Agilent BioTek Synergy H1

Multi-Mode Microplate Reader

INSTRUCTIONS FOR USE

ERRATA NOTICE: This document contains references to BioTek. Please note that BioTek is now Agilent. For more information, go to www.agilent.com/lifesciences/biotek

Document part number 8041005I
Revision D
Agilent Technologies, Inc.
August 2022

Contents

Preface 4
Copyright 4
Contact Information 4
Intended Use Statement....5
Incident Reporting 5
Quality Control 5
Safety Notices 5
Warnings and Precautions....5
Symbols....8
Conformance to Standards....11
EMC Information and Technical Description 12
Ingress Protection Code....12
Disposal....12
Installation....13
Important Information 13
Package Contents 13
Models 14
Unpack and Inspect the Reader....15
Select an Appropriate Location 16
Remove the Shipping Hardware 16
Install the Power Supply 17
Install the Gas Controller (if applicable) 17
Install the Dispense Module (if applicable) 17
Connect the Host Computer 20
Install Gen5 Software 20
Turn on the Reader....20
Establish Communication 20
Run a System Test....21
Verify/Set Dispenser Calibration Values....21
Verify Performance....22
Repackaging and Shipping Instructions 23
Getting Started 24
External Components 24
Internal Components....25
Gen5 Software 27
Dispense Module Control 30
Maintenance 31
Warnings and Precautions....31
Recommended Maintenance Schedule....32
Clean Exposed Surfaces 32
Inspect/Clean Excitation and Emission Filters (F Models) 32
Maintenance for Models with Injectors (D Models)....33
Decontamination 36
Instrument Testing 40
Recommended Qualification Schedule 40
System Test....41
Absorbance Tests....41
Fluorescence Tests....42
Luminescence Test 43

Injection System Testing....47
Specifications....54
General Specifications....54
Absorbance Specifications....55
Fluorescence Specifications (Mono-Based)....57
Fluorescence Specifications (Filter-Based)....58
Luminescence Specifications....58
Dispense/Read Specifications....59

Preface

Copyright

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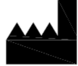
Contact Information



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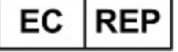

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Intended Use Statement

The Synergy H1 is a multi-mode microplate reader and intended to be used for the examination of clinical specimens to analyze their characteristics in relation to a variety of analytes including in human serum and cells.

Incident Reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority, or appropriate regulatory body, in the country or region in which the user is established.

Quality Control

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

Safety Notices

Pay special attention to the following safety notices in all product documentation.

WARNING A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

CAUTION A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

Warnings and Precautions

Electrical Hazards

WARNING **Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

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

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

WARNING **Service.** Only qualified technical personnel should perform service procedures on internal components.

CAUTION **Power Supply.** Use only the power supply shipped with the instrument and operate it within the range of line voltages listed on it.

Chemical/Environmental

WARNING

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

WARNING **Liquids.** Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, stop the program and turn off the instrument. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

CAUTION **Liquids.** Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

CAUTION **Environmental Conditions.** Do not expose the instrument to temperature extremes. For proper operation, temperature near the instrument should remain within the range in the ***Specifications*** section of this document. Performance may be adversely affected if temperatures fluctuate above or below this range.

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

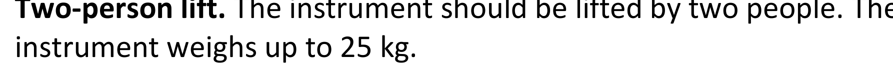
CAUTION **Lubricants.** Do not apply lubricants to moving parts. Lubricant on components in the carrier compartment will attract dust and other particles, which may cause the instrument to produce an error.

CAUTION **DMSO Concentration.** Dimethyl sulfoxide (DMSO) vapor can coat optical surfaces, which can trigger instrument self-test errors. Using DMSO assay concentrations of 2% or below is recommended. Limit long exposure in kinetic assays or incubated assays when possible. Agilent recommends increasing the frequency of Preventive Maintenance visits by a certified service technician to every six months and minimally every year when

running assays with DMSO, especially if the concentration is higher than 2%.

CAUTION Agilent BioTek instruments are designed for use in standard benchtop conditions. Operation in corrosive, caustic, or abrasive surroundings, like anaerobic chambers, can negatively affect performance and require increased service frequency, i.e., higher frequency of service than is covered by the instrument warranty.

Components



Two-person lift. The instrument should be lifted by two people. The instrument weighs up to 25 kg.

WARNING

Pinch Hazard. Some areas of the external dispense module can present pinch hazards when the instrument is operating. Keep hands and fingers clear of these areas when the instrument is operating.

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

CAUTION **Shipping Hardware.** All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

CAUTION **Filter Cube (F models).** The reader is delivered with a filter cube installed, and the reader's onboard software is configured with the filter and mirror values and their locations in that filter cube. When Gen5 communicates with the reader, it requests this information and stores the values in a Filter Cube table.

It is critical that the values in Gen5 and onboard the reader exactly match the contents of the installed filter cube. If you exchange the filter cube or modify its contents, you must update Gen5's Filter Cube table and send the new information to the reader.

The filter cube is accessed through a hinged door in the front of the instrument. Do not open the door to access the filter cube during instrument operation! Doing so may result in invalid data.

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

CAUTION **Service.** Only qualified technical personnel should perform service procedures on internal components.

Intended Product Use

WARNING **Software Quality Control.** The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct quality control checks could result in erroneous test data.

WARNING **Data Reduction.** No limits are applied to the raw measurement data. Data exported via computer control must be analyzed by the operator. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. Users must evaluate this instrument and PC-based software in conjunction with their specific assay(s). This evaluation must include the confirmation that performance characteristics for the specific assay(s) are met.

WARNING **Unspecified Use.** Failure to operate equipment according to the guidelines and safeguards specified in the product user documentation could result in a hazardous condition.

CAUTION Use of labware other than described in this document can result in positioning errors during program execution.

Symbols

	Caution
	Caution
	Warning; Biological hazard
	Warning; Pinch hazard
	Caution; carton exceeds 50lbs (22.5kg). When handling, two or more people are required.
	Disposal Notice: Dispose of the instrument according to Directive 2012/19/EU, "on waste electrical and electronic equipment (WEEE)" or local ordinances

Symbol	Description
	Consult instructions for use or consult electronic instructions for use
IVD	*In vitro* diagnostic medical device
CE	CE Marking — indicates compliance with the requirements of the In Vitro Diagnostic Regulation 2017/746
EC REP	Authorized representative in the European Community/European Union
	Manufacturer
	Date of manufacture
REF	Catalogue number
SN	Serial number
TÜV SÜD NRTL C US	TÜV SÜD – Type tested; production monitored
IP20	Ingress Protection - Product protected against solid objects up to 12 millimeters. Not protected from liquids.
40	This product complies with environmental protection use period as defined in People's Republic of China Electronic Industry Standard SJ/T11364-2006. Toxic or hazardous substances will not leak or mutate under normal operating conditions for 40 years.
UK CA	UK Conformity Assessed marking is a certification mark that indicates conformity with the applicable requirements for products sold within Great Britain.
	Temperature limit
%	Humidity limitation
UDI	Unique device identifier
#	Model number

	Importer

Conformance to Standards

The Synergy H1 meets the requirements of the following standards:

2014/35/EU – Low Voltage Directive

2014/30/EU – EMC Directive

2017/746 – In Vitro Diagnostic Regulation

2011/65/EU (with exemptions) and (EU) 2015/863 – RoHS Directives

2012/19/EU – WEEE Directive as amended by (EU) 2018/849

2006/42/EC of the European Parliament and of the Council of 17 May 2006 on machinery

Standard	Description
IEC QC 080000	IEC Quality Assessment System for Electronic Components (IECQ System) - Hazardous Substance Process Management (HSPM) System Requirements
UL 61010-1	UL Standard for Safety Electrical Equipment For Measurement, Control, and Laboratory Use; Part 1: General Requirements
EN 61010-1	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 1: General Requirements
EN 61010-2-010	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials
EN 61010-2-081	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
EN 61010-2-101	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment
CAN/CSA C22.2 No. 61010-1	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 1: General Requirements
CAN/CSA C22.2 No. 61010-2-010	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials

CAN/CSA C22.2 No. 61010-2-081	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
CAN/CSA C22.2 No. 61010-2-101	Safety Requirements for Electrical Equipment For Measurement, Control, and Laboratory Use – Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment

EMC Information and Technical Description

The Synergy H1 conforms to:

Emissions:

EN55011/CISPR 11, Class A
CFR Title 47 FCC Part 15 Subpart B, Class A
ICES-001, Issue 5, Class A (CAN ICES-001(A)/NMB-001(A))
ACMA AS/NZS CISPR 11, Class A

Immunity:

EN/IEC 61326-1 and 61326-2-6
ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL AND LABORATORY USE
PART 2-6: PARTICULAR REQUIREMENTS FOR (IVD) MEDICAL EQUIPMENT

Ingress Protection Code

IP 20. Protected against solid foreign objects of 12.5 mm diameter and greater. No protection against water.

Disposal

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

Installation

Important Information

Two-person lift. The instrument should be lifted by two people. The instrument weighs up to 25 kg.

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

- This chapter contains installation and setup tasks for the Synergy H1 and accessories. Perform the tasks in the order presented.
- Save all packaging materials. Be sure to use packaging materials supplied by the manufacturer when shipping the reader. Using other forms of commercially available packaging, or failing to follow the repackaging instructions, may void your warranty.
- During the unpacking process, inspect the packaging, reader, and accessories for shipping damage. If the reader is damaged, notify the carrier and your Agilent representative. Keep the shipping boxes and the packaging materials for the carrier's inspection.

Package Contents

- Synergy H1 instrument model per the sales order
- *Synergy H1 User Manual* on USB flash drive
- Power supply
- Power cord
- USB cable
- Optional accessories per the sales order, unless shipped separately

Models

Part Number	Absorbance	Filter Fluorescence and Filter Luminescence	Fixed BP Mono Fluorescence and Broadband Luminescence	Variable BP Mono Fluorescence and Broadband Luminescence	Dispense Ready	Gas Ready	Maximum Incubation Temperature
H1F-SI		√			√		45°C
H1FG-SI		√			√	√	45°C
H1M-SI	√		√		√		45°C
H1MF-SI	√	√	√		√		45°C
H1MFG-SI	√	√	√		√	√	45°C
H1MG-SI	√		√		√	√	45°C
SH1F-SI		√			√		45°C
SH1FG-SI		√			√	√	45°C
SH1M-SI	√		√		√		45°C
SH1MF-SI	√	√	√		√		45°C
SH1MFG-SI	√	√	√		√	√	45°C
SH1MG-SI	√		√		√	√	45°C
SH1M2-SI	√			√	√		70°C
SH1M2F-SI	√	√		√	√		70°C
SH1M2G-SI	√			√	√	√	70°C
SH1M2FG-SI	√	√		√	√	√	70°C

Unpack and Inspect the Reader

Two-person lift. The instrument should be lifted by two people. The instrument weighs up to 25 kg.

Save the packaging in case you need to ship the instrument for service/repair.

Cardboard panel

Foam corner (4)

Plastic bag

Accessories box

Instrument

Foam corner (4)

Shipping box

Select an Appropriate Location

Install the reader on a level, stable surface in an area where temperatures between 18°C and 40°C can be maintained. Leave at least 15 cm of space between the instrument's rear panel and any other object. This space ensures proper air flow in and out of the instrument.

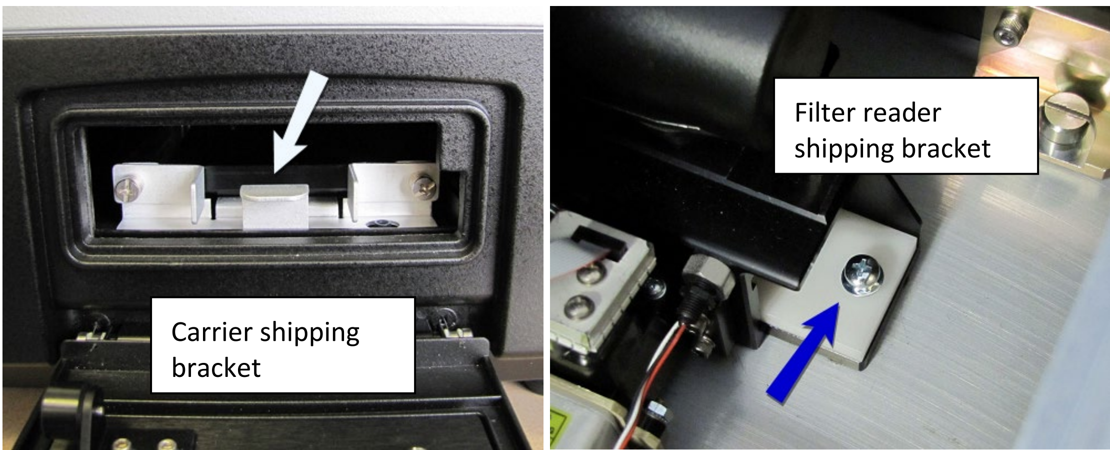
The reader is sensitive to extreme environmental conditions. Avoid the following:

- Excessive humidity. Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks. The humidity must be in the range of 10–85%, non-condensing.
- Excessive light. Bright light may affect the reader's optics and readings, reducing its linear range.
- Dust. Readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

Remove the Shipping Hardware

CAUTION **Shipping Hardware.** All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

1. Locate the shipping hardware, as shown in the photos below.
2. Pull down the microplate carrier access door. Using a screwdriver, remove the carrier shipping bracket.
3. If the instrument is equipped with the filter module: Open the top access door and use a screwdriver to remove the filter reader shipping bracket.
4. Store the shipping hardware with the original packaging for reuse in case you need to ship the instrument.



Install the Power Supply

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

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

CAUTION **Power Supply.** Use only the power supply shipped with the instrument and operate it within the range of line voltages listed on it.

1. If the instrument is on, turn it off.
2. Plug the power supply's cord into the power inlet on the rear of the reader. Connect the power cord to the power supply.
3. Plug the power cord into an appropriate power receptacle.

Install the Gas Controller (if applicable)

The gas controller is an external module to control CO_2 and O_2 concentrations inside the reading chamber. Refer to the *Gas Controller User Manual* for installation instructions.

Install the Dispense Module (if applicable)

Applies only to models equipped with injectors.

Place the dispense module on top of the reader (or on top of the gas controller, if equipped). Do not place the dispense module next to the reader.

1. Open the bag containing the injector tube and tips. Remove the clear plastic shrouds from the tubes.
2. Remove the two inlet tubes from their plastic canisters.
3. Identify the two syringe valves on the dispense module. Each is labeled with a left-pointing arrow.

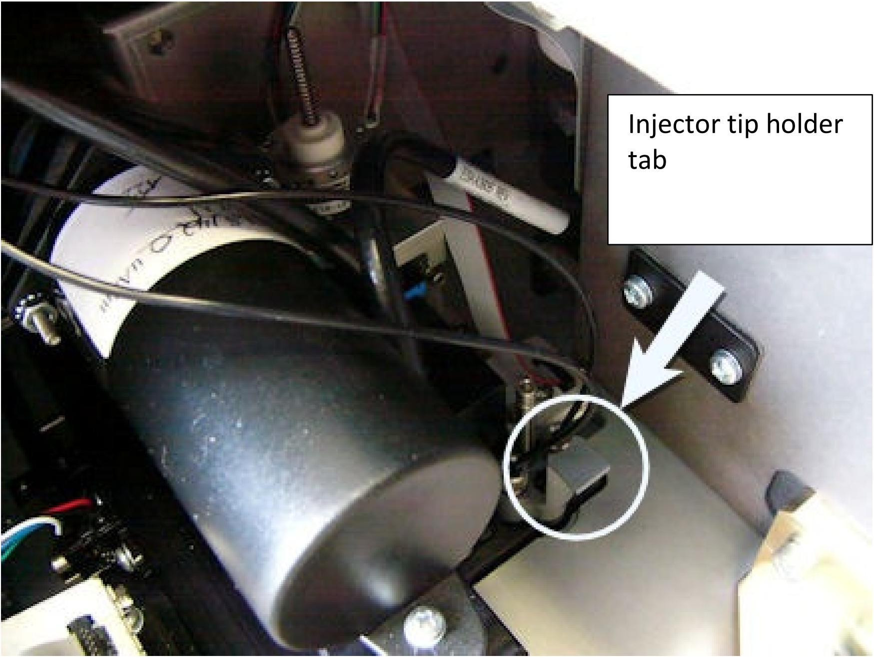
When installing the tubes, do not use any tools. Finger-tighten only.

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

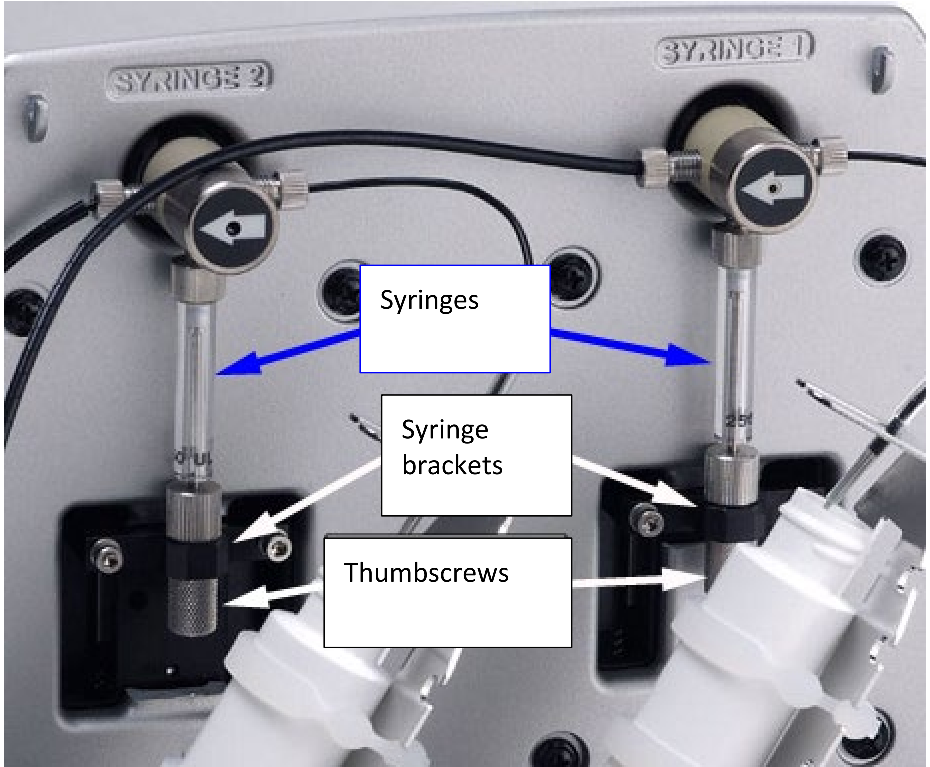
Be sure to install the tubing into the correct ports. Otherwise, injected fluid may miss the intended well.

7. Remove the tubing feed-through cover from the top of the reader (2 screws). Store the cover and screws with the shipping hardware in case the reader needs to be shipped again.
8. Thread the injector tip holder, with outlet tubing connected to both ports, through the hole in the top of the reader.
9. Open the reader's top door, and, holding the injector tip holder by the tab, insert the injector tips into the appropriate holes inside the reader.

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



10. Place the tubing feed-through cover over the hole in the top of the reader, and finger-tighten the thumbscrews to secure it.
11. Remove the two syringes from their protective boxes. They are identical and interchangeable.
12. Install the syringes:
    - Hold the syringe vertically with the threaded end at the top.
    - Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten.
    - Pull down the bottom of the syringe until it rests inside the hole in the bracket.
    - Pass a thumbscrew up through this hole and thread it into the bottom of the syringe. Hold the syringe to prevent it from rotating while tightening the thumbscrew. Finger-tighten.



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

Connect the Host Computer

The Synergy H1 is equipped with a USB communication port, located on the back of the reader. Connect one end of the supplied communication cable to the USB port on the reader and the other end to an appropriate port on the host computer.

Install Gen5 Software

The Synergy H1 is controlled by Gen5 software running on a host computer. Follow instructions supplied with Gen5 to install the necessary software.

- Ensure the computer meets the minimum system requirements as described in the *Gen5 Instructions for Use*.
- You must have administrator privileges to install Gen5. Log in to Windows as “Administrator” or consult your IT department for assistance.

Turn on the Reader

1. If Gen5 is open, close it now.
2. The power switch is located on the lower-left corner of the front panel; turn on the Synergy H1. The reader performs a System Test. When the test is completed, the reader extends the microplate carrier. If the test fails, contact Technical Support.

Establish Communication

1. Start Gen5 and log in if prompted.
2. From the main screen, select **System > Instrument Configuration**.
3. Click **Add Reader** and select **Synergy H1**. Click **OK**.
4. Perform one of the following steps, as applicable:
    - Select **Plug & Play**. (A reader must be connected to the computer and turned on to appear in the Available Plug & Play Readers list.)
    - Select **Com Port** and select the computer’s COM port to which the reader is connected. (If using the USB cable, the information can be found via Windows Control Panel, under Ports in the Hardware/Device Manager area of System Properties.)
5. Click **Test Comm**. Gen5 attempts to communicate with the reader. If the communication attempt is successful, return to Gen5’s main screen.

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

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

- Try a different COM port.
- If using the USB cable, did you install the driver software?

If you remain unable to get Gen5 and the reader to communicate with each other, contact Technical Support.

Run a System Test

1. Turn on the incubator:
   - In Gen5, select **System > Instrument Control > Synergy H1**.
   - Click the **Pre-Heating** tab. Enter a Requested temperature of at least 37°C and then click **On**.
   - Wait until the incubator reaches the set point before continuing.
2. Select **System > Diagnostics > Run System Test**. Select your reader if prompted and click **OK**.
3. When the test is complete, a dialog requesting additional information appears. Enter the information (if required) and click **OK**.
4. The results report appears. The text should show "SYSTEM TEST PASS." If it shows "SYSTEM TEST FAILS" contact Technical Support.
5. Turn off the incubator.

Verify/Set Dispenser Calibration Values

Applies only to models equipped with injectors.

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

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

1. If you have not already done so, turn on the instrument and establish communication with Gen5.
2. In Gen5, go to **System > Instrument Configuration**, select the **Synergy H1**, and click **View/Modify**.
3. Click **Setup** and select the Dispenser 1 tab.
4. Click **Get Volumes.**
5. Compare the Calibration Volumes in the dialog with the Syringe #1 values on the rear panel of the dispense module.

   If the values match, skip to step 6.

   If there is a mismatch:

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

6. Select the **Dispenser 2** tab and repeat steps 4–5 for Dispenser 2.

Verify Performance

As applicable for your Synergy H1 model, refer to the *Instrument Testing* section for test procedures for:

- Absorbance Tests
- Fluorescence Tests
- Luminescence Test
- Injection System Tests

Repackaging and Shipping Instructions

Two-person lift. The instrument should be lifted by two people. The instrument weighs up to 25 kg.

Shipping Hardware. All shipping hardware must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

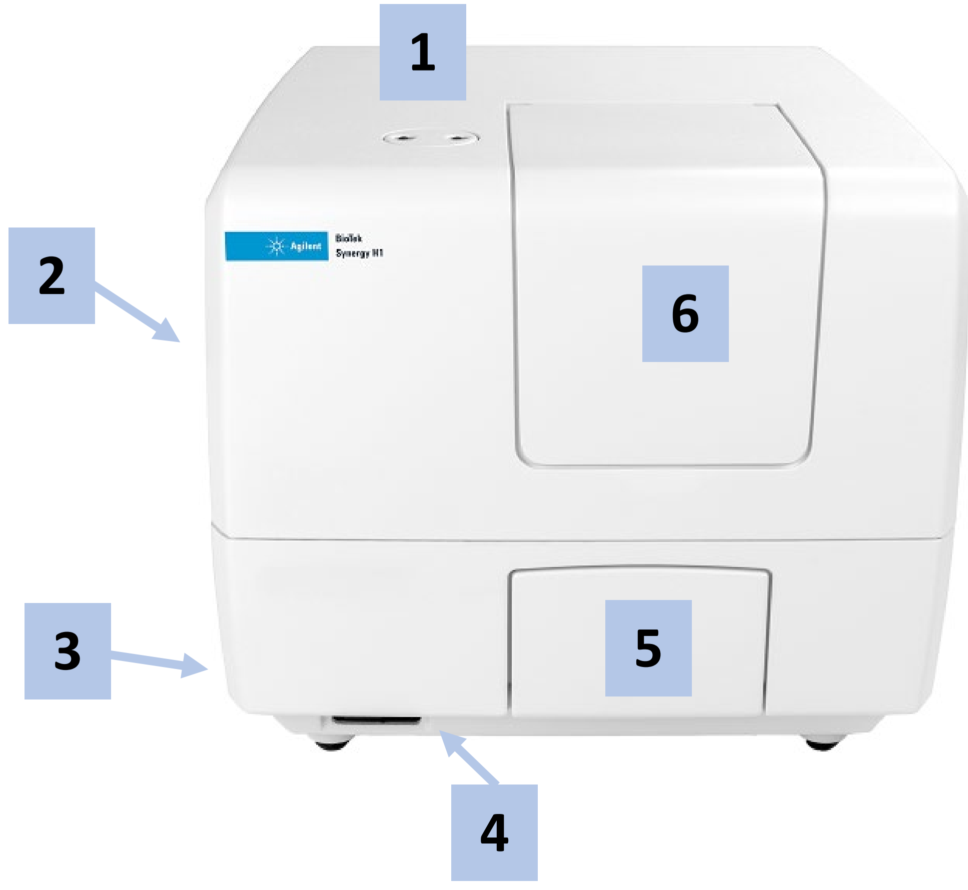
Please read the information provided below before preparing the Synergy H1 for shipment.

- Contact Technical Support before returning equipment for service.
- Decontamination prior to shipment is required by the U.S. Department of Transportation regulations.
- If the reader has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the instrument during shipping, handling, and servicing. The Maintenance chapter contains decontamination instructions.
- Ensure the microplate carrier is empty. Spilled fluids can contaminate the optics and damage the instrument.
- Install the shipping hardware.
- The instrument’s packaging design is subject to change. If the instructions in this document do not apply to the packaging materials you are using, contact Technical Support for guidance.
- Be sure to use packaging materials supplied by the manufacturer. Other forms of commercially available packaging are not recommended and can void the warranty.
- If the packaging materials have been damaged or lost, or if the same set has been used more than four times, order replacement materials.

1. Decontaminate the reader and, if attached, the dispense module. Disconnect the dispense module. If applicable, remove the tip priming trough from the microplate carrier.
2. Retract the microplate carrier. Turn off and unplug the reader.
3. Install the carrier shipping bracket and, if applicable, the filter reader shipping bracket. See page 16.
4. Place the accessories in the accessories box and then seal the box with tape.
5. Place the instrument in a plastic bag. Place the instrument in the shipping box with foam corners.
6. Place the accessories box in the shipping box. Seal the box with tape.

Getting Started

External Components



1	Entry for the dispense outlet tubes and injectors (if equipped).
2	The reader's back panel contains the communication and dispense module ports and the input for the power supply.
3	The tubing for the gas controller module is in the bottom tray of the reader (if equipped).
4	Power switch and microplate carrier eject button.
5	Light-blocking microplate carrier access door.
6	Access door for the filter cube and internal components.

Internal Components

Filter Cube (F models)

CAUTION

Filter Cube (F models). The reader is delivered with a filter cube installed, and the reader's onboard software is configured with the filter and mirror values and their locations in that filter cube. When Gen5 communicates with the reader, it requests this information and stores the values in a Filter Cube table.

It is critical that the values in Gen5 and onboard the reader exactly match the contents of the installed filter cube. If you exchange the filter cube or modify its contents, you must update Gen5's Filter Cube table and send the new information to the reader.

The filter cube is accessed through a hinged door in the front of the instrument. Do not open the door to access the filter cube during instrument operation! Doing so may result in invalid data.

Synergy H1 "F" models are equipped with a filter cube that contains excitation and emission filters, mirrors, and, if required, polarizing filters.

Excitation and emission filters are used for obtaining fluorescence and luminescence measurements. The excitation filter selects the band of light to which the sample will be exposed. The emission filter selects the band of light with the maximum fluorescence signal of the sample, to be measured by the photomultiplier tube (PMT).

For filter-based, top-reading fluorescence analysis, the Synergy H1 uses mirrors to direct the excitation and emission light paths. Mirrors are required for fluorescence polarization (FP) measurements to direct light to the sample, because fibers cannot carry polarized light. Mirrors also provide increased gain/sensitivity for fluorescence intensity (FI) and time-resolved fluorescence (TRF) measurements. The filter cube stores up to two mirrors and there are two possible mirror types:

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

Filters and mirrors are stored in a filter cube. If you run different types of fluorescence or luminescence assays, you can replace the entire filter cube with a different one; this is the recommended option. Alternatively, you can install different filters or mirrors in the cube.

Injection System (D models)

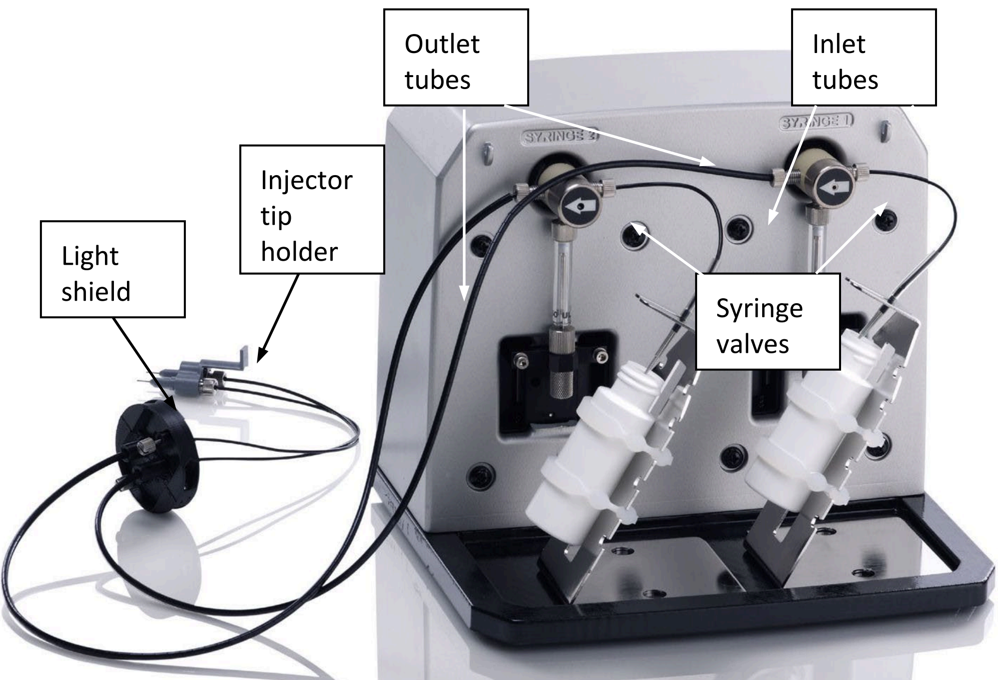
The tubing and injectors should be cleaned at least every three months.

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

If a syringe is leaking, it may need to be replaced.

Dispense Module

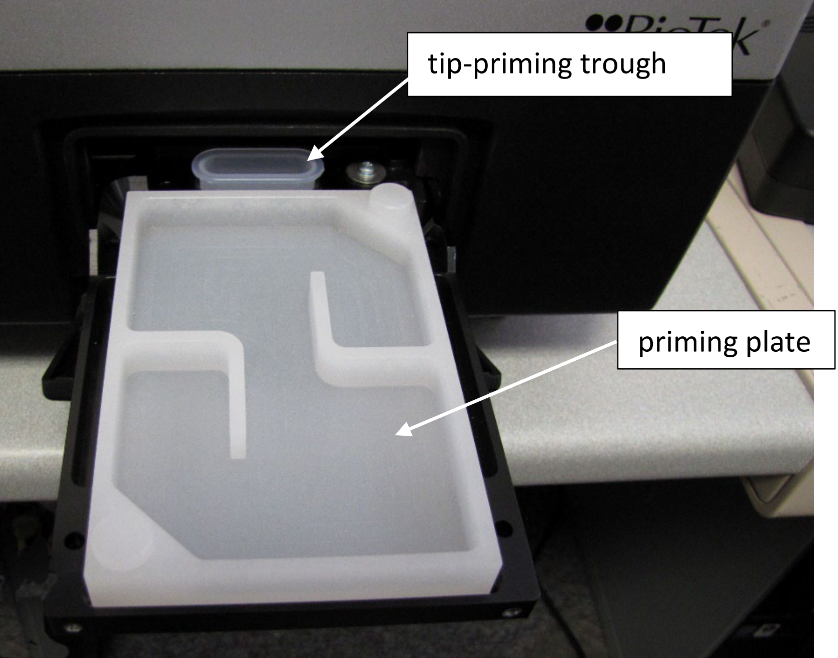
The dispense module sits on top of the reader and pumps fluid from the reagent bottles to injectors located inside the instrument. Fluid is injected into one well at a time. The injectors support plate types from 6- to 384-well plates.



Priming the Injection System

Before running a Dispense assay, prime the system with the reagent or dispensing fluid. In addition, tip priming can be performed at the start of the assay and, sometimes, just before each dispense to a well. The tip prime compensates for any fluid loss at the injector tip due to evaporation since the last dispense. All priming activities are controlled via Gen5.

Both types of primes require the priming plate and tip-priming trough installed in the carrier.



- Do not perform tip priming when using tall plates. Generally, plates with fewer than 96 wells are too tall for error-free tip priming; and, tip priming is rarely required for these larger-volume plates.
- The priming plate should be empty before priming, and it should contain fluid after priming.
- If the injection system is not adequately primed, air bubbles can get trapped in the system and affect injection volumes. Air bubbles in the system can also result in fluid spraying or scattering inside the reader.
- Avoid continuous contact with harsh chemicals. Rinse the fluid path with deionized water after contact with any strong acid, base, or solvent.
- For information on the materials used in the injection system, refer to Injection System - Chemical Compatibility Technical Note on the USB flash drive supplied with the Synergy H1.

Gen5 Software

Gen5 supports all Synergy H1 reader models. Use Gen5 to control the reader, the dispense module (if equipped), and BioStack (if equipped); perform data reduction and analysis on the measurement values; print/export results; and more. This section provides brief instructions for working with Gen5 go create protocols and experiments and read plates. Refer to the *Gen5 Instructions for Use* and the Gen5 Help system for more information.

Define Filter Cubes in Gen5 and on the Reader

CAUTION

Filter Cube (F models). The reader is delivered with a filter cube installed, and the reader's onboard software is configured with the filter and mirror values and their locations in that filter cube. When Gen5 communicates with the reader, it requests this information and stores the values in a Filter Cube table.

It is critical that the values in Gen5 and onboard the reader exactly match the contents of the installed filter cube. If you exchange the filter cube or modify its contents, you must update Gen5's Filter Cube table and send the new information to the reader.

The filter cube is accessed through a hinged door in the front of the instrument. Do not open the door to access the filter cube during instrument operation! Doing so may result in invalid data.

Some Synergy H1 models are delivered with filter cubes installed, and the reader's onboard software is configured with the filter and mirror values and their locations in that filter cube. When Gen5 communicates with the reader, it "asks" for this information and then stores the values in a Filter Cube table.

If you exchange or modify the filter cubes, you must update the Gen5 Filter Cube table and send the information to the reader.

1. From the Gen5 main view, select **System > Instrument Configuration**. Highlight **Synergy H1**, click **View/Modify**, and then click **Setup**.
2. If this is a new filter cube, enter a unique name to identify the cube and then enter a name for Filter Set 1.
3. If applicable, check the **Fluorescence Polarization Cube** box.
4. Define/modify settings for the excitation and emission filters:
   - Select **Band Pass**, **Long Pass**, or **Short Pass**, as appropriate for each filter type.

Band Pass	A standard interference filter with a defined central wavelength and bandwidth
Long Pass	Cutoff filter that transmits longer wavelengths and blocks shorter wavelengths
Short Pass	Cutoff filter that transmits shorter wavelengths and blocks longer wavelengths

   - Select **PLUG** to indicate the presence of a plug.
   - Select **HOLE** to indicate an empty location.
5. Select the mirror type and enter the excitation and emission ranges. (Note the "M" value on the filter cube label is the cutoff (nm).)

If **Fluorescence Polarization Cube** is checked, only Filter Set 1 is required for definition. The filters and mirrors of Filter Set 2 must be identical to those for Filter Set 1 for FP.

6. Define Filter Set 2, if necessary.
7. Click **Send Values** to transfer the information to the reader.
8. When finished, click **Close**.

Creating Protocols and Experiments

In Gen5, a protocol contains instructions for controlling the reader and (optionally) 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.

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

1. In the Gen5 Task Manager, select the **Protocols** icon and click **Create New**.
2. Open the Procedure dialog (double-click **Procedure** in the menu tree).
3. Select an appropriate Plate Type.
4. Add steps to shake or heating the plate, dispense fluid, read the plate, and more.
5. Click **Validate** to verify that the attached reader supports the defined steps, and then click **OK**.
6. Optionally, perform the next steps to analyze and report the results:
   - Open the Plate Layout dialog and assign blanks, samples, controls, and/or standards to the plate.
   - Open the Data Reduction dialog to add data reduction steps. Categories include Transformation, Well Analysis, Curve Analysis, and more.
   - Create a report or export template, via one of the Report/Export Builder options.
7. Select **File > Save** and give the protocol an identifying name.

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

1. In the Gen5 Task Manager, select the **Experiments** icon and click **Create using an existing protocol**.
2. Select the desired protocol and click **OK**.
3. Select a plate in the menu tree and select **Plate > Read Plate #** or click the **Read New** icon.
4. When the read is complete, measurement values appear in Gen5.
5. Select **File > Save** and give the experiment an identifying name.

Dispense Module Control

Applies only to models equipped with injectors.

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

Prime

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

1. Place the priming plate on the carrier.
2. Fill the supply bottle with a sufficient volume of the fluid to be used for the prime and the assay. Insert the appropriate inlet tube into the bottle.
3. Select **System > Instrument Control > Synergy H1** and click the **Prime** tab.
4. Select the Dispenser number (1 or 2) associated with the supply bottle.
5. Enter the Volume to be used for the prime. The minimum recommended prime volume is 2000 µL.
6. Select a prime Rate, in µL/second.
7. Click **Prime** to start the process. When finished, carefully remove the priming plate from the carrier and empty it.

If the priming plate is empty, the prime volume was too low.

Purge

To conserve reagent, Gen5 provides the option to purge fluid from the system back into the supply bottle.

1. Select **System > Instrument Control > Synergy H1** and click the **Prime** tab.
2. Select the Dispenser number (1 or 2) associated with the supply bottle.
3. Enter the desired purge Volume in µL (e.g., 2000).
4. Select a prime Rate in µL/second.
5. Click **Purge** to start the process.

Maintenance

Warnings and Precautions

WARNING **Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

WARNING **Liquids.** Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, stop the program and turn off the instrument. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

CAUTION **Liquids.** Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

CAUTION **Lubricants.** Do not apply lubricants to moving parts. Lubricant on components in the carrier compartment will attract dust and other particles, which may cause the instrument to produce an error.

CAUTION **DMSO Concentration.** Dimethyl sulfoxide (DMSO) vapor can coat optical surfaces, which can trigger instrument self-test errors. Using DMSO assay concentrations of 2% or below is recommended. Limit long exposure in kinetic assays or incubated assays when possible. Agilent recommends increasing the frequency of Preventive Maintenance visits by a certified service technician to every six months and minimally every year when running assays with DMSO, especially if the concentration is higher than 2%.

WARNING **Potential Biohazards.** 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.

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.

Recommended Maintenance Schedule

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

Clean Exposed Surfaces

Exposed surfaces may be cleaned (not decontaminated) with a cloth moistened (not soaked) with water or water and a mild detergent, and then water.

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, contact Technical Support.

Inspect/Clean Excitation and Emission Filters (F Models)

Agilent recommends inspecting the filters for dust and other debris every three months. You will need:

- Isopropyl, ethyl, or methyl alcohol
- 100% pure cotton balls or high-quality lens-cleaning tissue
- Cloth gloves
- Magnifying glass

Do not touch the filters with your bare fingers.

1. Inspect the glass filters for speckled surfaces or a “halo” effect. If you have any concerns, contact Technical Support.

2. Using cotton balls or lens-cleaning tissue moistened with a small amount of high-quality alcohol, clean each filter by lightly stroking its surface in one direction. Ensure that the filters remain in their current locations.
3. Use a magnifying glass to inspect the surface; remove any loose threads left by the cotton ball.

Maintenance for Models with Injectors (D Models)

Flush/Purge the Fluid Path

Applies only to models with injectors.

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

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

1. Fill two supply bottles with deionized or distilled water. Insert the supply (inlet) tubes into the bottles.
2. Place the priming plate on the carrier.
3. Select **System > Instrument Control > Synergy H1**.
4. Click the **Prime** tab and select Dispenser 1.
5. Set the Volume to 5000 µL. Keep the default prime rate.
6. Click **Prime** to start the process. When the process is complete, carefully remove the priming plate from the carrier and empty it.
7. Repeat the process for Dispenser 2.

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

To purge the fluid from the system:

1. Place the inlet tubes in empty supply bottles or a beaker.
2. Select **System > Instrument Control > Synergy H1**.
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.

Empty/Clean the Tip Priming 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.

Clean the Priming Plate

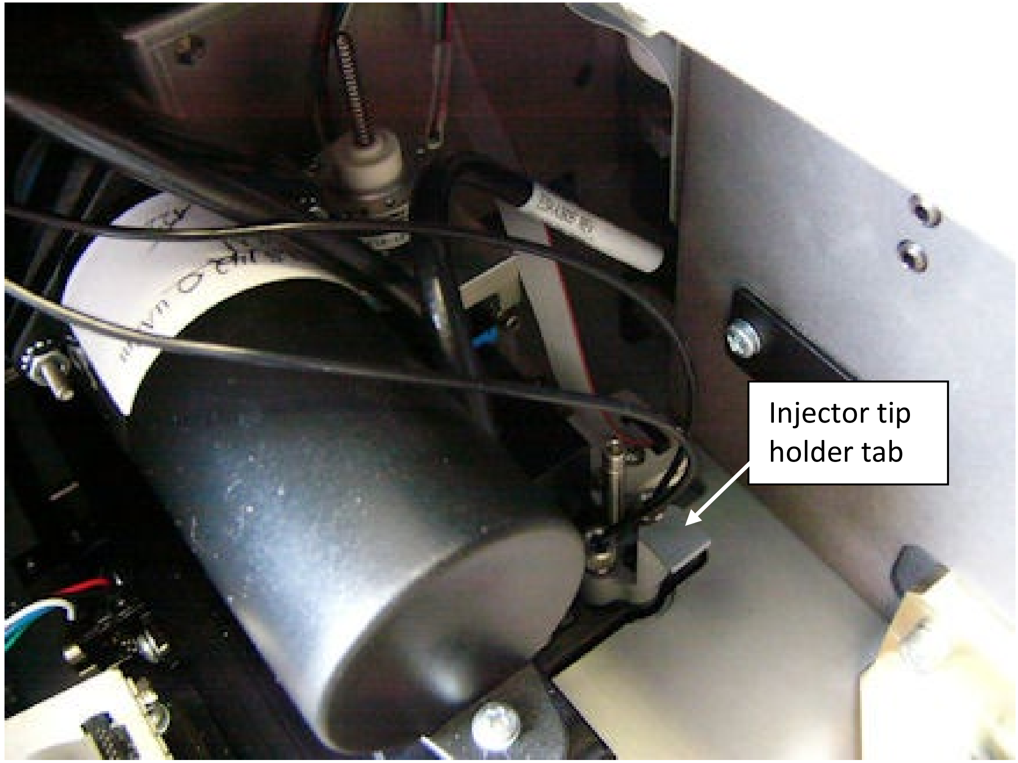
Clean the priming plate regularly to prevent bacteria growth and residue buildup. Wash the plate in hot soapy water, using a small brush to clean in the corners if necessary. Rinse thoroughly and allow it to dry completely.

Clean the Dispense Tubes and Injectors

Required Materials

- Protective gloves
- Safety glasses
- Mild detergent
- Clean, lint-free cotton cloths
- Deionized or distilled water
- Stylus (part number 2872304)

Remove the Dispense Tubes and Injector Holders



1. Open the door on the front of the reader.
2. Grasp the injector tip holder by the tab and pull it up out of its socket.
3. Using your fingers, remove the thumbscrews securing the light shield to the top of the reader and slide the shield up the outlet tubes/
4. Slide the injector tip holder through the hole in the top of the reader.
5. Turn each tube's thumbscrew counterclockwise and gently pull each tube from its injector tip.
6. On the dispense module, turn each outlet tube's thumbscrew counterclockwise to disconnect it from the injector.

Clean the Dispense Tubes and Injectors

1. Some reagents can crystallize and clog the tubing and injectors. Daily flushing and purging can help to prevent this, but more rigorous cleaning may be necessary if reagent has dried in the tubing or injectors.
2. To clean the dispense tubes, soak them in hot, soapy water to soften and dissolve any hardened particles. Flush each tube by holding it vertically under a stream of water.
3. To clean the injectors:
   - Gently insert the stylus into each injector tip to clear any blockages.
   - Stream water through the pipe to be sure it is clean. If the water does not stream out, soak in hot soapy water and then reinsert the stylus.

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

Decontamination

WARNING **Internal Voltage.** Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument.

CAUTION **Liquids.** Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact Technical Support.

- The Synergy H1 requires decontamination prior to shipping, storage, and disposal.
- 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.
- Agilent 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 Agilent 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.

Required Materials

- Sodium hypochlorite ($NaClO$)
- 70% isopropyl alcohol (as an alternative to sodium hypochlorite)
- 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 All Models

1. Turn off and unplug the reader.
2. Prepare a disinfecting aqueous solution of 0.5% sodium hypochlorite. If the effects of sodium hypochlorite are a concern, 70% isopropyl alcohol may be used.
3. Moisten a clean, lint-free cloth with the disinfecting solution, then thoroughly wring it out so that liquid does not drip from it. Do not soak the cloth.
4. Open the door and slide out the plate carrier. Wipe the plate carrier and all exposed surfaces of the instrument.
5. Wait 20 minutes.
6. Moisten a cloth with deionized or distilled water and wipe all surfaces of the instrument that have been cleaned with the disinfecting solution.
7. Use a clean, dry lint-free cloth to wipe all wet surfaces.
8. Discard the used gloves and cloths, using a biohazard trash bag and an approved biohazard container.

Procedure for Models with the Dispense Module

If disinfecting with sodium hypochlorite, be sure to flush repeatedly with deionized water to ensure that no sodium hypochlorite is carried over. After disinfecting with sodium hypochlorite, perform the rinse procedure provided on page 38.

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.

Decontaminate the Fluid Lines

1. Place a beaker with 20 mL of an aqueous solution of 0.5% sodium hypochlorite solution or 70% isopropyl alcohol near SYRINGE 1 on the dispense module.
2. Place the SYRINGE 1 inlet tube in the beaker.
3. If you have not already done so, detach the dispense module's outlet tubes from the instrument. Place the ends of the outlet tubes in an empty beaker and set the beaker 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 10,000 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 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 Tubing and Injectors

Perform the procedures described earlier to access, remove, and clean the internal tubing and injectors. When finished, replace the internal components and the reader’s shroud.

Decontaminate 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 an aqueous solution of 0.5% sodium hypochlorite or 70% isopropyl alcohol for 20 to 30 minutes.
   - If decontaminating in sodium hypochlorite solution, remove the trough and plate, and thoroughly rinse with deionized water.
   - If decontaminating with alcohol, remove the trough and plate and let them air-dry.
4. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Instrument Testing

Recommended Qualification Schedule

The following schedule is recommended for a Synergy H1 used two to five times per week:

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

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

** Perform the FI tests (and FP and TRF if applicable for your reader model) using a Fluorescence Test Plate (PN 1400501) or the Fluorescence Liquid Test procedures described in this chapter.

System Test

Each time the Synergy H1 is turned on, it performs a series of tests.

If any test fails, the reader beeps repeatedly and the LED on the power switch flashes. If this occurs, press the carrier eject button to stop the beeping. If necessary, start another system test to try to retrieve an error code from the reader.

1. Turn on the reader and launch Gen5.
2. If necessary, set Gen5's wavelength table to the six wavelengths you most frequently use.
3. If your assays use incubation, enable temperature control and allow the incubator to reach its set point before running the system test. To access this feature, select **System > Instrument Control > Synergy H1** and click the **Pre-Heating** tab.
4. Select **System > Diagnostics > Run System Test**.
5. When the test is complete, a dialog will appear, requesting additional information. Enter the information and then click **OK**.
6. The test report will appear; it should show "SYSTEM TEST PASS". If it shows "SYSTEM TEST FAIL" contact Technical Support.
7. Turn off the incubator.

Absorbance Tests

Absorbance Test Plate Part Number 7260522 uses NIST-traceable neutral density filters to confirm absorbance specifications in the visible range (400–800 nm). This test plate also contains precision-machined holes to verify mechanical alignment.

Absorbance Test Plate Part Number 7260551 uses NIST-traceable neutral density filters to confirm absorbance specifications in the UV range (340 nm).

Define the Absorbance Test Plate Parameters

Before the Absorbance Plate Test can be performed, the wavelength settings and the calibration data for each wavelength selected must be initially entered into Gen5. Use the data sheet included with the Absorbance Test Plate for the following:

- **S**elect **System > Diagnostics > Test Plates > Add/Modify Plates**, then click **Add**. Click **Help** for guidance when setting the wavelengths and entering the OD and peak wavelength values.

Run the Absorbance Plate Test

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

If any result shows “FAIL”, try the following and rerun the test. If the test continues to fail, contact Technical Support.

- Make sure the information entered into Gen5 matches the Test Plate’s Certificate.
- Verify that the Test Plate is within its calibration certification period. If it is out of date, contact Agilent to schedule a recertification.
- Ensure that the Test Plate is correctly seated in the microplate carrier.
- Check the alignment (corner) holes on the Test Plate to ensure they are clear of debris.
- Check the filters on the Test Plate to ensure they are clean. If necessary, clean them with lens paper. Do not remove the filters from the test plate, and do not use alcohol or other cleaning agents.

Fluorescence Tests

For models with fluorescence capability, a solid-state Fluorescence Test Plate (Part Number 1400006) is used to test the fluorescence system.

The Fluorescence Test Plate simplifies the process of conducting fluorescence intensity, fluorescence polarization, and time-resolved fluorescent tests. The test plate is solid and immune to the pipetting errors, evaporation issues, and costs experienced with conventional liquid tests.

The test plate package includes Synergy H1-specific Gen5 protocols designed for use with the test plate. The protocols include embedded Microsoft Excel spreadsheets to automatically calculate results and determine pass/fail.

The package also contains a user manual that describes the test methods, helps you get started with using the plate, and provides information for cleaning and maintaining the

test plate. The manual also provides troubleshooting tips and information on the annual recalibration program.

Results Analysis

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

Corners Test	%CV < 3.0
Linearity Test	R2 >= 0.9500
Sensitivity Tests:	
Filters, Top, SF	Detection Limit <= 10 pM
Monochromators, Top, SF	Detection Limit <= 20 pM
Monochromators, Bottom, SF	Detection Limit <= 20 pM
Filters, Top, MUB	Detection Limit <= 160 pg/mL
Monochromators, Top, MUB	Detection Limit <= 160 pg/mL
Monochromators, Bottom, MUB	Detection Limit <= 160 pg/mL
Time-Resolved Fluorescence (TRF) Test	Detection Limit <= 250 fM
Fluorescence Polarization (FP) Test	Mean PHPR > 340 mP, STD PLPR < 5 mP

Luminescence Test

For models with luminescence capability, a Harta Luminometer Reference Microplate is used to test the luminescence system. The test plate is LED-based and NIST-traceable.

Materials

- Harta Luminometer Reference Microplate, Part Number 8030015 (includes adapter Part Number 8042263 for this reader)
- Gen5 protocol(s) described on page 44.

Procedure

1. Turn on the Harta reference plate using the I/O switch on the back of the plate.
2. Check the plate's battery by pressing the test button on the back of the plate and ensuring that the test light turns on. If the light does not turn on, replace the battery.
3. Place the adapter on the reader's microplate carrier, and then place the Harta reference plate on top of the adapter.

4. In Gen5, create an experiment based on the Synergy H1 F-LumTest_Harta.prt (for filter-based luminescence) or Synergy H1 M-LumTest_Harta.prt (for monochromator-based luminescence) protocol, and read the plate.
5. Calculate and evaluate results as described under *Results Analysis* below.
6. When finished, turn off the Harta reference plate to preserve battery life.

Results Analysis

1. Determine if the plate's battery is still functioning properly. If A8 > (0.2 * A7), the battery is good. Otherwise, it requires replacement.
2. On the Harta plate's calibration certificate, locate the NIST measurement for the A2 position and convert to attomoles: (A2 NIST measurement*0.02884)
3. Calculate the signal-to-noise ratio:
   (A2–Mean of the buffer cells)/(3 * Standard deviation of buffer cells)
4. Calculate the detection limit:
   A2 NIST measurement in attomoles/signal-to-noise ratio
   - If the reader is equipped with the low-noise PMT, the detection limit must be **<= 75 amol** to pass.
   - If the reader is equipped with the red-shifted PMT, the detection limit must be **<= 500 amol** to pass.

Gen5 Protocol Reading Parameters

Synergy H1 F-LumTest_Harta.prt

Parameter	Setting
Plate Type:	If present, "8030015 – w/o 8032028 adapter"; otherwise "Costar 96 black opaque"
Delay Step:	3 minutes
Read Step 1:	
Detection Method:	Luminescence
Read Type:	Endpoint
Optics Type:	Filters
Step Label:	"Reference well A2"
Read Well:	A2
Filter Sets:	1
Excitation:	Plug
Emission:	Hole
Gain:	135
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard

Read Height:	7.00 mm
Read Step 2:	
Detection Method:	Luminescence
Read Type:	Endpoint
Optics Type:	Filters
Step Label:	“Background”
Read Wells:	D1–G4
Filter Sets:	1
Excitation:	Plug
Emission:	Hole
Gain:	135
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Standard
Read Height:	7.00 mm
Read Step 3:	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	“Battery Check”
Read Wells:	A7–A8
Filter Sets:	1
Excitation:	Plug
Emission:	Hole
Gain/Sensitivity:	60
Integration Time:	0:01.00 MM:SS.ss
Delay After Plate Movement:	350 msec
Dynamic Range:	Extended
Read Height:	10.00 mm

Synergy H1 M-LumTest_Harta.prt

The same as the filter-based test, with these exceptions:

Parameter	Default Setting
Read Step 1	
Optics Type:	Luminescence Fiber
Filter Sets:	<none>
Gain:	150
Read Height:	1.00 mm
Read Step 2	
Optics Type:	Luminescence Fiber
Read Wells:	F1–G12

Filter Set:	<none>
Gain:	150
Read Height:	1.00 mm
Read Step 3	
Optics Type:	Luminescence Fiber
Filter Set:	<none>
Gain:	80
Read Height:	1.00 mm

Injection System Testing

For models equipped with injectors and an external dispense module, the tests describe below assure that the injection system performs to specification.

Test Method

The Accuracy Test is a measure of the mean volume per well for multiple dispenses. The actual weight of the dispensed fluid is compared to the expected weight and must be within a certain percentage to pass. Pass/Fail criteria depends on the per-well volume dispensed: 2.0% for 80 µL, 5.0% for 20 µL, and 20.0% for 5 µL.

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

Gen5 Parameters

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

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

Synergy H1 Disp 1 Test.prt and **Synergy H1 Disp 2 Test.prt** (for use with models with Absorbance capability)

Parameter	Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Wells A1–H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at 275 µL/sec
Plate Out,In	Comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A5–H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at 250 µL/sec
Plate Out,In	Comment: Weigh the plate (20 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A9–H12

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

Synergy H1 Disp 1 Test No Read.prt and **Synergy H1 Disp 2 Test No Read.prt** (for use with models without Absorbance capability)

Parameter	Default Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Wells A1–H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at 275 µL/sec
Plate Out,In	Comment: Weigh the plate (80 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Wells A5–H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at 250 µL/sec
Plate Out,In	Comment: Weigh the plate (20 uL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.

Dispense Step	Dispenser <1 or 2> Wells A9–H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at 225 µL/sec
Plate Out,In	Comment: Weigh the plate (5 uL test). RECORD the weight, TARE the balance. PIPETTE 150 µL/well of DI water into all 12 columns. Place the plate back on the carrier. Click OK to perform the Read steps.
Read Step	Define a brief Read step for a single well. The measurement value will not be used. The step is only necessary because Gen5 requires a Read step with dispense protocols.

Synergy H1 Disp Test Other Reader.prt (for use with an Agilent BioTek absorbance-capable reader other than Synergy H1)

Parameter	Default Setting
Shake Step	<medium intensity> for 15 seconds
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: <as appropriate for the reader type> Step label: 80 ul Read Wells: A1..H4 Wavelengths, 2: 405 nm, 750 nm Speed: Normal
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: <as appropriate for the reader type> Step label: 20 and 5 ul Read Wells: A5..H12 Wavelengths, 2: 630 nm, 750 nm Speed: Normal
Data Reduction	Define two Delta OD transformations: 405-750 nm for the 80 ul Read step, columns 1-4 630-750 nm for the 20 and 5 ul Read step, columns 5-12

Test Procedure

Materials

- Absorbance reader with capability of reading at 405, 630, and 750 nm. The reader must have an accuracy specification of ±1.0% ±0.010 OD or better and a repeatability specification of ±1.0% ±0.005 OD or better. The Synergy H1 may be used if it supports Absorbance and has passed the Absorbance Plate Test.
- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200 µL hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250-mL beaker
- New 96-well, clear, flat-bottom microplates
- Green Test Dye Solution (BTI #7773003) undiluted
- Gen5 protocols listed below (as applicable for your reader model) and described in the previous section

  For models with Absorbance capabilities:

  Synergy H1 Disp 1 Test.prt
  Synergy H1 Disp 2 Test.prt

  For models without Absorbance capabilities:

  Synergy H1 Disp 1 Test No Read.prt
  Synergy H1 Disp 2 Test No Read.prt

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

  Synergy H1 Disp Test Other Reader.prt

Test Procedure for Models with Absorbance Capability

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000 µL. This prevents the water from diluting the dye.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. Create an experiment based on the Synergy H1 Disp 1 Test protocol.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

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

7. Initiate a plate read. Gen5 will prompt you to empty the tip priming trough.
8. When ready, proceed with the experiment. The sequence is as follows:
   - 80 µL/well is dispensed to columns 1–4.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 20 µL/well is dispensed to columns 5–8.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 5 µL/well is dispensed to columns 9–12.
   - When prompted, remove the plate and weigh it. Record the weight.
   - Manually pipette 150 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.
   - Place the plate on the carrier for the shake and read steps.
9. When the experiment is complete, save the file with an identifying name.
10. Remove the plate from the carrier and set it aside.
11. Repeat the procedure using the Synergy H1 Disp 2 Test protocol and a new microplate.
12. When the tests are complete:
    - Prime both dispensers with at least 5000 µL of deionized water to flush out the dye solution.
    - See the Results Analysis section.

Test Procedure for Models without Absorbance Capability

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

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

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000 µL. This prevents the water from diluting the dye.

3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. Create an experiment based on the Synergy H1 Disp 1 Test No Read protocol.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

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

7. Initiate a plate read. Gen5 will prompt you to empty the tip priming trough.
8. When ready, proceed with the experiment. The sequence is as follows:
   - 80 µL/well is dispensed to columns 1–4.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 20 µL/well is dispensed to columns 5–8.
   - When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
   - 5 µL/well is dispensed to columns 9–12.
   - When prompted, remove the plate and weigh it. Record the weight.
   - Manually pipette 150 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.
   - Carefully set the plate aside.
9. Close the experiment without saving it.

If you are not using an Agilent BioTek absorbance reader, read the plate using the parameters described in the table above. Perform the calculations and determine pass/fail according to the instructions in the Results Analysis section.

10. If you are using an Agilent BioTek absorbance reader, configure Gen5 to communicate with the reader.
11. Create an experiment based on the Other Reader protocol and read the plate.
12. When the experiment is complete, save the file with an identifying name.
13. Remove the plate from the carrier and set it aside.
14. Repeat the procedure using the Synergy H1 Disp 2 Test No Read protocol and a new microplate.

15. When the tests are complete:
    - Prime both dispensers with at least 5000 µL of deionized water to flush out the dye solution.
    - Refer to the instructions in the Results Analysis section.

Results Analysis

When the experiment for one injector is complete, 32 delta OD values are reported for each of the three dispense volumes. The pass/fail criteria for each set of 32 wells with the same dispense volume is based on the calculated coefficient of variation (% CV) and Accuracy % Error.

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

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

   ((ActualWeight–ExpectedWeight)/ExpectedWeight)* 100

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

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

If any tests fail, prime the fluid lines and rerun the tests. If the tests fail again, the injectors may require cleaning. If tests continue to fail, contact Technical Support.

Specifications

General Specifications

Microplates	
The Synergy H1 accommodates standard 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry, and the Take3 and Take3 Trio Micro-Volume Plates. **Maximum Plate Height:** • Absorbance mode: plates up to 20.30 mm high • Fluorescence/Luminescence modes: plates up to 22.6 mm high	
Hardware and Environmental	
Light Source	Xenon flash light source
Dimensions	46.4 cm x 37.5 cm x 33 cm
Weight	< 25 kg
Environment	Operational temperature range 18°C to 40°C Storage temperature range -25°C to 50°C
Humidity	Operational: 10% to 85% relative humidity (non-condensing) Storage: 10% to 80% relative humidity (non-condensing)
Power Consumption	"M2" models are powered from an external 250W (minimum), 24VDC power supply compatible with 100-240 volts AC @50-60Hz. The other models are powered from an external 130W (minimum), 24VDC power supply compatible with 100-240 volts AC @50-60Hz.
Incubation	Range: For M2 models with incubator assembly PN 2070501 installed and basecode PN 1910200 version 1.02 or higher, the maximum incubation temperature is 70°C. For all other models/configurations, the maximum incubation temperature is 45°C. Uniformity: ±0.5°C at 37°C, tested with Innovative Instruments, Inc. temperature test plate Top and bottom incubation controlled via software-adjustable gradient.

<table>
<tr><td>Plate Shaking</td><td>Linear:
• Amplitude: 1 mm to 6 mm in 1-mm steps
• Frequency*: ~18 Hz to ~6 Hz
Orbital Slow:
• Amplitude: 1 mm to 6 mm in 1-mm steps
• Frequency*: ~10 Hz to ~3 Hz
Orbital Fast:
• Amplitude: 1 mm to 6 mm in 1-mm steps
• Frequency*: ~14 Hz to ~5 Hz
Double Orbital Slow:
• Amplitude: 1 mm to 6 mm in 1-mm steps
• Frequency*: ~10 Hz to ~3 Hz
Double Orbital Fast:
• Amplitude: 1 mm to 6 mm in 1-mm steps
• Frequency*: ~14 Hz to ~5 Hz
* Frequency is based on the amplitude selected.</td></tr>
</table>

Absorbance Specifications

<table>
<tr><th colspan="2">Accuracy, Linearity, Repeatability</th></tr>
<tr><td colspan="2">All qualifications were conducted using 96-/384-well, flat-bottom microplates. For the performance described here, the Gain on the Optics Test should be <= 8.0.</td></tr>
<tr><td>Measurement Range: 0.000 to 4.000 OD</td><td>Resolution: 0.0001 OD</td></tr>
<tr><td colspan="2">Accuracy
96-well plate, normal read speed
0–2 OD: ±1% ±0.010 OD, delay after plate movement = 100 ms
2–2.5 OD: ±3% ±-0.010 OD, delay after plate movement = 100 ms
384-well plate, normal read speed
0–2 OD: ±2% ±0.010 OD, delay after plate movement = 100 ms
2–2.5 OD: ±5% ±-0.010 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed
0–1 OD: ±1% ±0.010 OD</td></tr>
</table>

Linearity 96-well plate, normal read speed 0–2 OD: ±1% ±0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: ±3% ±0.010 OD, delay after plate movement = 100 ms 384-well plate, normal read speed 0–2 OD: ±2% ±0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: ±5% ±0.010 OD, delay after plate movement = 100 ms 96-well and 384-well plate, sweep read speed 0–1 OD: ±1% ±0.010 OD
Repeatability 96-well and 384-well plate, normal read speed 0–2 OD: ±1% ±0.005 OD, delay after plate movement = 100 ms 2–2.5 OD: ±3% ±0.005 OD, delay after plate movement = 100 ms 96-well and 384-well plate, sweep read speed 0–1 OD: ±2% ±0.010 OD

Optics	
Wavelength range	230 to 999 nm
Wavelength accuracy	± 2 nm
Wavelength repeatability	± 0.2 nm
Bandpass	< 4 nm (230–285 nm), < 8 nm (>285 nm)
Detector	Photodiodes (2)

Fluorescence Specifications (Mono-Based)

The Synergy H1 reads fluorescence intensity with monochromators from the top and bottom of 6- to 384-well plates. All detection limit (DL) requirements are measured by the “two-point” method, which gives the limit of detection at a signal-to-noise ratio of one where noise is defined as three times the standard deviation of the background wells.

The following requirements apply to 96-well plate reads. All tests are conducted in a Greiner clear-bottom Sensoplate. All tests use 100 flashes or fewer per data point and 350 msec delay before read with 200 µL in each well.

Monochromator-based Fluorescence	
Excitation range	250–700 nm (with low-noise PMT); 250–900 nm (with red-shifted PMT)
Emission range	250–700 nm (with low-noise PMT); 300–700 nm for emission scans (up to 900 nm with red-shifted PMT)
Bandpass	Fixed: <=18 nm (Excitation and Emission) Variable: From 9 nm to 50 nm in 1 nm increments (both excitation and emission)

Sodium Fluorescein in phosphate buffered saline (PBS)
DL <= 20 pM top read
DL <= 20 pM bottom read
Excitation 485 nm
Emission 528 nm

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

Fluorescence Specifications (Filter-Based)

Sensitivity	
The following specifications apply to 96-well read formats	
3 mm fixed optical probe, Top reading	DL ≤ 10 pM (3 pM typical) solution of Sodium Fluorescein in PBS Excitation 485/20, Emission 528/20, 510 nm mirror Corning Costar 96-well solid black plate DL ≤ 0.16 ng/mL (0.91 nM typical) solution of Methylumbelliferone in CBB Excitation 360/40, Emission 460/40, 400 nm mirror Corning Costar black strips

Time-Resolved Fluorescence	
96-well plate	DL ≤ 250 fM (100 fM typical) Excitation 360/40 nm, Emission 620/40 nm, 400 nm mirror
Integration Interval	20 to 16,000 µs
Delay	0 to 16,000 µs
Granularity	1-µs steps

Fluorescence Polarization	
96-well plate	5 mP standard deviation at 1 nM Sodium Fluorescein Excitation 485/20 nm, Emission 528/20 nm, 510 nm mirror Excitation range: 330 to 700 nm (UV-transparent polarizing filter) Emission range: 400 to 700 nm

Luminescence Specifications

≤ 75 amol/well DL ATP in a 96-well plate (low-noise PMT), 20 amol typical

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

Dispense/Read Specifications

Applies only to models equipped with injectors

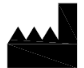
Plate Type	Both injectors dispense to standard height 6-, 12-, 24-, 48-, 96-, and 384-well microplates .
Detection Method	Absorbance, Fluorescence (FI, FP, TRF), Luminescence
Volume Range	5–1000 µL with a 5–20 µL tip prime
Accuracy	±1 µL or 2.0%, whichever is greater
Precision	≤2.0% for volumes of 50–200 µL ≤4.0% for volumes of 25–49 µL ≤7.0% for volumes of 10–24 µL ≤10.0% for volumes of 5–9 µL

In This Book

This document contains installation, operation, maintenance, and qualification information for all models of the Synergy H1.

Document Revision History			
Part Number	Revision	Date	Modifications
8041005I	D	August 2022	Added a CAUTION statement regarding DMSO Concentration. Added a CAUTION statement regarding the expected benchtop conditions.

Original Language – EN



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