cloth with water, or with water and mild detergent. **Do not soak the cloth**.
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. Wipe all exposed surfaces of the dispense module (if used).
5. If detergent was used, wipe all surfaces with a cloth moistened with water.
6. Use a clean, dry cloth to dry all wet surfaces.
7. Reassemble the instrument as necessary.

- **Models with injectors:** If the Tip Priming Trough overflows, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. If overflow is significant, you may have to remove the shroud of the instrument to better access the surface beneath the carrier.
- See page 62 for instructions on removing the shroud.
- See page 76 for instructions for cleaning the surface beneath the carrier.

Inspect/Clean Excitation and Emission Filters

Applies only to Synergy HTX models with fluorescence and luminescence capability.

Laboratory air is used to cool the lamp, and the filters can become dusty as a result. The filters should be inspected and cleaned at least every three months. You will need:

- Isopropyl, ethyl, or methyl alcohol
- Lens-cleaning tissue

❖ Do not touch the filters with your bare fingers!

To inspect and clean the excitation and emission filters:

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 slide the filter wheel's supporting metal bracket straight out of the compartment.

❖ **Chapter 3, Getting Started** contains illustrations for identifying the filter wheels and their unique characteristics. This chapter 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. Clean the filters using lens-cleaning tissue moistened with a small amount of isopropyl, ethyl, or methyl alcohol. Ensure that the filters remain in their current locations.
5. Replace the filter wheel brackets in their respective positions and replace the thumbscrews. Close the hinged door.

Flush/Purge the Fluid Path

Applies only to Synergy HTX models with injectors.

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

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

To flush the fluid path:

1. Fill two supply bottles with deionized or distilled water. Insert the supply (inlet) tubes into the bottles.
2. Place the priming plate on the carrier.
3. From Gen5's main screen, select **System > Instrument Control.** Select the appropriate reader if prompted.
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**. Select the appropriate reader if prompted.
3. Click the **Prime** tab and select **Dispenser 1**.
4. Set the **Volume** to 2000 µL.
5. Click **Purge** to start the process.

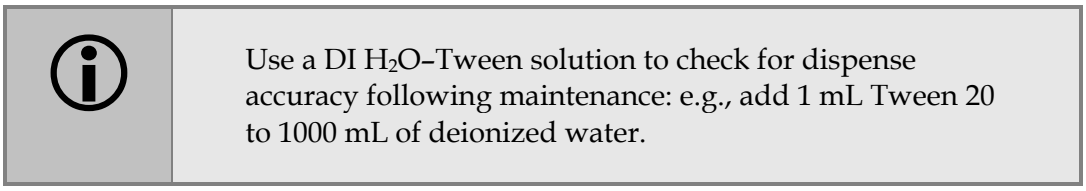
   When the purge is complete, repeat the process for **Dispenser 2**.

> ❖ After purging the system, you may wish to run a quick Dispense protocol to visually verify the dispense accuracy. See the next page for instructions for creating the protocol.

Running a Dispense Protocol (Optional)

Applies only to Synergy HTX models with injectors.

After flushing/purging the system (page 57) and before running an assay that requires dispense, take a moment to visually inspect the dispensing accuracy.



❖ Select a Plate Type in the Protocol that matches the plate you are using.

1. Create a new protocol and then select **Protocol > Procedure**.
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).
   - Select a **Rate** (adjust the rate to support the dispensing volume).
   - Click **OK** to close the dialog and add the Dispense step to the list.
3. Add another Dispense step with the same parameters, selecting **Dispenser 2**.
4. Add a quick Read step with the following parameters (Gen5 requires that a Read step follow the Dispense step):
   - Define a **partial plate** read on just one well (e.g., A1)
   - Set the Detection Method to **Absorbance**
   - Set the Read Type to **Endpoint**
   - Set the Read Speed to **Normal**
   - Select any **wavelength**
5. Click **OK** to close the dialog and add the Read step to the list.
6. Click **OK** to close the Procedure.
7. Save the protocol with an identifying name, such as "Dispense Observation."
8. Create a new experiment to run the Dispense Observation protocol.
9. Initiate the plate read and follow the prompts.

10. When the procedure is complete, visually assess the fluid level in the wells for accuracy. If the well volume appears to be unevenly distributed, clean the internal dispense tubes and injector heads as described in **Cleaning Internal Components** on page 60.

Empty/Clean the Tip Priming Trough

Applies only to Synergy HTX models with injectors.

The tip priming trough is a small, removable priming cup located in the left rear of the microplate carrier, used for performing the Tip Prime. The trough holds about 1.5 mL of liquid and must be periodically emptied and cleaned by the user.

To empty/clean the tip prime trough:

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.

When starting a Gen5 experiment that includes dispensing, Gen5 will prompt you to empty the tip prime trough. Follow the instructions provided.

Clean the Priming Plate

Applies only to Synergy HTX models with injectors.

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

Clean the Internal Components

Applies only to Synergy HTX models with injectors.

The Synergy HTX's internal components that require routine cleaning include:

- Optical probes
- Surface beneath the microplate carrier
- Internal dispense tubes and injector heads

The internal 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.

❖ The procedures in this section should be performed in succession. Start with **Removing the Reader's Shroud** and execute the procedures that meet your needs, in the order in which they are presented. Finish with **Reassembling the Components**.

We recommend running a System Test in Gen5 before and after performing these cleaning procedures. This will verify that all systems are functioning properly and allow you to compare results before and after maintenance.

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

Required Materials

Warning! Always wear protective gloves and safety glasses when performing cleaning/maintenance procedures.

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:

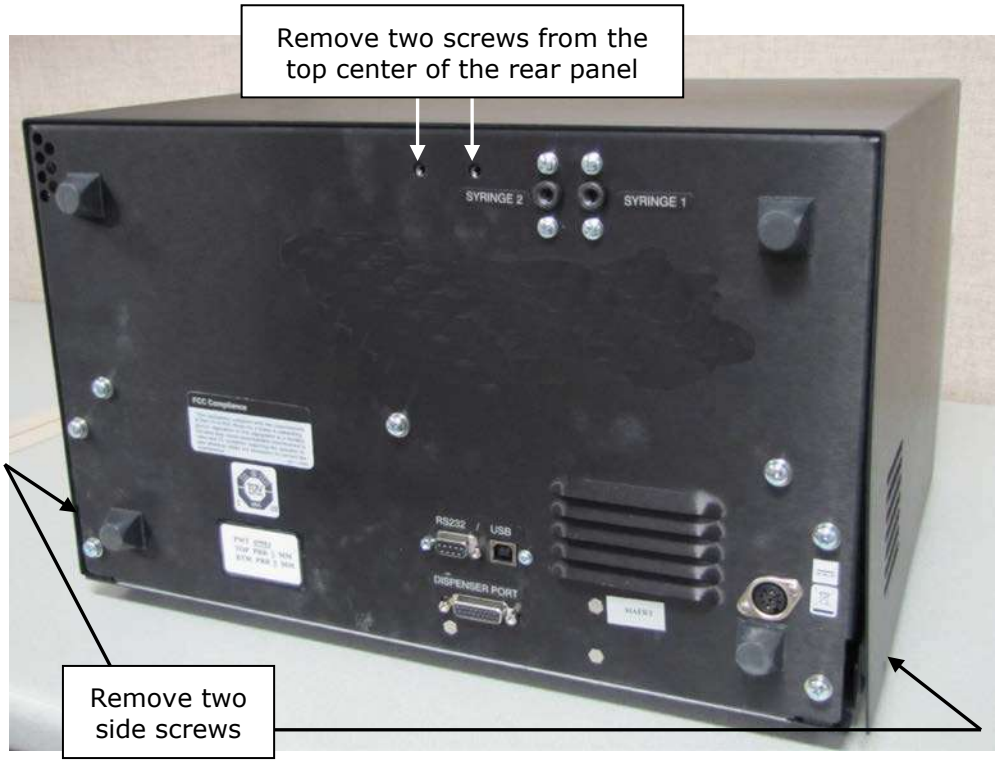
- Clean cotton swabs
- Isopropyl alcohol
- Lens-cleaning tissue

Removing the Reader's Shroud

Caution! Before removing the shroud: Purge the dispense module (see page 57 for instructions), and then turn off and disconnect the reader from its power supply, the PC, and the dispense module.

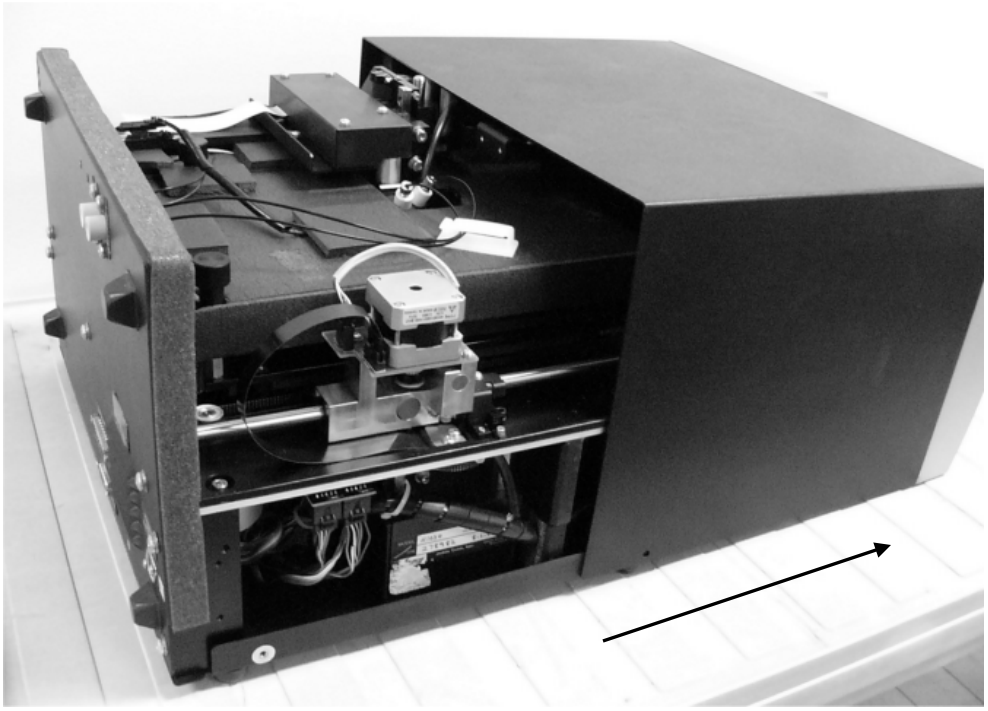
The reader's shroud (cover) must be removed to expose the internal components.

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



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

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



Removing the Internal Tubes and Injector Heads

Take a moment to identify the components described in this section:

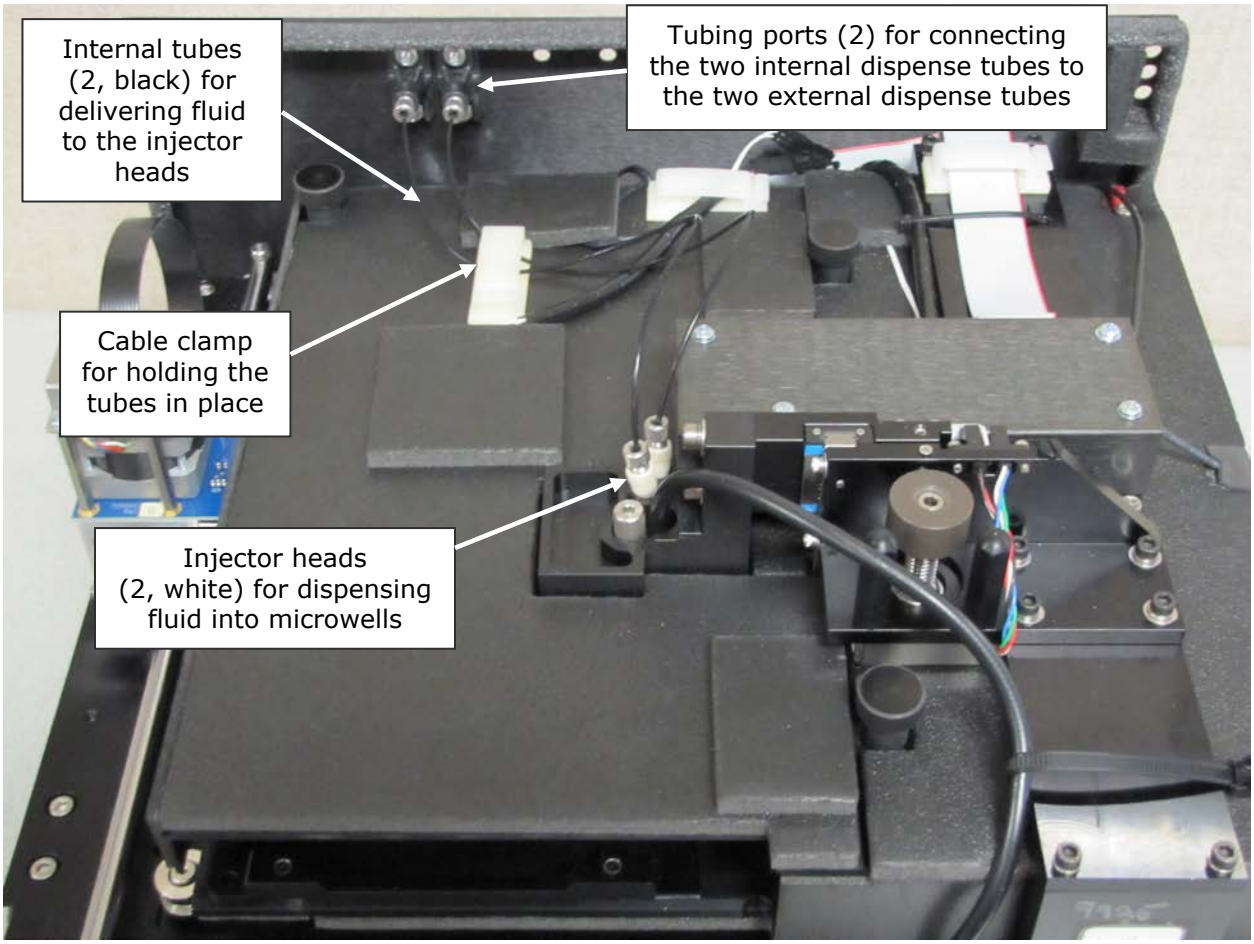
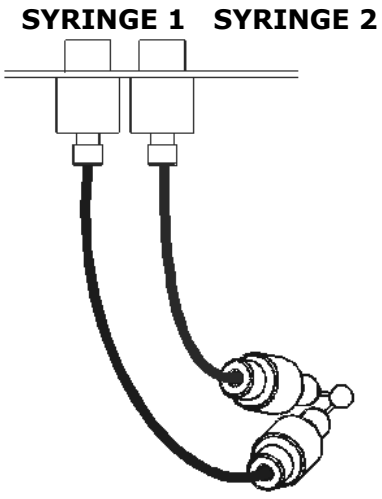


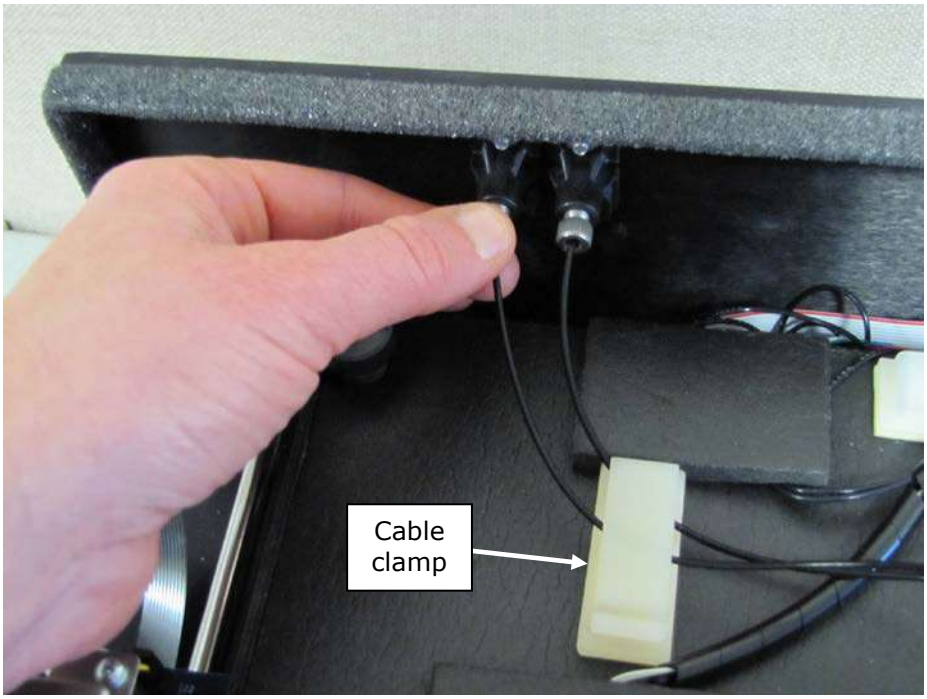
Figure 20: Internal components for the injection system

Important! When reinstalling the internal dispense tubes, be sure to align the tubing ports with the injector heads as shown in this diagram. Look for the **SYRINGE 1** and **SYRINGE 2** labels on the instrument's rear panel.

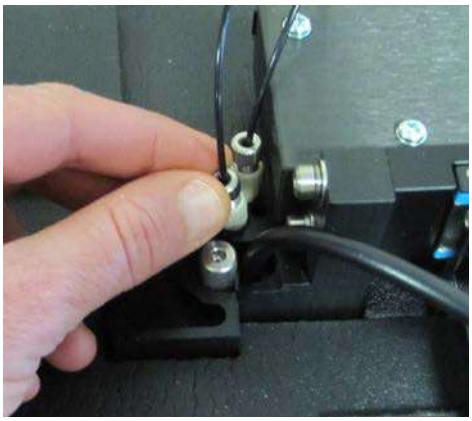


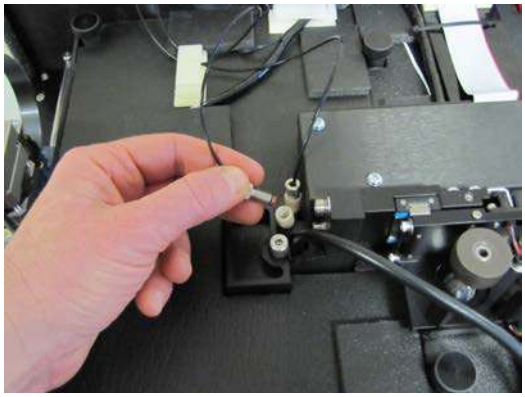
Perform these steps to remove both sets of internal dispense tubes and injector heads:

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.

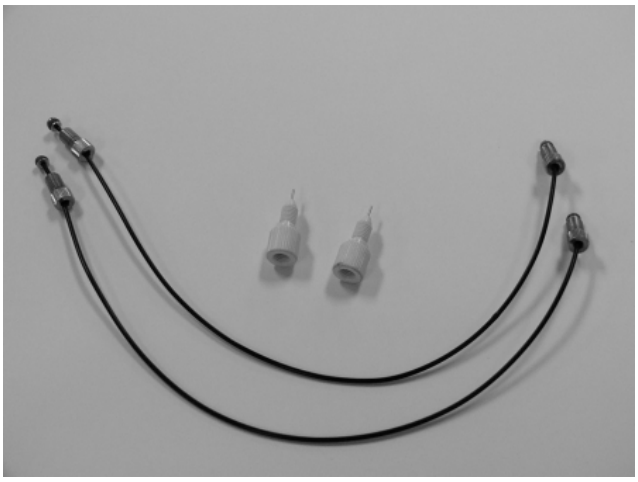




4. Turn the injector heads counterclockwise and gently pull them out of their sockets.

❖ Be sure to seat the injector tips securely when reinstalling. See the photo on page 77.

Cleaning the Internal Tubes and Injector Heads



As discussed on page 52, 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.

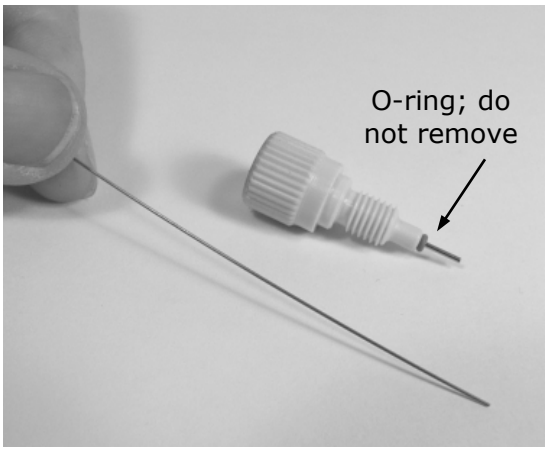
To clean the tubes:

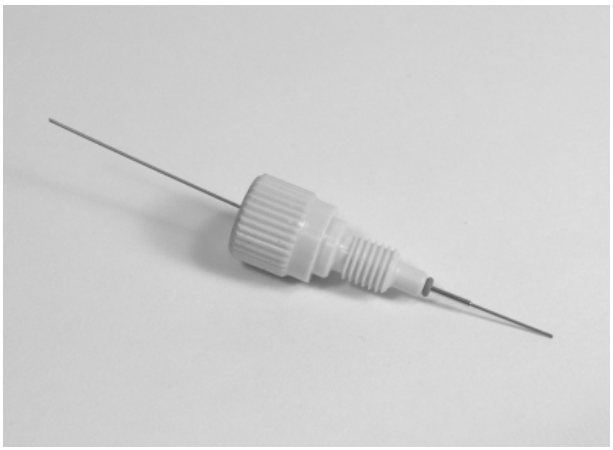
1. Soak the internal tubes in hot soapy water to soften and dissolve any hardened particles.
2. Flush each tube by holding it vertically under a stream of water from a faucet.

To clean the injector heads:

❖ Do not remove the o-ring from the injector head (see photos below).

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





Cleaning 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 the 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 the "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 62 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

Take a moment to identify the components discussed in this section:

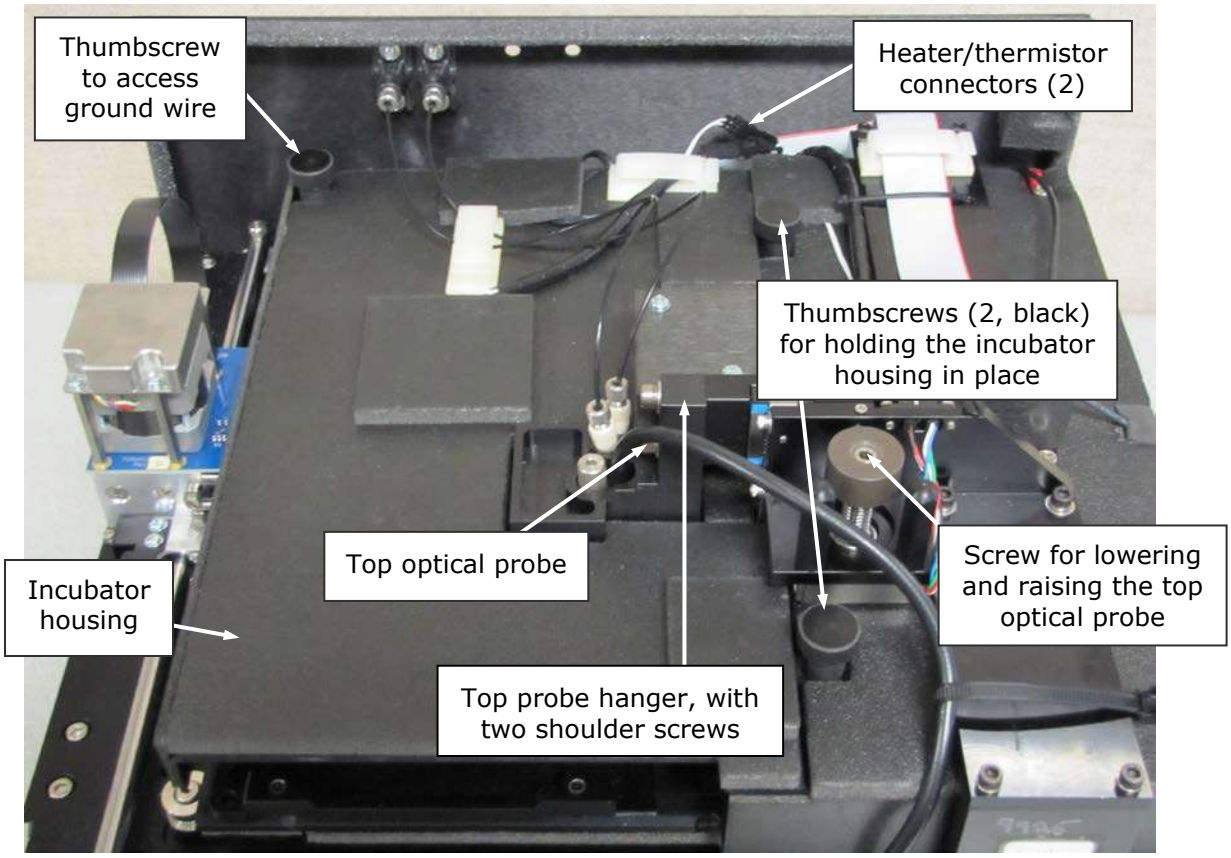
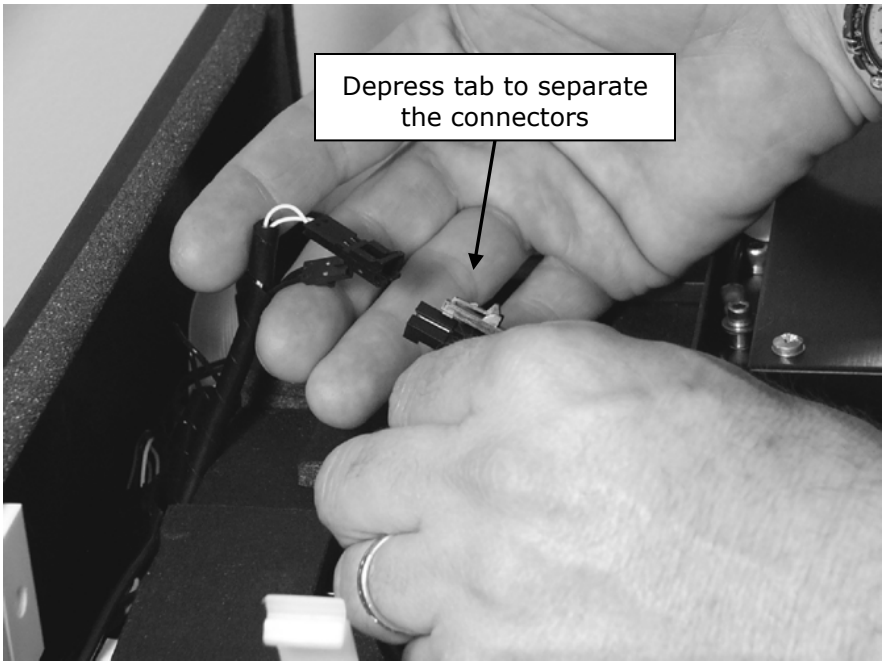


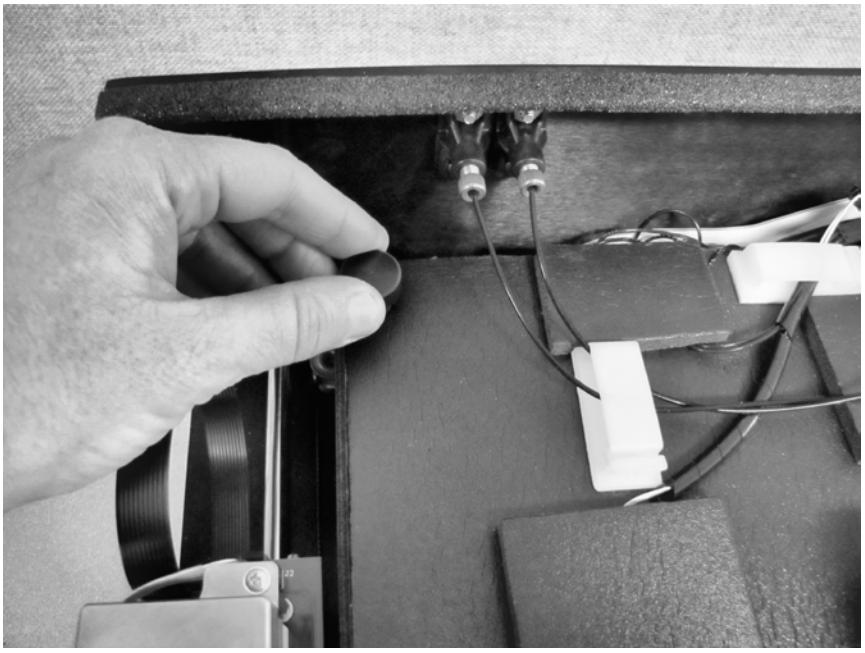
Figure 21: Internal components to be removed/adjusted for cleaning the optic probes

Once the shroud has been removed and the internal tubes and injector heads have been removed and cleaned (see page 67), follow these instructions 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 (pictured below) and separate the connectors.



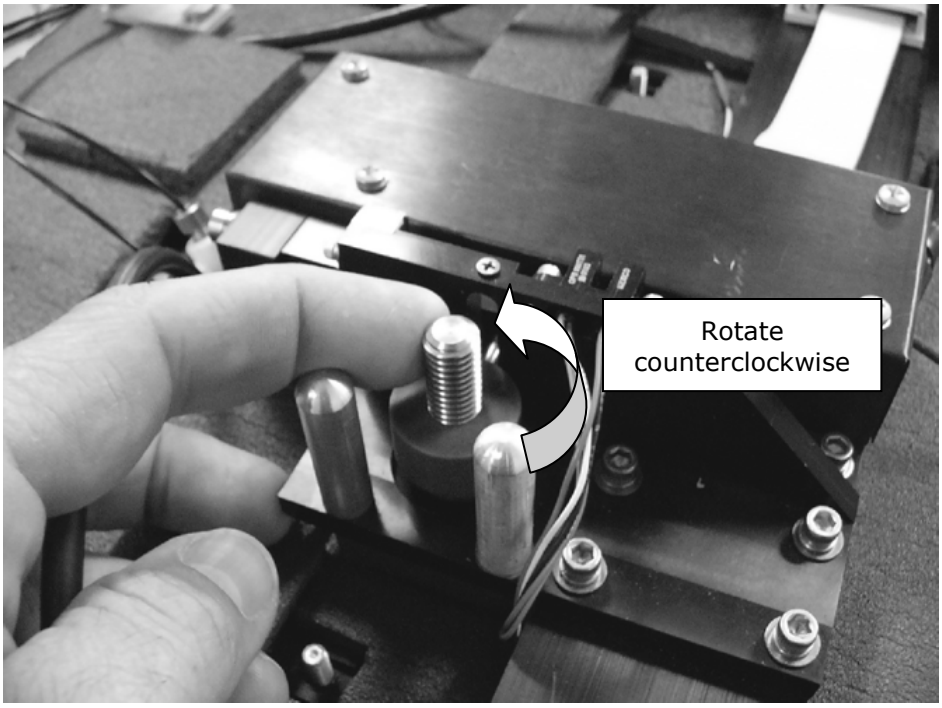
2. Remove the thumbscrew located in the left rear of the instrument and set it aside. This exposes the ground wire.



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

4. Locate the two black thumbscrews that hold the incubator housing in place. Remove both of them 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.)

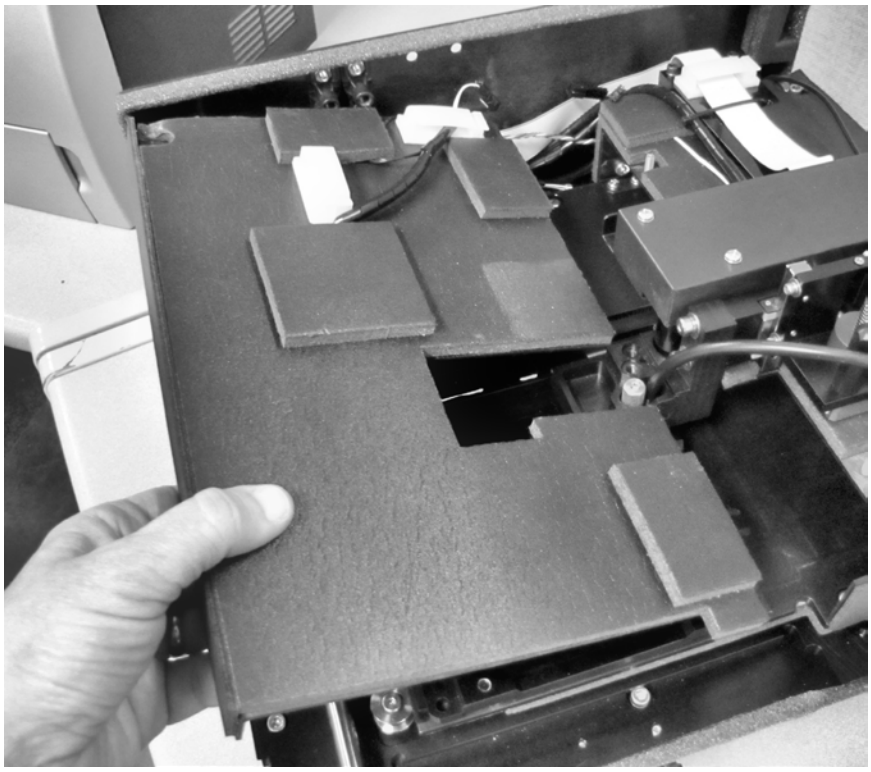


6. Gently lift the left side of the incubator housing and carefully slide it out.

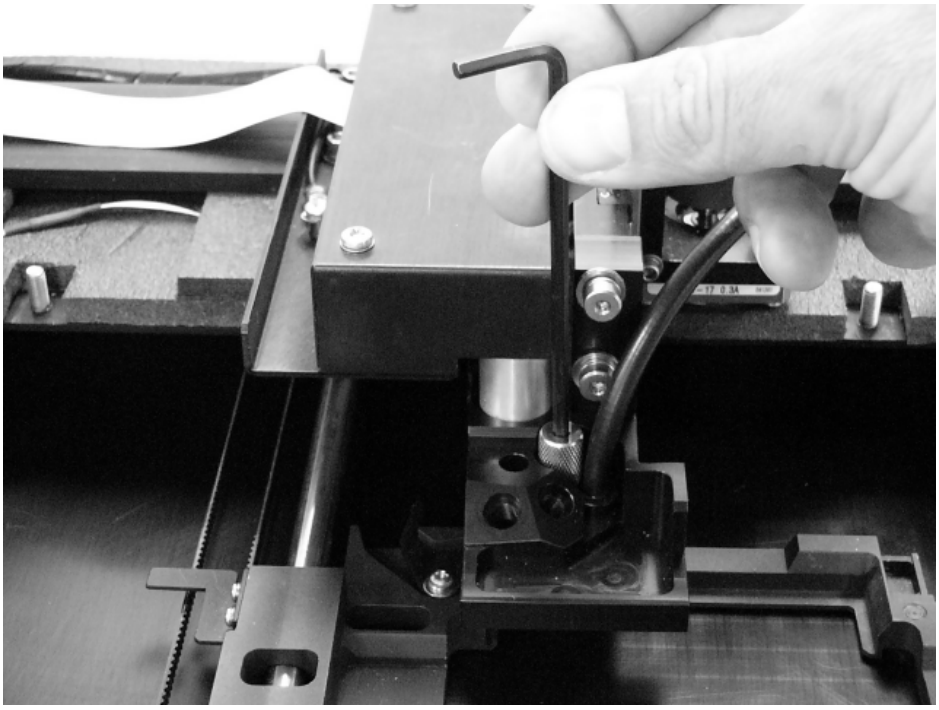
Note:

When replacing the incubator housing, the two "forks" on its right side should wrap around the holding screws.

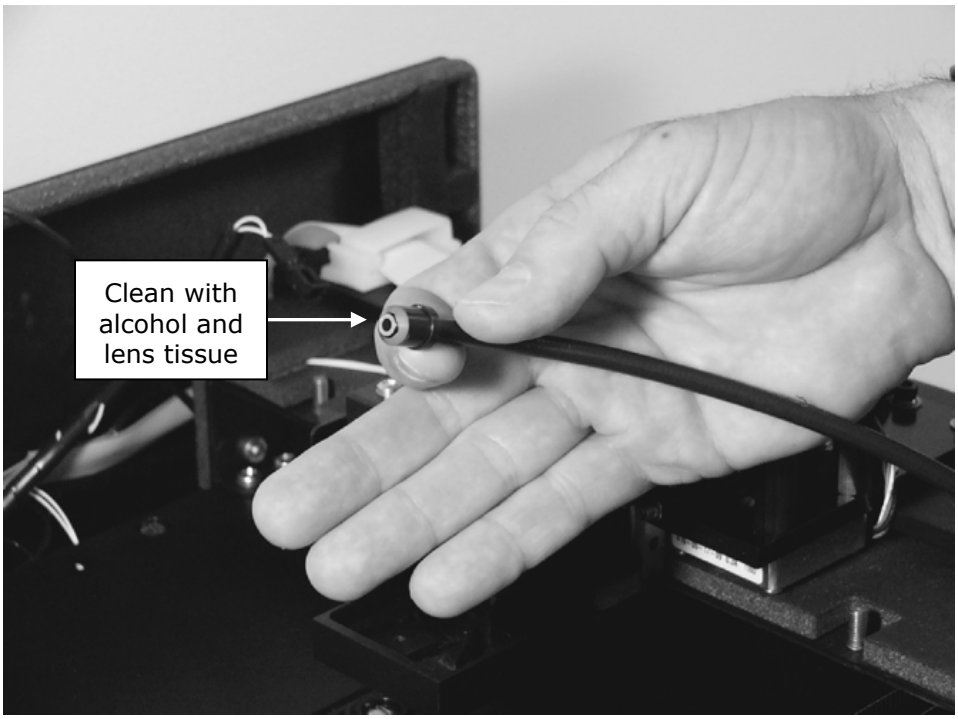
The forks should not slide under the fixed foam housing.



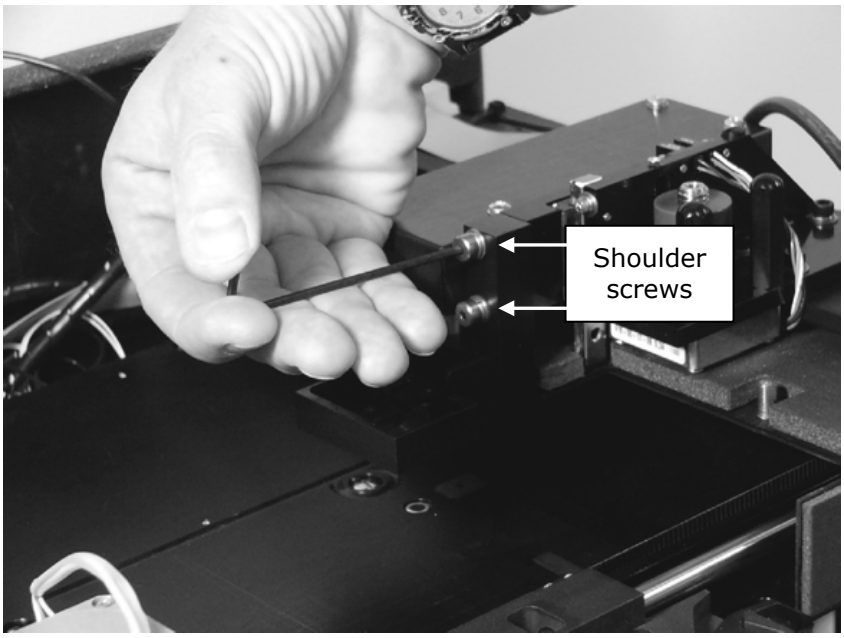
7. Use a 1/8" hex key to remove the top optical probe's holding screw.



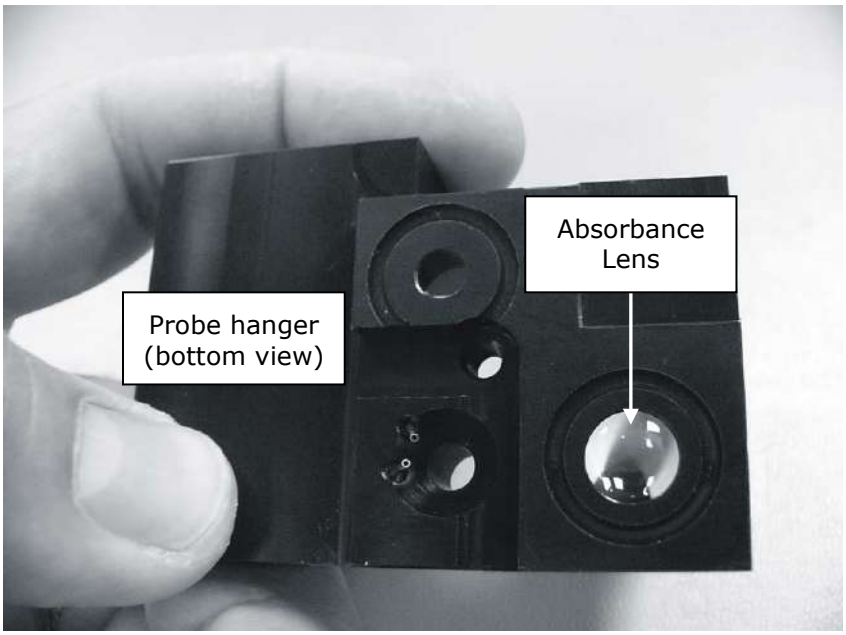
8. Gently pull the optical probe up and out of its socket to expose it for cleaning. Soak the end of the probe in alcohol for one minute **maximum**. Wipe with lens-cleaning tissue and set aside.



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.



10. Drop the top probe hanger down 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.



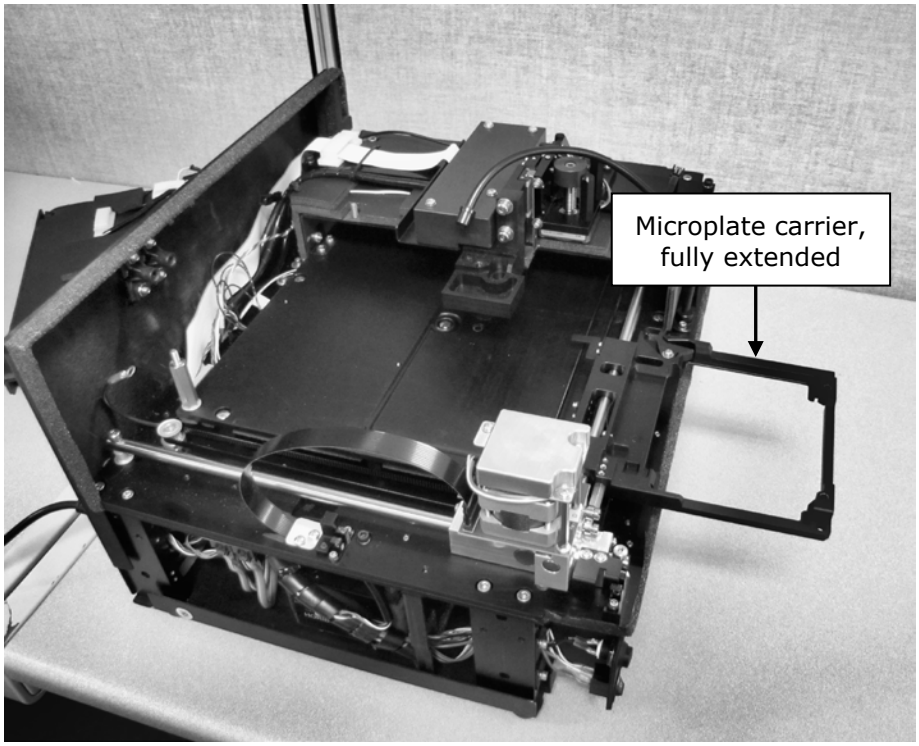
❖ **Important!** When cleaning the absorbance lens with the swab, apply very little pressure to the lens! Applying too much pressure can push the lens out of its holder; reinstallation must be performed by BioTek service personnel. If the lens does fall out, contact BioTek TAC.

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

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

Cleaning the Reader's Internal Surface

1. If you have not already done so, unplug the instrument and remove its shroud (see page 62 for instructions). Follow the instructions under **Cleaning 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 (steps 1 through 6).
2. Manually slide the microplate carrier to the left to engage the support pin, and then away from the center surface.



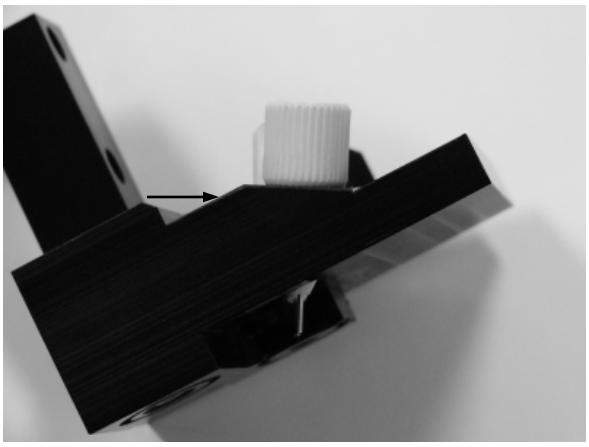
3. Moisten (**do not soak**) a clean cotton cloth with alcohol, water, or with water and mild detergent. Wipe all sides of the plate carrier. Wipe the instrument's horizontal surface.

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.

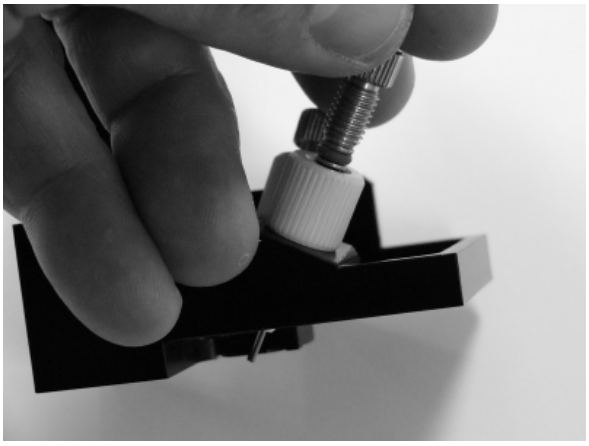
Reassembling the Components

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.

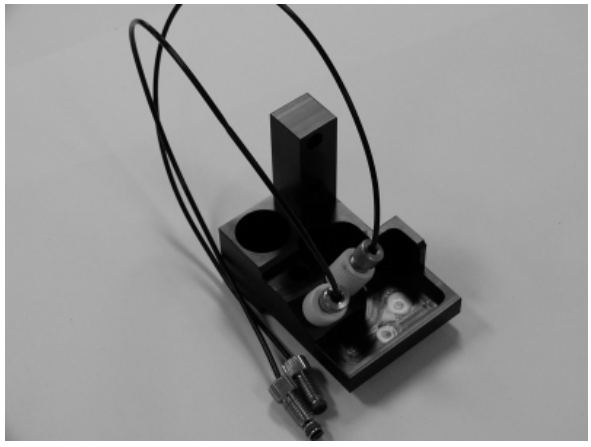
1. Slide the microplate carrier back into the instrument, page 76.
2. Insert the two injector heads into their sockets in the top probe hanger. **Do not touch the absorbance lens with your fingers!** Ensure that the injector heads are properly seated in the hanger. The knurled plastic should sit flush against the hanger surface, as shown below.



3. Attach the two internal dispense tubes to the injector heads, as shown below. **Do not overtighten the thumbscrews!**



Here is the top probe hanger ready for reinstallation, with injector heads and internal dispense tubes attached:



4. Replace the top probe hanger and shoulder screws (using the 3/32" hex key), page 74.

5. Insert the top optic probe into its socket and replace its holding screw (using the 1/8" hex key), page 73.
6. Replace the incubator housing and two thumbscrews, pages 72 and 71. Do not slide the two "forks" on the housing's right side *under* the fixed foam housing.
7. Replace the groundwire and its thumbscrew, page 70.
8. Reconnect the heater and thermistor wires, page 70. Be sure to connect wires of the same color.
9. Attach the two internal dispense tubes to the tubing ports, taking care to align the correct port with the correct injector head, page 65.
10. Slide the two internal dispense tubes into the cable clamp and close the clamp, page 65.
11. Review the steps you just performed to make sure the components have been properly reassembled.
12. Slide the shroud onto the instrument, page 63.
13. Replace the four screws to securely attach the shroud to the base, page 62.

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 appendix contains procedures for decontaminating all models of the Synergy HTX.

Purpose

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

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. Decontamination is required by the U.S. Department of Transportation regulations.

Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.

	BioTek Instruments, Inc. recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither BioTek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the Biohazard(s) they handle.
	Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, and nose. Eating and drinking while decontaminating instruments is not advised.
	Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when performing the decontamination procedure.

Required Materials

For all Synergy 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

Additional materials for models with injectors:

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

Procedure for Models without Injectors

	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 "wet" cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department.
	**Important!** 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). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.

❖ Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10.0% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; if this is the case, prepare a 1:10 dilution.

3. Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth.**
4. Manually open the plate carrier door; 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.

Routine Procedure for Models with Injectors

❖ Perform this **Routine Procedure** when all systems are functioning normally on the Synergy HTX with Injectors. If you are unable to prime the Synergy HTX due to a system failure, perform the **Alternate Procedure** described on page 89.

If disinfecting with sodium hypochlorite (bleach), be sure to flush repeatedly with deionized water to ensure that no bleach is carried over. After disinfecting with sodium hypochlorite, perform the rinse procedure provided on page 87.

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

Clean Exposed Surfaces

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

❖ Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10.0% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; if this is the case, prepare a 1:10 dilution.

3. Manually open the plate carrier door; slide out the plate carrier.
4. Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth.**
5. Wipe the plate carrier and the exposed surfaces of the external dispense module.
6. Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.

8. Reassemble the instrument as necessary.
9. If the dispense module is installed, detach the outlet tubes from the rear panel of the instrument. If it is not installed, attach just the dispense module's communication cable to the instrument. Remove the supply bottles and their holders.
10. Perform the procedures described below through page 88 to decontaminate the fluid lines in the dispense module, the internal tubing and injector heads, and the tip priming trough and priming plate.

Decontaminate the Fluid Lines

1. Place a beaker with 20 mL of 0.5% sodium hypochlorite solution **or** 70% isopropyl alcohol near SYRINGE 1 on the dispense module.
2. Place the SYRINGE 1 inlet tube in the beaker.
3. If you have not already done so, detach the dispense module's outlet tubes from the instrument's rear panel. Place the ends of the outlet tubes in an empty beaker and set the beaker on the work surface.
4. Launch Gen5, select **System > Instrument Control,** and click the **Prime** tab.
5. Select **Dispenser 1**, enter a Volume of **5000 µL**, and keep the default dispense **Rate.**
6. Place the priming plate on the carrier (it is not used, but the reader requires its presence).
7. Run two prime cycles, for a total of 10000 µL.
8. Pause for 20 to 30 minutes to allow the solution to disinfect the tubing.
9. Remove the inlet tube from the beaker of disinfectant solution.
10. From the Reader Control dialog, change the Volume to **1000 µL.**
11. Run one prime cycle, to flush the disinfectant out of the fluid lines.
12. Empty the beaker containing the outlet tubes. Put the tubes back in.
13. Important! If sodium hypochlorite (bleach) was used, perform **Rinse the Fluid Lines** on the next page.

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

Rinse the Fluid Lines

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

1. Place a beaker containing at least 30 mL of deionized water on the dispense module.
2. Place the SYRINGE 1 or 2 inlet tube in the beaker.
3. If you have not already done so, place the outlet tubes in an empty beaker.
4. From the Reader Control dialog, select **Dispenser 1** or **2**, set the Volume to **5000 µL**, and keep the default dispense **Rate**.
5. Run five prime cycles, for a total of 25000 µL.
6. Pause for 10 minutes and then run one prime cycle with 5000 µL. This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
7. Empty the beaker containing the outlet tubes.
8. Wipe all surfaces with deionized water.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Clean the Internal Tubing and Injector Heads

Turn to **Chapter 4, Preventive Maintenance** and perform the following procedures to access, remove, and clean the internal tubing and injector heads:

- **Required Materials**
- **Removing the Reader's Shroud**
- **Removing the Internal Tubes and Injector Heads**
- **Cleaning the Internal Tubes and Injector Heads**

When finished, replace the internal components and the reader's shroud.

Clean the Tip Priming Trough and Priming Plate

1. Remove the tip priming trough from the left rear of the instrument's microplate carrier (see below).
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 20 to 30 minutes.
4. If decontaminating in bleach solution, remove the trough and plate, and thoroughly rinse with DI water.

   If decontaminating with alcohol, remove the trough and plate and let them air-dry.
5. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

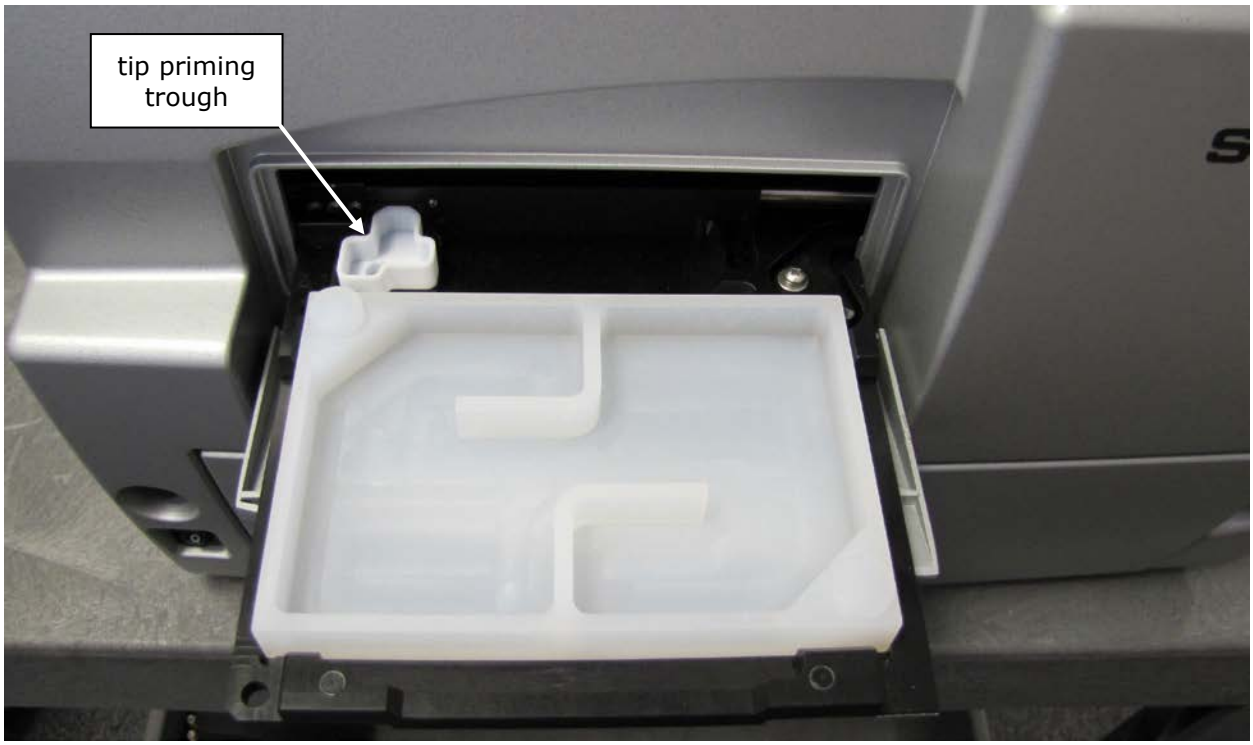


Figure 33: Tip priming trough and priming plate

Alternate Procedure for Models with Injectors

If you are unable to prime the Synergy HTX due to a system failure, decontaminate the instrument and the Dispense Module as follows:

1. Turn to **Chapter 4, Preventive Maintenance** and perform the following procedures to remove the shroud and remove/clean the internal tubes and injector heads. When finished, leave the shroud off the reader and proceed to step 2 below.
   - **Required Materials**
   - **Removing the Reader's Shroud**
   - **Removing the Internal Tubes and Injector Heads**
   - **Cleaning the Internal Tubes and Injector Heads**
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.

> ❖ Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10.0% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; if this is the case, prepare a 1:10 dilution.

3. Slide the microplate carrier out of the instrument.
4. Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth.**
5. Use the cloth to wipe:
   - All surfaces of the shroud
   - All surfaces of the plate carrier
   - The instrument's rear panel
   - The exposed surfaces of the dispense module, including the syringe valves
6. Remove the external tubing and the syringes from the dispense module and soak them in the bleach or alcohol solution. **Wait for 20 minutes.**

> ❖ **To remove the syringes:** In Gen5, click **System > Instrument Control**. On the Prime tab, click **Maintenance**. Pull down the syringe bracket until it stops. 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 and store it in its original packaging (see **Chapter 2, Installation**).

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 wet surfaces on the instrument and the Dispense module.
10. Reassemble the instrument and dispense module as necessary.
11. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Appendix B

Error Codes

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

Error Codes Overview

When a problem occurs during operation of the Synergy HTX, an error codes appears in Gen5. Error codes typically contain four characters, such as "2101," and in most cases are accompanied by descriptive text. With many errors, the instrument will beep repeatedly; press the carrier eject button to stop this alarm.

Some problems can be solved easily, whereas others 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 should run a system test for diagnostic purposes. In Gen5, select **System > Diagnostics > Run System Test**. Having the system test report before calling the BioTek's Technical Assistance Center can speed the resolution of the error.

If an error message appears while an experiment is in process and after having received measurement data, it is your responsibility to determine if the data is valid.

Contact Info: BioTek Service/TAC

Use this appendix to diagnose problems and solve them if possible. If you need further assistance, record the following information and contact BioTek's Technical Assistance Center:

- Ensure the error code is repeatable.
- Note the conditions (i.e., what the instrument was doing, was a protocol running, etc.) when the error occurred.
- Write down the serial number of the instrument and run and print a self-test.

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

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

Fax: 802-654-0638

E-mail: tac@biotek.com

> ❖ For errors that are displayed during operation of the Synergy HTX with the 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, of 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 Probable Causes:
0200	**24VDC dropped below safe level.** • External power supply has failed. • Verify connection to AC mains. • Power supply connection to instrument is loose or broken. Contact BioTek TAC.
150x	**Temperature is out of range.** **External 24-volt power supply is low.** • x=1: Zone 1 • x=2: Zone 2 • x=3: Zones 1 and 2 • x=4: Zone 3 • x=5: Zones 1 and 3 • x=6: Zones 2 and 3 • x=7: Zones 1, 2, and 3 • x=8: Zone 4 • x=9: Zones 1 and 4 • x=A: Zones 2 and 4 • x=B: Zones 1, 2, and 4 • x=C: Zones 3 and 4 • x=D: Zones 1, 3, and 4 • x=E: Zones 2, 3, and 4 • x=F: Zones 1, 2, 3, and 4 Contact BioTek TAC.
152x	**One or more incubator zones are defective.** • x=1: Zone 1 • x=2: Zone 2 • x=3: Zones 1 and 2 • x=4: Zone 3 • x=5: Zones 1 and 3 • x=6: Zones 2 and 3 • x=7: Zones 1, 2, and 3 • x=8: Zone 4 • x=9: Zones 1 and 4 • x=A: Zones 2 and 4

Code	Description and Probable Causes:
	• x=B: Zones 1, 2, and 4 • x=C: Zones 3 and 4 • x=D: Zones 1, 3, and 4 • x=E: Zones 2, 3, and 4 • x=F: Zones 1, 2, 3, and 4 Turn the incubator on and wait at least 10 minutes for it to stabilize. Contact BioTek TAC.
2101	**Plate dimensions incorrect. Row count < 1 or > 99.** Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.
2102	**Plate dimensions incorrect. Column count < 1 or > 99.** Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.
2109	**Plate dimensions incorrect. Plate height > 28.575 mm.** Review plate type defined in protocol. Ensure counts or dimensions do not exceed limits. Contact BioTek TAC.
230x	**Plug not found in filter wheel.** x=2: excitation filter wheel x=3: emission filter wheel 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.
2313	**Empty hole not found in emission filter wheel.** Protocol contains an empty hole in the emission filter wheel, but it was not found. Verify that an empty hole is in the emission filter wheel and that this is accurately reflected in the filter table.
2326	**TRF cartridge not installed.** Protocol calls for TRF, but the TRF block is not installed in the excitation filter wheel slot. Verify that the TRF block is physically installed and that this is accurately reflected in the filter table.
2327	**Excitation filter wheel not installed.** Verify that the excitation filter wheel is physically installed.
240x	**Read area of the plate will not fit in the inside open area of the carrier.** x=3: First row position + Y offset < 2.54 mm or > 83.57 mm x=4: Low row position + Y offset < 2.54 mm or > 83.57 mm x=5: First column position + X offset < 4.57 mm or > 120.65 mm x=6: Last column position + X offset < 4.57 mm or > 120.65 mm

Code	Description and Probable Causes:
	x=7: Plate width < 84.15 mm or > 86.11 mm x=8: Plate length < 125.73 mm or > 128.40 mm 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.
2B0x	**Syringe failure.** x=1: Syringe failed to reach home sensor (optical sensor should be on). x=2: Syringe moved off home sensor, but sensor didn't changes state (optical sensor should be off). x=3: Syringe clean position too far from home sensor. x=4: Steps to clear sensor at runtime deviated from value saved when homing (verify error). Protocol definition is incorrect or reader is being controlled by incorrectly programmed third-party software. Syringe was not installed correctly or was not cleaned, preventing a move to home sensor. Contact BioTek TAC.
2B0A	**Plate not in carrier for system prime operation.** Place priming plate in carrier.
2C01 2C05 2C07	**Dispenser configuration incorrect.** **Volume calibration data is invalid.** **Volume calibration is needed to override defaults.** Verify that the dispenser calibration values have been loaded into Gen5. See the Installation chapter in the *Synergy HTX Operator's Manual*. Contact BioTek TAC.
2D09	**Tip prime volumes specified could overflow the tip priming trough.** Empty the tip priming trough. Contact BioTek TAC.
2D0A	**Tip prime trough or plate is full or may overflow.** Empty the tip priming plate.
2D15	**Invalid kinetic interval selected for plate mode/plate synchronous mode.** Enter a valid kinetic interval. Call BioTek TAC.
2D16	**Assay missed scheduled start of read (well synchronous mode).** Verify computer setup. Hibernate or sleep mode should not be enabled. Contact BioTek TAC.
2D22	**Invalid volume selected for tip prime.** Select a valid volume. Call BioTek TAC.
2D23	**Invalid volume selected for dispense.** Select a valid volume. Call BioTek TAC.

Code	Description and Probable Causes:
2D24	**Invalid rate selected for dispense.** Select a valid rate. Call BioTek TAC.
2D28	**Dispenser module not attached.** Verify the cable is connected. Call BioTek TAC.
2D2A	**Dispense not primed successfully.** Reinitialize the dispenser, then repeat the prime operation. Contact BioTek TAC.
2D46	**Invalid wavelength specified.** Wavelength must be between 230 and 999 nm.
3700 3710 3800 3810	**Absorbance reference channel failed noise test.** **Absorbance measurement channel failed noise test.** **Absorbance reference channel failed offset test.** **Absorbance measurement channel failed offset test.** Humidity is outside the environmental specification of instrument. Note the specification in the operator's manual and move to an area with lower humidity. Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
390Y 391Y	**Absorbance reference channel dark range outside of limits (measurement < 100 counts), where Y= readset.** **Absorbance measurement channel dark range outside of limits, where Y = readset.** Humidity is outside the environmental specification of instrument. Note the specification in the operator's manual and move to an area with lower humidity. Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
3900 3910	**Absorbance reference channel dark range outside of limits.** **Absorbance measurement channel dark range outside of limits.** Humidity is outside the environmental specification of instrument. Note the specification in the operator's manual and move to an area with lower humidity. Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.

Code	Description and Probable Causes:
3E0y	**Absorbance reference channel saturated during one of the following steps:** **Absorbance Blank Data Collection, y=readset #** **Absorbance Gain Calibration** **Absorbance Blank Data Collection** **Absorbance Spectral Scan** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spill in chamber or dirty absorbance optics.
3E1y	**Absorbance measurement channel saturated during one of the following steps:** **Absorbance Blank Data Collection, y=readset #** **Absorbance Gain Calibration** **Absorbance Blank Data Collection** **Absorbance Spectral Scan** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spill in chamber or dirty absorbance optics.
3F0y	**Absorbance reference signal out of range:** **Absorbance Read Process, y = readset#** **Absorbance Optics Test** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spill in chamber or dirty absorbance optics.
3F1y	**Absorbance measurement out of range:** **Absorbance Read Process, y = readset #** **Absorbance Optics Test** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spill in chamber or dirty absorbance optics.
3F00	**Absorbance reference correction value out of range:** **Absorbance Spectral Scan** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured. Check for spill in chamber or dirty absorbance optics.
4xxx	**PMT well overload** Sensitivity too high. Chemistry too concentrated. Verify that the physical filter configuration matches the Gen5 Filter Table.
4810	**PMT measurement offset test failure (offset is < 700 or > 2450 counts).** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.

Code	Description and Probable Causes:
4A0X	**PMT gain out of range, x = readset.** Too much light in read chamber. Ensure the instrument enclosure is completely installed and secured.
4B10 4B11 4B12 4B15 4B18	**PMT measurement value is too low.** **Failed high-voltage PMT test.** **Failed low-voltage PMT test.** **Failed well overload test for absorbance or fluorescence.** **Failed background overload test.** 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 not light leakage.
4E0X 4E11 4E12	**Flash-on reference value at full scale during the flash fluorescence read (x=readset #).** **PMT test failed at 750 volts.** **PMT test failed at 500 volts.** Too much light in chamber. Door not closed completely. Ensure that the Gen5 Filter Table accurately reflects the physical configuration of the excitation or emission filter wheels. Ensure that instrument case is completely installed and secured. Sensitivity set too high; try adjusting sensitivity setting.
4E18	**PMT saturation detected.** Too much light in chamber. Door not closed completely. Ensure the Gen5 Filter Table accurately reflects the physical configuration of the excitation and emission filter wheels. Ensure the instrument case is completely installed and secured. Sensitivity set too high. Try adjusting sensitivity 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 overlaps between excitation and emission positions 2 and 3.

Code	Description and Probable Causes:
4F0X	**Fluorescence signal out of range (too low).** Too much light in chamber. Door not closed completely. Ensure the Gen5 Filter Table accurately reflects the physical configuration of the excitation and emission filter wheels. Ensure the instrument case is completely installed and secured. Sensitivity set too high. Try adjusting sensitivity 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 overlaps between excitation and emission positions 2 and 3.
5000 5200	**Carrier x-axis failed to home.** Shipping screw may be installed. An object may be obstructing the path.
5001 5201	**Carrier y-axis failed to home.** Y-axis rails are dusty or rusty. Dirt in the roller bearings is causing them to jam. An object may be obstructing the path.
5002 5202	**Excitation filter wheel axis failed to home.** Filter wheel not inserted correctly. Filter wheel obstructed. Filter not clipped in. Gear teeth of filter wheel binding with gear teeth of the motor. Remove filter wheel, spin wheel by hand, and reinsert.
5003 5203	**Emission filter wheel axis failed to home.** Filter wheel not inserted correctly. Filter wheel obstructed. Filter not clipped in. Gear teeth of filter wheel binding with gear teeth of the motor. Remove filter wheel, spin wheel by hand, and reinsert.
5006 5206	**Probe z-axis failed to home.** Shipping bracket not removed.
5402 5403	**Excitation filter wheel failed positional verify.** **Emission filter wheel failed positional verify.** Ensure filter cartridge is fully inserted.
5700 5701	**Carrier x-axis obstructed.** **Carrier y-axis obstructed.** Axis may have hit probe z-axis. Tip priming trough is not correctly inserted.

Code	Description and Probable Causes:
5702 5703	**Excitation filter wheel obstructed.** **Emission filter wheel obstructed.** Ensure filter cartridge is fully inserted.
5706	**Probe z-axis obstructed.** 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 5709	**Dispenser syringe 1 obstructed.** **Dispenser syringe 2 obstructed.** Verify nothing is blocking the syringe drive.
5800 5801	**Carrier x-axis obstructed.** **Carrier y-axis obstructed.** Shipping screw still installed. An object may be obstructing the carrier's path.
5A00 5A01	**Carrier x-axis obstructed.** **Carrier y-axis obstructed.** Plate has hit something. Plate cover not accounted for when creating plate dimension file. Tip prime trough dislodged.
5B00	**Plate height violation.** Plate is inside chamber when it should be outside. • The read was aborted and "home all axes" not performed. • 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.