



Agilent BioTek Synergy HTX  
Multi-Mode Microplate Reader  
**User Manual**



# Notices

## Document Identification

1341000N

Revision E, October 2024

## Copyright

© Agilent Technologies, Inc. 2024

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Agilent Technologies, Inc.  
5301 Stevens Creek Blvd.  
Santa Clara, CA 95051  
USA

## Instrument Manufacturer



Manufactured by  
Agilent Technologies, Inc.  
5301 Stevens Creek Blvd.  
Santa Clara, CA 95051  
USA



## Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

## Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

## Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers.

Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

## Hazard Notices

### DANGER

A DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.

### WARNING

A WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury.

### CAUTION

A CAUTION is used with the safety alert symbol, indicating a hazardous situation which, if not avoided, could result in minor or moderate injury.

### NOTICE

A NOTICE indicates a situation which, if not avoided, will result in damage to or destruction of the instrument or data.

# Contents

---

<b>Notices</b>	<b>i</b>
<b>1 General Information</b>	<b>1</b>
Contact Information	2
Worldwide Sales and Support	2
Technical Support and Service	2
Customer Care	2
Intended Use Statement	3
Quality Control	3
Hazard Notices	3
Safety Hazards	4
Electrical Hazards	4
Biohazards	4
Component Hazards	4
Intended Product Use	5
Instrument and Data Hazards	5
Chemical Hazards	5
Environmental Hazards	5
Component Hazards	5
Intended Product Use	6
Symbols	7
Electromagnetic Compatibility (EMC) Information	11
Emission	11
Immunity	12
Disposal	12
<b>2 Introduction</b>	<b>13</b>
Product Description	14
Instrument Models	15
Package Contents	15
Optional Accessories	16
Materials for Conducting Liquid Tests	17
Technical Support	18
<b>3 Installation</b>	<b>19</b>
Important Pre-Installation Information	20
Unpack and Inspect the Reader	20
Select an Appropriate Location	22
Remove the Shipping Hardware	22

---

Install the Fluorescence Lamp Assembly .....	23
Install the Power Supply .....	24
Unpack and Inspect the Dispense Module .....	25
Install the Dispense Module .....	26
Dispense Module Components .....	26
Install the Dispenser .....	27
Install the Software on the Host Computer .....	29
Install Gen5/Gen6 .....	29
Install the USB Driver .....	30
Connect the Host Computer and Reader .....	30
Turn on the Reader .....	30
Start the Software and Test Communications .....	30
Gen5 .....	30
Gen6 .....	31
Troubleshooting .....	31
Set Dispenser Calibration Values .....	32
Gen5 .....	32
Gen6 .....	32
Run a System Test .....	33
Test the Injection System .....	33
Operational/Performance Qualification .....	34
Repackaging and Shipping Instructions .....	34
Repackage the Instrument .....	35
Prepare the Dispense Module for Shipment .....	38
<b>4 Getting Started .....</b>	<b>41</b>
External Components .....	42
Internal Components .....	44
Lamp Assembly and Filter Wheel Access .....	44
Excitation and Emission Filter Wheels .....	45
Installing the Time-Resolved Cartridge .....	47
Configuring the System for Luminescence Measurements .....	48
Injection System .....	48
Getting Started with Gen5 Software .....	51
Viewing and Modifying Filter Wheel Information in Gen5 .....	51
Protocols and Experiments .....	52
Getting Started with Gen6 Software .....	53
Viewing and Modifying Filter Wheel Information in Gen6 .....	53
Experiments .....	54
Dispense Module Control .....	55

---

Prime .....	55
Purge .....	56
Plate Shaking Options .....	56
Recommendations for Optimum Performance .....	57
General .....	57
Using 384-Well Microplates .....	57
Luminescence Measurements .....	58
Models with Injectors .....	58
Incubation and Partial Plates .....	58
<b>5 Periodic Maintenance .....</b>	<b>59</b>
Periodic Maintenance Overview .....	60
Daily Cleaning for the Dispense Module .....	60
Recommended Maintenance Schedule .....	61
Maintenance Hazards .....	62
Clean Exposed Surfaces .....	63
Materials .....	63
Procedure .....	63
Inspect/Clean Excitation and Emission Filters .....	64
Required Materials .....	64
Procedure .....	64
Flush/Purge Fluid Path .....	65
Run a Dispense Protocol (Optional) .....	66
Empty/Clean the Tip Priming Trough .....	67
Clean the Priming Plate .....	67
Clean the Internal Components (As Applicable) .....	67
Required Materials .....	67
Remove the Reader's Shroud .....	68
Remove the Internal Dispense Tubes and Injector Heads .....	69
Clean the Dispense Tubes and Injector Heads .....	72
Clean the Optical Probes .....	73
Clean the Reader's Internal Surface .....	80
Reassemble the Components .....	83
Performance Check .....	84
<b>6 As Needed Maintenance .....</b>	<b>85</b>
Decontamination .....	86
Materials .....	87
Procedure for Models without the Dispense Module .....	87
Procedure for Models with the Dispense Module .....	87

---

Routine Procedure .....	88
Alternate Procedure .....	90
Dispense Module, Syringe Replacement .....	90
Syringe Maintenance Position .....	90
Replace the Syringe .....	91
<b>7 Instrument Qualification .....</b>	<b>92</b>
Instrument Qualification Overview .....	93
IQ/OQ/PQ Description .....	93
Recommended Qualification Schedule .....	94
System Test .....	95
Test Setup .....	95
Plate Shaker Test .....	97
Absorbance Testing Overview .....	98
Absorbance Plate Tests .....	98
Test Method .....	98
Requirements .....	99
Setup .....	99
Test Procedure .....	100
Troubleshooting .....	101
Absorbance Liquid Tests .....	102
Test Methods .....	102
Gen5 Protocol Parameters .....	103
Gen6 Experiment Parameters .....	104
Absorbance Liquid Test 1 .....	106
Absorbance Liquid Test 2 .....	107
Absorbance Liquid Test 3 (optional) .....	108
Results Analysis .....	110
Troubleshooting .....	112
Fluorescence Testing Overview .....	112
Fluorescence Plate Test .....	112
Requirements .....	113
Test Procedure .....	113
Results Analysis .....	113
Fluorescence Liquid Tests .....	114
Test Methods .....	114
Gen5 Protocol Parameters .....	114
Gen6 Experiment Parameters .....	119
Required Materials .....	123
Test Solutions .....	124

---

Pipette Map .....	125
Test Procedure .....	126
Results Analysis .....	127
Troubleshooting .....	128
Alternate/Supplemental Tests Using Methylumbellifерone (MUB) .....	129
Luminescence Test .....	132
Test Method .....	132
Plate Layout .....	132
Gen5 Protocol Parameters .....	133
Gen6 Experiment Parameters .....	135
Required Materials .....	136
Test Procedure .....	137
Results Analysis .....	137
Troubleshooting .....	138
Dispense Module Tests .....	138
Test Method .....	138
Gen5 Protocol Parameters .....	139
Gen6 Experiment Parameters .....	142
Required Materials .....	144
Alternate Test Solutions .....	145
Test Procedure for Models with Absorbance Capability .....	145
Test Procedure for Models without Absorbance Capability .....	146
Results Analysis .....	147
Troubleshooting .....	148
<b>Appendix A: Specifications .....</b>	<b>149</b>
General Specifications .....	150
Environmental Conditions .....	150
Dispense/Read Specifications .....	151
Absorbance Specifications .....	151
Performance .....	152
Fluorescence Specifications .....	153
Optics .....	153
Sensitivity .....	153
Read Timing .....	153
Time-Resolved Fluorescence .....	153
Luminescence Specifications .....	154
<b>Appendix B: Error Conditions .....</b>	<b>155</b>
Error Codes Overview .....	156

---

Error Codes .....	156
<b>Appendix C: Safety Information .....</b>	<b>165</b>
Safety Notices .....	166
Safety Hazards .....	167
Electrical Hazards .....	167
Biohazards .....	171
Component Hazards .....	171
Intended Product Use .....	174
<b>In This Book .....</b>	<b>177</b>

# 1 General Information

This chapter gives important information about safety, hazards, and contact information.

---

Contact Information .....	2
Intended Use Statement .....	3
Quality Control .....	3
Hazard Notices .....	3
Safety Hazards .....	4
Instrument and Data Hazards .....	5
Symbols .....	7
Electromagnetic Compatibility (EMC) Information .....	11
Disposal .....	12

## 1 General Information

### Contact Information

## Contact Information

---



Agilent Technologies, Inc.  
5301 Stevens Creek Blvd.  
Santa Clara, CA 95051

### Worldwide Sales and Support

[www.agilent.com/en/contact-us/page](http://www.agilent.com/en/contact-us/page)

### Technical Support and Service

Service Toll-Free US and Canada: (800) 227-9770

[www.agilent.com/en/support](http://www.agilent.com/en/support)

Email: [bio.tac@agilent.com](mailto:bio.tac@agilent.com)

Instrument service and repair is available worldwide at one of our international service centers and in the field at your location.

### Customer Care

Email: [bio.CustomerCare@agilent.com](mailto:bio.CustomerCare@agilent.com)

## 1 General Information

### Intended Use Statement

## Intended Use Statement

---

The Synergy HTX is a multi-mode microplate reader and intended to be used for the examination of specimens to analyze their characteristics and impact on a variety of analytes.

## 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.

## Hazard Notices

---

Raadpleeg Bijlage C voor informatie in andere talen.

Reportez-vous à l'annexe C pour obtenir des informations dans d'autres langues.

Informationen in anderen Sprachen finden Sie in Anhang C.

Fare riferimento all'Appendice C per informazioni in altre lingue.

Consulte el Apéndice C para obtener información en otros idiomas.

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



**DANGER** A DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.



**WARNING** A WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury.



**CAUTION** A CAUTION is used with the safety alert symbol, indicating a hazardous situation which, if not avoided, could result in minor or moderate injury.



**NOTICE** A NOTICE indicates a situation which, if not avoided, will result in damage to or destruction of the instrument or data.

## 1 General Information

### Safety Hazards

## Safety Hazards

---

### Electrical Hazards

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

**⚠WARNING** **Power Cords.** Do not replace detachable Mains power cords with inadequately rated cords. Always replace with power cords purchased from Agilent.

**⚠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.

**⚠WARNING** **Disconnection.** The electrical connection at the back of the instrument is the primary disconnection point. The instrument should be positioned to allow access to the power cord for easy disconnection.

**⚠CAUTION** **EMC.** The shielding and length of USB and other port cables are critical to Electromagnetic Compatibility performance. Use only the cables provided from Agilent.

### Biohazards

**⚠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.

### Component Hazards

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

## 1 General Information

### Instrument and Data Hazards

#### ⚠️ WARNING

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

#### ⚠️ WARNING

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

#### ⚠️ CAUTION

**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.

## Intended Product Use

#### ⚠️ 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.

## Instrument and Data Hazards

---

### Chemical Hazards

#### NOTICE

**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%.

### Environmental Hazards

#### NOTICE

**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.

#### NOTICE

**Environment.** 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, that is, higher frequency of service than is covered by the instrument warranty.

### Component Hazards

#### NOTICE

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

## 1 General Information

### Instrument and Data Hazards

#### NOTICE

**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.

#### NOTICE

**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.

#### NOTICE

**Fluorescence Lamp Assembly.** Do not touch the glass lenses. Fingerprints on the condenser lens or heat absorber may negatively affect performance.

#### NOTICE

**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.

## 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.

#### NOTICE

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

## 1 General Information

### Symbols

## Symbols

---

Veiligheidssymbolen

Symboles de sécurité

Sicherheitssymbole

Simboli di sicurezza

Símbolos de seguridad



Caution.

Voorzichtig.

Attention.

Achtung.

Attenzione.

Precaución.



Warning; Biological hazard.

Waarschuwing; biologisch gevaar.

Avertissement : Risque biologique.

Warnung; biologische Gefahr.

Avvertenza, rischio biologico.

Advertencia: peligro biológico.



Warning; Pinch hazard.

Waarschuwing; beknelingsgevaar.

Avertissement : risque de pincement.

Warnung; Quetschgefahr.

Avvertenza, rischio di pizzicamento.

Advertencia: peligro de atrapamiento.



Warning; Hot surface.

Waarschuwing; heet oppervlak.

Avertissement : surface chaude.

Warnung; heiße Oberfläche.

Avvertenza, superficie molto calda.

Advertencia: superficie caliente.

## 1 General Information

### Symbols



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

Kennisgeving van verwijdering: Verwijder het instrument volgens Richtlijn 2012/19/EU betreffende afgedankte elektrische en elektronische apparatuur (AEEA) of lokale verordeningen.

Avis concernant la mise au rebut : mettez l'instrument au rebut conformément à la directive 2012/19/EU portant sur les déchets d'équipement électrique et électronique (DEEE) ou aux dispositions locales.

Entsorgungshinweis: Entsorgen Sie das Gerät gemäß der Richtlinie 2012/19/EU „für Elektro- und Elektronik-Altgeräte (WEEE)“ bzw. den Landesvorschriften.

Avviso per lo smaltimento: smaltire lo strumento in base alla Direttiva 2012/19/EU, sui "rifiuti di apparecchiature elettriche ed elettroniche (WEEE)" o le ordinanze locali.

Aviso de eliminación: elimine el instrumento de conformidad con la Directiva 2012/19/UE sobre residuos de aparatos eléctricos y electrónicos (RAEE) o las ordenanzas locales.



CE Marking – This product complies with all the applicable EU Directives and Regulations.

CE-markering – Dit product voldoet aan alle toepasselijke EU-richtlijnen en -verordeningen.

Marquage CE : ce produit est conforme à toutes les directives et règlementations européennes qui s'appliquent.

CE-Kennzeichnung – Dieses Produkt erfüllt die Anforderungen aller geltenden EU-Richtlinien und -Verordnungen.

Marcatura CE: questo prodotto è conforme a tutte le Direttive e i Regolamenti EU applicabili.

Marcado CE: Este producto cumple todas las directivas y normativas técnicas aplicables de la UE.

Date of manufacture.



Productiedatum.

Date de fabrication.

Herstellungsdatum.

Data di produzione.

Fecha de fabricación.



This product has been evaluated and certified by TÜV SÜD and complies with the applicable safety standards for US and Canada.

Dit product is beoordeeld en gecertificeerd door TÜV SÜD en voldoet aan de geldende veiligheidsnormen voor de VS en Canada.

Ce produit a été évalué et certifié par TÜV SÜD, et est conforme aux normes de sécurité qui s'appliquent pour les États-Unis et le Canada.

Dieses Produkt wurde vom TÜV SÜD geprüft und zertifiziert und erfüllt die anwendbaren Sicherheitsnormen für die USA und Kanada.

Questo prodotto è stato valutato e certificato da TÜV SÜD ed è conforme agli standard di sicurezza applicabili negli Stati Uniti e in Canada.

Este producto ha sido evaluado y certificado por TÜV SÜD y cumple las normas de seguridad aplicables en EE. UU. y Canadá.

## 1 General Information

### Symbols



This product complies with environmental protection use period as defined in People's Republic of China Electronic Industry Standard SJ/T11364. Toxic or hazardous substances will not leak or mutate under normal operating conditions for 40 years.

Dit product voldoet aan de milieubeschermsgebruiksperiode zoals gedefinieerd in de Electronic Industry Standard SJ/T11364 van de Volksrepubliek China. Giftige of gevaarlijke stoffen zullen onder normale bedrijfsomstandigheden gedurende 40 jaar niet lekken of muteren.

Ce produit est conforme à la période d'utilisation dans le cadre de la protection de l'environnement telle que définie par la norme de l'industrie électronique de la République populaire de Chine SJ/T11364. Les substances toxiques ou dangereuses ne fuiront pas ou ne subiront pas de mutation dans des conditions de fonctionnement normales pendant 40 ans.

Dieses Produkt entspricht der Umweltschutz-Nutzungsdauer gemäß der Definition im Electronic Industry Standard SJ/T11364 der Volksrepublik China. Giftige oder gefährliche Stoffe werden unter normalen Betriebsbedingungen 40 Jahre lang nicht austreten oder mutieren.

Questo prodotto è conforme al periodo di utilizzo della protezione ambientale come definito nello Standard del settore elettronico della Repubblica Popolare Cinese SJ/T11364. Le sostanze tossiche o pericolose non fuoriescono o non subiscono mutazioni in condizioni operative normali per 40 anni.

Este producto cumple con el periodo de uso de protección ambiental según el estándar SJ/T11364 de la República Popular China para la industria electrónica. Las sustancias tóxicas o peligrosas no se filtrarán ni mutarán en condiciones de funcionamiento normales durante 40 años.



UK Conformity Assessed marking is a certification mark that indicates conformity with the applicable requirements for products sold within Great Britain.

De 'UK Conformity Assessed'-markering is een certificeringsmerk dat aangeeft dat producten die in Groot-Brittannië worden verkocht, voldoen aan de toepasselijke eisen.

Le marquage UK Conformity Assessed est une marque de certification qui indique la conformité aux exigences applicables aux produits vendus en Grande-Bretagne.

Die Kennzeichnung „UK Conformity Assessed“ ist ein Zertifizierungszeichen, das die Konformität mit den geltenden Anforderungen für in Großbritannien verkaufte Produkte anzeigt.

Il marchio UKCA (conformità valutata del Regno Unito) è un marchio di certificazione che indica la conformità ai requisiti applicabili per i prodotti venduti in Gran Bretagna.

El marcado UKCA (UK Conformity Assessed) es una marca de certificación que indica la conformidad con los requisitos aplicables para los productos vendidos en Gran Bretaña.



This symbol indicates product conformity with applicable Technical Regulations of EAEU.

Dit symbool geeft aan dat het product voldoet aan de toepasselijke technische voorschriften van EAEU.

Ce symbole indique la conformité du produit aux règlements techniques applicables de l'EAEU.

Dieses Symbol zeigt die Produktkonformität mit den geltenden technischen Vorschriften der EAWU an.

Questo simbolo indica la conformità del prodotto ai regolamenti tecnici applicabili dell'EAEU.

Este símbolo indica la conformidad del producto con los reglamentos técnicos aplicables de la UEEA.

## 1 General Information

### Symbols



Product complies with applicable EMC regulations in Australia and New Zealand.

Het product voldoet aan de toepasselijke EMC-voorschriften in Australië en Nieuw-Zeeland.

Le produit est conforme aux réglementations CEM applicables en Australie et en Nouvelle-Zélande.

Das Produkt entspricht den geltenden EMV-Vorschriften in Australien und Neuseeland.

Il prodotto è conforme alle normative EMC applicabili in Australia e Nuova Zelanda.

El producto cumple con las regulaciones EMC aplicables en Australia y Nueva Zelanda.



Korea Certification (KC) mark signifies Korea product compliance mark for safety and EMC/Radio/SAR of electrical and electronic equipment. The EMC requirements are applied to Agilent products.

Korea Certification (KC)-merkstaat voor Korea-productconformiteitsmerk voor veiligheid en EMC/Radio/SAR van elektrische en elektronische apparatuur. De EMC-eisen worden toegepast op Agilent-producten.

La marque de certification coréenne (KC) signifie la marque de conformité des produits coréens pour la sécurité et l'EMC/Radio/SAR des équipements électriques et électroniques. Les exigences CEM s'appliquent aux produits Agilent.

Das Korea-Zertifizierungszeichen (KC) bezeichnet das koreanische Produktkonformitätszeichen für Sicherheit und EMV/Funk/SAR von elektrischen und elektronischen Geräten. Die EMV-Anforderungen gelten für Agilent-Produkte.

Il marchio Korea Certification (KC) indica il marchio di conformità del prodotto Corea per la sicurezza e EMC/Radio/SAR di apparecchiature elettriche ed elettroniche. I requisiti EMC vengono applicati ai prodotti Agilent.

La marca de certificación de Corea (KC) significa la marca de cumplimiento de productos de Corea para la seguridad y EMC / Radio / SAR de equipos eléctricos y electrónicos. Los requisitos de EMC se aplican a los productos Agilent.



Temperature limit.

Temperatuur limiet.

Limite de temperatura.

Temperaturgrenze.

Limite di temperatura.

Límite de temperatura.

Humidity limitation.

Vochtigheidsbeperking.

Limitation d'humidité.

Feuchtigkeitsbegrenzung.

Limitazione dell'umidità.

Limitación de humedad.

## 1 General Information

### Electromagnetic Compatibility (EMC) Information

# Electromagnetic Compatibility (EMC) Information

---

This product conforms to:

## Emission

EN 55011/CISPR 11: Group 1, Class A

**Group 1 ISM equipment:** group 1 contains all Industrial, Scientific and Medical (ISM) equipment in which there is intentionally generated and/or used conductively coupled radio-frequency energy which is necessary for the internal functioning of the equipment itself.

**Class A equipment** is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

- Relocate the radio or antenna.
- Move the device away from the radio or television.
- Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
- Make sure that all peripheral devices are also certified.
- Make sure that appropriate cables are used to connect the device to peripheral equipment.
- Consult your equipment dealer, Agilent Technologies, Inc., or an experienced technician for assistance.

Changes or modifications not expressly approved by Agilent Technologies, Inc. could void the user's authority to operate the equipment.

## 1 General Information

### Disposal

## Immunity

IEC 61326-1/EN IEC 61326-1.

This product is intended to be used in a Basic Electromagnetic Environment with the following test requirements applied:

Test Items	Basic Standards	Test Limits	Performance Criteria
Electrostatic discharge immunity	IEC 61000-4-2	4kV Contact Discharge; 8kV Air Discharge	B
Radiated frequency immunity	IEC 61000-4-3	3V/m (80MHz-1GHz); 3V/m (1.4GHz-6.0GHz)	A
Electrical fast transient/burst immunity	IEC 61000-4-4	1kV (AC, 5kHz or 100kHz); 0.5kV (I/O, 5kHz or 100kHz)	B
Surge immunity	IEC 61000-4-5	±2kV (Line to Ground); ±1kV (Line to Line)	B
Conducted immunity	IEC 61000-4-6	3V (150kHz to 80MHz)	A
Magnetic field immunity	IEC 61000-4-8	3A/m (50Hz, 60Hz)	A
Voltage dips, short interruptions and voltage variations immunity	IEC 61000-4-11	0% half-cycle; 0% full-cycle; 70% 25/30 cycles; 0% 250/300 cycles	B B C C

### CAUTION

**EMC.** The shielding and length of USB and other port cables are critical to Electromagnetic Compatibility performance. Use only the cables provided from Agilent.

## Disposal

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

## 2 Introduction

This chapter introduces the Synergy HTX.

---

Product Description .....	14
Package Contents .....	15
Optional Accessories .....	16
Technical Support .....	18

## 2 Introduction

### Product Description

## Product Description

---

The Synergy HTX is a single-channel, Multi-Mode Microplate Reader available with absorbance, fluorescence, and luminescence detection. It is computer-controlled using Gen5/Gen6 software for all operations including data reduction and analysis. Synergy HTX is robot accessible and compatible with the BioStack Microplate Stacker.

The Synergy HTX supports the reading of 6-, 12-, 24-, 48-, 96-, and 384-well microplates with standard 128 x 86 mm geometry, as well as the Take3 and Take3 Trio Micro-Volume Plates.<sup>1</sup>

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/Gen6's Read Height reading parameter.

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

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

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.

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.

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.

#### NOTICE

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

#### NOTE

See [Appendix A Specifications](#) for performance and technical specifications.

---

<sup>1</sup>Gen6 software does not support Take3 or Take3 Trio. Gen6 users can access these functions through the Take3 app.

## 2 Introduction

### Package Contents

## Instrument Models

The instrument's part number indicates its capabilities:

Part Number	Absorbance	Fluorescence	Luminescence	Alpha	Incubation	Shaking	Dispense Ready	TRF
S1L-SN			•		•	•	•	
S1A-SN	•				•	•	•	
S1LA-SN	•		•		•	•	•	
S1LF-SN		•	•	•	•	•	•	
S1LFA-SN	•	•	•	•	•	•	•	
S1LFTA-SN	•	•	•	•	•	•	•	•

## Package Contents

### NOTE

Package contents and manufacturer part numbers are subject to change.

Item	Part Number
Synergy HTX User Manual	1341000N
Power supply	76061
Power cord	Varies by country
USB cable	75108
RS-232 serial cable	75034
Wrench	7772028
Fluorescence lamp assembly, if applicable to your reader model (Note: The replacement lamp assembly is PN 7080500)	7080501
Storage bag and fastener strips	-----
Time-Resolved Fluorescence cartridge assembly ("T" models only)	7090523
Optional accessories per the sales order, unless shipped separately.	

## 2 Introduction

### Optional Accessories

## Optional Accessories

#### NOTE

Item availability and part numbers are subject to change.

Optional Dispense Module with the following accessories:	Part Number
Outlet tubes (2, plus 2 spare) from dispense module to reader	7082120
Inlet tubes (2) from supply bottles to syringe drives	7082121
250 $\mu$ L syringes (2)	7083000
Syringe thumbscrews (2)	19511
Priming plate	8042202
Injector tip priming trough	1342017
Dispense module communication cable	75107
Dispense module front cover	7082137
Supply bottles (2, 30 mL)	7122609
Supply bottle holder assemblies (2)	7090564
Injector tip cleaning stylus and storage bag	2872304

Item	Part Number
Absorbance Test Plate (400–800 nm)	7260522
Absorbance Test Plate (340 nm)	7260551
Fluorescence Test Plate	1400501
Luminescence Test Plate (Harta Luminometer Reference Microplate) (includes microplate carrier adapter part number 8032028 for Synergy HTX)	8030015
Synergy HTX Product Qualification (IQ-OQ-PQ) package	1340508
Take3 Micro-Volume Plate*	TAKE3
Take3 Trio Micro-Volume Plate*	TAKE3TRIO
BioCell Adapter Plate	7270512
BioCell Quartz Vessel	7272051
Terasaki Adapter Plate	7330531
Additional fluorescence filters	Contact Agilent for part numbers and availability
Gen5/Gen6 software or upgrade	Contact your local dealer for details

\*Gen6 software does not support Take3 or Take3 Trio. Gen6 users can access these functions through the Take3 app. Part numbers TAKE3-SN and TAKE3TRIO-SN include both a plate and the app. To order the Take3 app by itself, use part number 1810010.

#### NOTE

The Synergy HTX is compatible with the BioStack Microplate Stacker. The BioStack rapidly and systematically transfers microplates to and from the instrument's microplate carrier.

## 2 Introduction

### Optional Accessories

## Materials for Conducting Liquid Tests

### NOTE

Manufacturer part numbers are subject to change.

Item	Part Number
<b>Absorbance Liquid Tests</b>	
Agilent Wetting Agent Solution	7773002
Agilent QC Check Solution #1 (25 mL)	7120779
Agilent QC Check Solution #1 (125 mL)	7120782
Phosphate-Buffered Saline (PBS) tablets, pH 7.2-7.6	Sigma #P4417
β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, reduced form)	98233 or Sigma #N6785-10VL (pre-weighed, 10 mg vials) or Sigma #N 8129 (bulk material)
<b>Fluorescence Liquid Tests</b>	
<i>Test Kits</i>	
Kit for FI tests using Sodium Fluorescein	7160013
Kit for FI tests using Methylumbelliferon	7160012
<i>Individual Materials</i>	
Sodium Fluorescein Powder, 1 mg vial	98155
Methylumbelliferon, 10 mg vial	98156
Carbonate-Bicarbonate Buffer (CBB) capsules	98158 or Sigma #3041
Phosphate-Buffered Saline (PBS) tablets, pH 7.2-7.6	Sigma #P4417
Sodium Borate, pH 9.18	Fisher Scientific #159532 or equivalent
<b>Injection System Tests</b>	
Green Test Dye	7773003

# Technical Support

---

## NOTE

See also [Contact Information](#).

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 instrument's basecode software part number and version and other instrument settings:
  - **Via Gen5:** Select **System > Instrument Configuration > Synergy HTX > View/Modify > Instrument Settings > Send Email to TAC.**
  - **Via Gen6:** Select **Configure > Synergy HTX > Instrument Settings > Send Email to TAC.**
- For troubleshooting assistance or instruments needing repair: the specific steps that produce your problem and any error codes displayed in Gen5/Gen6. See also [Appendix B Error Conditions](#).
- A text file of the diagnostic history of the instrument
  - **Via Gen5:** Select **System > Diagnostics > History**, then select the appropriate file and click **Export**.
  - **Via Gen6:** Select **Diagnostics > History**, then select the appropriate file and click **Export**.

## NOTE

Running a system test when a problem occurs provides valuable information for Technical Support. See [Chapter 7 Instrument Qualification](#) for instructions. When the test is complete, click **Save As** in Gen5/Gen6 to save a text file of the system test report, which can be emailed to Technical Support.

If you need to return an instrument to Agilent for service or repair, please contact Technical Support for instructions. See [Repackaging and Shipping Instructions](#).

## 3 Installation

This chapter includes instructions for unpacking and setting up the Synergy HTX and accessories, as applicable. Instructions are also included for preparing the Synergy HTX for shipment.

---

Important Pre-Installation Information .....	20
Unpack and Inspect the Reader .....	20
Select an Appropriate Location .....	22
Remove the Shipping Hardware .....	22
Install the Fluorescence Lamp Assembly .....	23
Install the Power Supply .....	24
Unpack and Inspect the Dispense Module .....	25
Install the Dispense Module .....	26
Install the Software on the Host Computer .....	29
Connect the Host Computer and Reader .....	30
Turn on the Reader .....	30
Start the Software and Test Communications .....	30
Set Dispenser Calibration Values .....	32
Run a System Test .....	33
Test the Injection System .....	33
Operational/Performance Qualification .....	34
Repackaging and Shipping Instructions .....	34

## Important Pre-Installation Information

---

### NOTICE

**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 HTX and accessories. Perform the tasks in the order presented, skipping those that do not apply to your reader's configuration.
- Save all packaging materials. Be sure to use packaging materials supplied by the manufacturer when shipping the instrument. 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, instrument, and accessories for shipping damage. If the instrument is damaged, notify the carrier and your Agilent representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. Agilent will arrange for repair or replacement.

## Unpack and Inspect the Reader

---

### NOTE

Refer to the illustrations following the procedure when performing these steps.

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

### 3 Installation

#### Unpack and Inspect the Reader

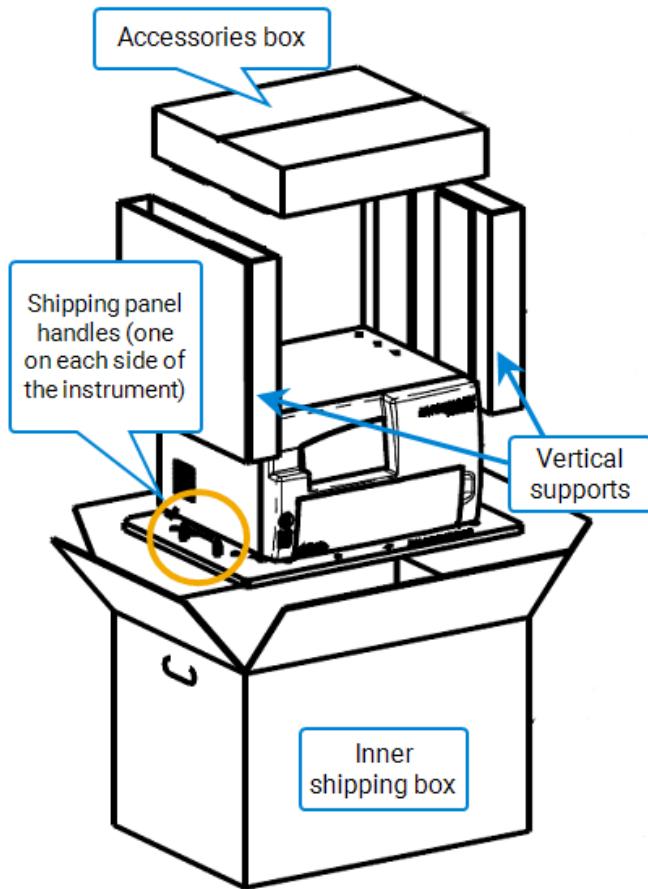


Figure 3-1: Unpacking the reader.

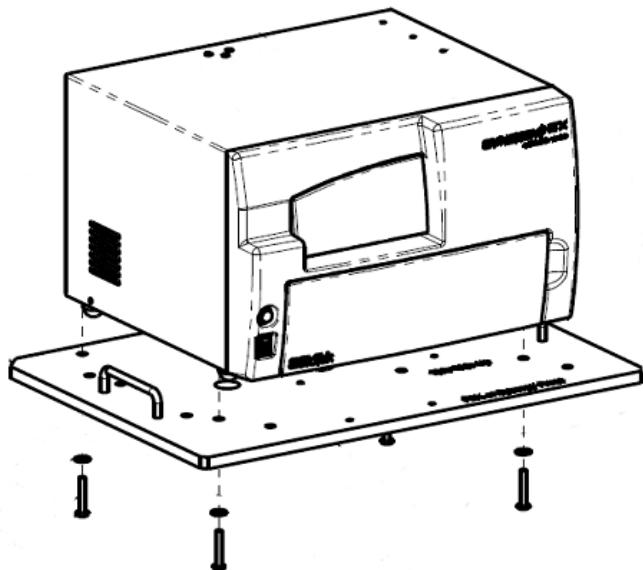


Figure 3-2: Removing the shipping panel.

### 3 Installation

#### Select an Appropriate Location

##### NOTICE

**Environment.** 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, that is, higher frequency of service than is covered by the instrument warranty.

Install the reader on a level, stable surface. Select an area where temperatures between 18°C (64.4°F) and 40°C (104°F) can be maintained. Leave at least six inches 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 ambient light.* Bright light may affect the reader's optics and readings, reducing its linear range.
- *Dust.* Readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

##### NOTE

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

## Remove the Shipping Hardware

##### NOTICE

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

##### NOTE

If necessary, contact Agilent to order a replacement bolt (part number 1342008) or o-ring (part number 49259).

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

### 3 Installation

#### Install the Fluorescence Lamp Assembly

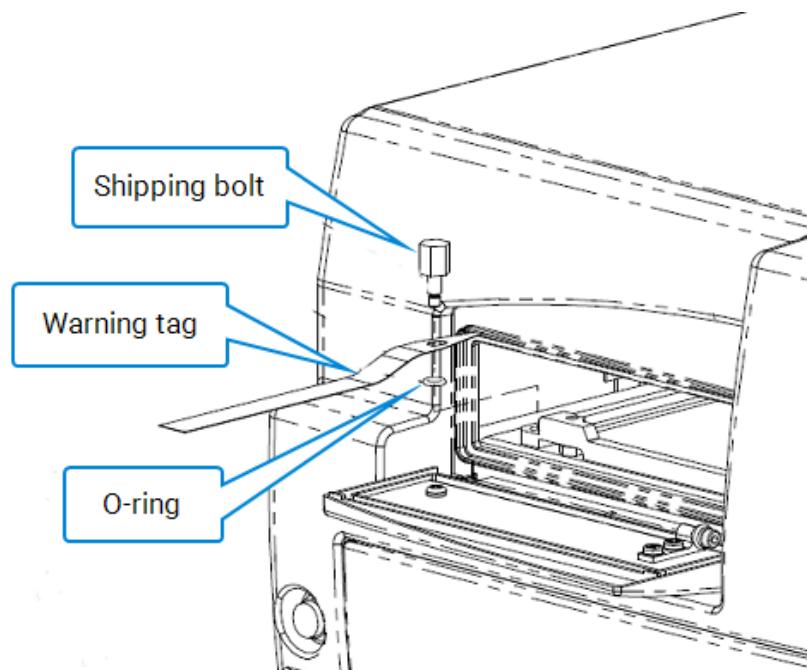


Figure 3-3: Removing the microplate carrier shipping bolt.

## Install the Fluorescence Lamp Assembly

---

*Applies only to models with fluorescence capability*

**NOTICE**

**Fluorescence Lamp Assembly.** Do not touch the glass lenses. Fingerprints on the condenser lens or heat absorber may negatively affect performance.

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

### 3 Installation

#### Install the Power Supply

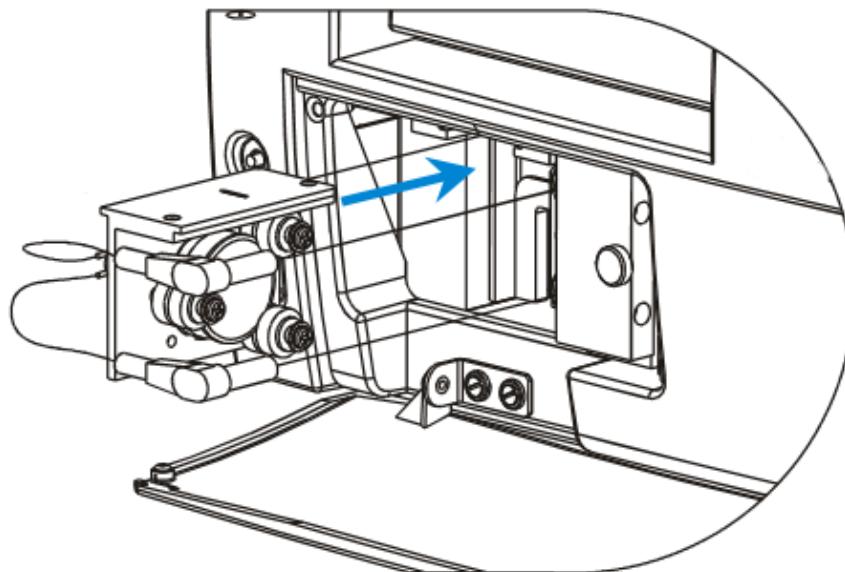


Figure 3-4: Installing the fluorescence lamp assembly.

**NOTE**

The replacement lamp part number is 7080500.

## 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.

**⚠ WARNING**

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

**⚠ WARNING**

**Power Cords.** Do not replace detachable Mains power cords with inadequately rated cords. Always replace with power cords purchased from Agilent.

**⚠ WARNING**

**Disconnection.** The electrical connection at the back of the instrument is the primary disconnection point. The instrument should be positioned to allow access to the power cord for easy disconnection.

- 1 Locate the power inlet on the back of the reader at the base.
- 2 Plug the rounded end of the external power supply's cord into the power inlet.
- 3 Connect the power cord to the power supply and plug it into an appropriate power receptacle.

### 3 Installation

#### Unpack and Inspect the Dispense Module

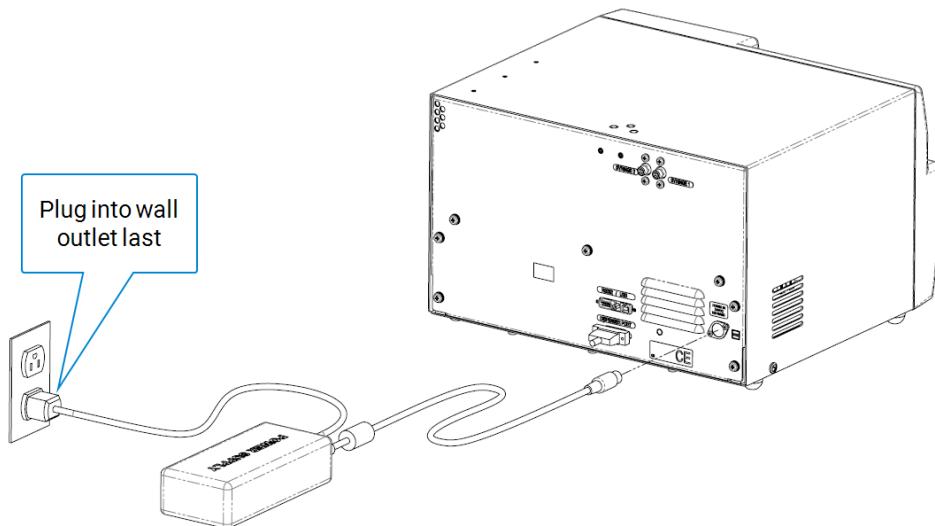


Figure 3-5: Connecting the instrument to power.

## Unpack and Inspect the Dispense Module

---

*Applies only to models equipped with injectors*

- Save all packaging materials. If you need to ship the dispense module to Agilent for repair or replacement, you must use the supplied materials. Using other forms of commercially available packaging, or failing to follow the repackaging instructions, may *void the warranty*.
- During the unpacking process, inspect the dispense module for shipping damage. If the dispense module is damaged, notify the carrier and your Agilent representative. Keep the shipping boxes and the packaging materials for the carrier's inspection.

See also the figures in [Prepare the Dispense Module for Shipment](#) for more information.

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

### 3 Installation

#### Install the Dispense Module

## Install the Dispense Module

*Applies only to models equipped with injectors*

**NOTICE**

**Dispenser Tubing.** Do not bend the dispenser inlet or outlet tubes. Bending may cause the tubes to become permanently warped or pinched, which leads to obstructed liquid flow.

### Dispense Module Components

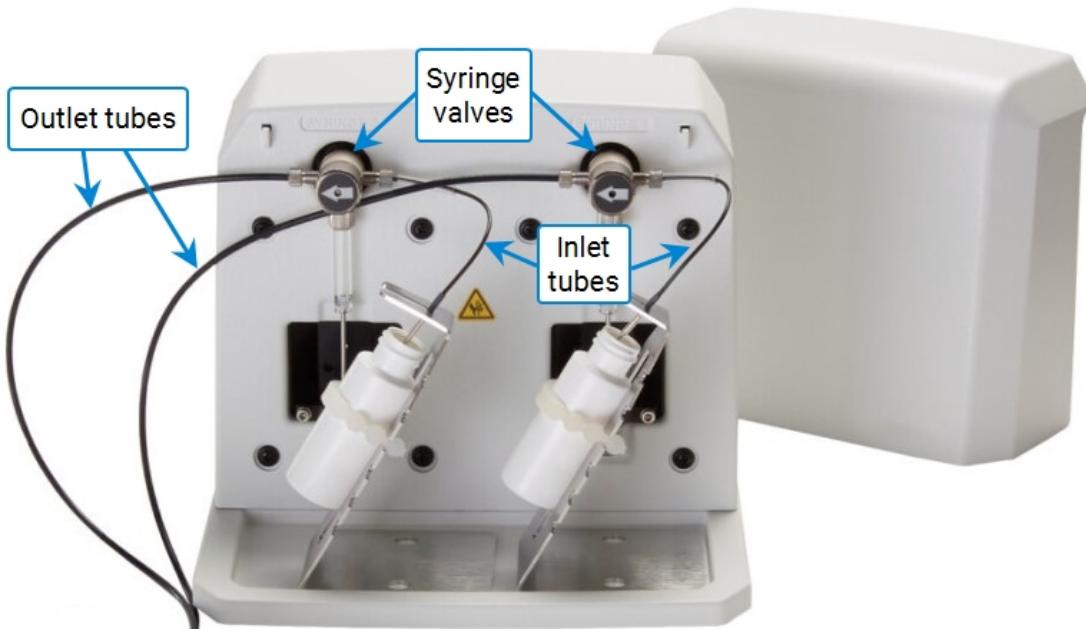


Figure 3-6: Dispense module.

### 3 Installation

#### Install the Dispense Module

## Install the Dispenser

- 1 Place the dispense module to the left side or on top of the reader.



Figure 3-7: Dispense module on top of the reader.

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



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

#### IMPORTANT

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

### 3 Installation

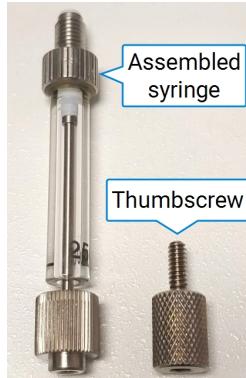
#### Install the Dispense Module



##### IMPORTANT

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

- 12 Remove the two syringes and two thumbscrews from their boxes. They are identical and interchangeable.
  - Each syringe should already be assembled in one piece. If there are two separate pieces, assemble them now: insert the white tip of the syringe plunger into the barrel of the syringe and gently push it all the way into the barrel.



**Figure 3-8:** Assembled syringe and thumbscrew.

- 13 Install the syringes, referring to the following figure:
  - a Hold the syringe vertically with the threaded end at the top.
  - b Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
  - c Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
  - d Pass a thumbscrew up through this hole and thread it into the bottom of the syringe. Hold the syringe to prevent it from rotating while tightening the thumbscrew. Finger-tighten only.

### 3 Installation

#### Install the Software on the Host Computer

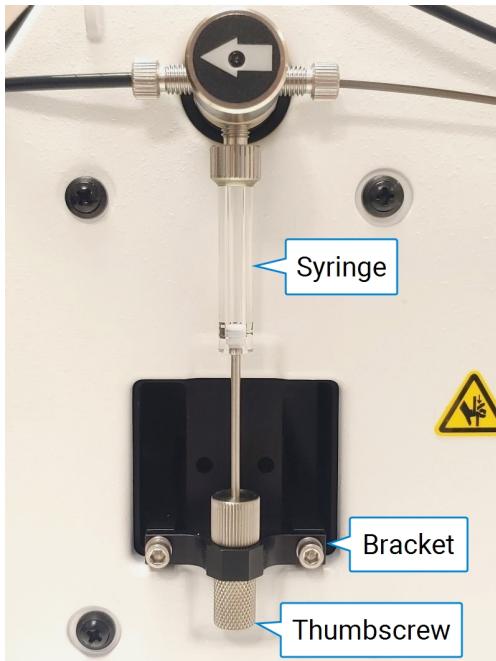


Figure 3-9: Syringe installation.

- 14 Locate the dispense module cable. Plug one end into the port on the left side of the dispense module. Plug the other end into the **DISPENSER PORT** on the rear of the reader.
- 15 Locate the injector-tip-cleaning stylus, packaged in a small cylinder. Attach the cylinder to the back of the dispense module for storage.



Figure 3-10: Tip-cleaning stylus in storage cylinder.

**NOTE**

Perform a visual inspection or a Performance Qualification test after reconnecting the tubes.

## Install the Software on the Host Computer

### Install Gen5/Gen6

Refer to instructions provided with the Gen5/Gen6 software for minimum system requirements, installation, and registration.

Gen5 software versions 3.11 and higher require Windows 10 or Windows 11. Gen6 requires Windows 10 or Windows 11.

You must have administrator privileges to install Gen5/Gen6. Log in to Windows as "Administrator" or consult your IT department for assistance.

### 3 Installation

Connect the Host Computer and Reader

#### Install the USB Driver

Refer to the instructions provided with the software to install the USB driver. The driver must be installed for the reader to communicate with the computer.

**NOTE**

Gen6 automatically installs the USB driver.

#### Connect the Host Computer and Reader

---

**CAUTION**

Ensure you set up the computer, keyboard, and mouse ergonomically to minimize physical strain.

The Synergy HTX is equipped with two communication ports on the back of the reader, USB and Serial (RS-232). The USB and Serial cables are included in the accessories tray.

**NOTE**

The USB cable is the preferred method of connection due to its ease of use.

- 1 Turn off the computer. If the reader is on, turn it off.
- 2 Connect one end of the cable to the appropriate port on the reader.
- 3 Connect the other end of the cable to the appropriate port on the computer.
- 4 Turn on the computer.

#### Turn on the Reader

---

- 1 If Gen5/Gen6 is open, close it now.
- 2 Locate the power switch on the lower-left corner of the front panel and turn on the Synergy HTX. The reader performs a System Test. When the test is completed, the reader extends the microplate carrier.
- 3 The carrier eject button, located next to the reader's power switch, is used to extend/retract the microplate carrier. Test the button by pressing it a few times when the System Test is complete. When the carrier is extended, pressing the button draws the carrier into the reading chamber and closes the loading door. When the carrier is in the chamber, pressing the button opens the loading door and fully extends the carrier.

#### Start the Software and Test Communications

---

On the host computer, start the software and log in if prompted. The default System Administrator password is **admin**.

##### Gen5

- 1 If prompted to add a reader, click **Yes**. Otherwise, select **System > Instrument Configuration > Add Reader**.

### 3 Installation

#### Start the Software and Test Communications

- 2 Set the Reader Type to **Synergy HTX** and select **OK**.
- 3 Perform one of the following steps, as applicable:
  - Select **Plug & Play**.
    - A Synergy HTX must be connected via USB to the computer and turned on to appear in the Available Plug & Play Readers list.
  - Set the **Com Port** to the COM port on the computer to which the reader is connected.
    - You must define the COM port when using the RS-232 Serial cable.
    - This information can be found via the Windows **Device Manager**.
- 4 Click **Test Communications** to verify that Gen5 can communicate with the Synergy HTX.
- 5 If the communication attempt is successful, Gen5 displays a success message. Return to the main screen.
- 6 You will soon perform a System Test. To prepare for this test:
  - a If the plate carrier is extended, press the carrier eject button to draw the carrier into the chamber.
  - b Select **System > Instrument Control > Synergy HTX**.
  - c Select the **Pre-Heating** tab, enter a Requested Temperature of at least **37°C**, and select **On**.

## Gen6

- 1 From the Gen6 Home screen, click **Configure**.
- 2 On the Instrument Configuration screen, select **Synergy HTX** from the drop-down list and click **Add**.
- 3 Click **Test Communications** to verify that Gen6 can communicate with the Synergy HTX.
- 4 If the communication attempt is successful, Gen6 displays a success message. Return to the Home screen.
- 5 You will soon perform a System Test. To prepare for this test:
  - a Select **Incubate** from the Instrument Control area of the Gen6 Home screen.
  - b Enter a Requested Temperature of at least **37°C** and select **On**.

## Troubleshooting

If the communication attempt is not successful, consider 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?
- Was the reader allowed to fully complete its power-up self-test before initiating communication with Gen5/Gen6?
- Gen5: Try a different COM Port or use Plug & Play.
- Gen5: Did you install the USB driver software?

If you remain unable to get Gen5/Gen6 and the reader to communicate with each other, contact Technical Support.

*Applies only to models equipped with injectors*

Before using the external dispense module with the Synergy HTX, you must set its calibration values in Gen5/Gen6. If you have not already done so, turn on the instrument and establish communication with Gen5/Gen6.



**IMPORTANT** The calibration values for both dispensers (#1 and #2) are printed on labels affixed to the rear of the dispense module. Each label lists six target calibration values (e.g., 200, 80, 40) with their actual measured values (e.g., 199.3, 79.7, 39.9). You enter the *measured* calibration values into Gen5/Gen6.

## Gen5

- 1 In Gen5, select **System > Instrument Configuration**, select the **Synergy HTX**, and select **View/Modify**.
- 2 Select **Setup** and select the **Dispenser 1** tab.
- 3 Select **Get Volumes**.
- 4 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 5.
  - If there is a mismatch:
    - a Press **CTRL+SHIFT+M** to enter maintenance mode for the Dispenser 1 window.
    - b Enter the syringe calibration values from the corresponding label on the rear of the dispense module.
    - c Select **Send Volumes** and then select **Get Volumes** to verify that the entered values were sent to the reader.
- 5 Select the **Dispenser 2** tab and repeat steps 3 and 4 for Dispenser 2.

## Gen6

- 1 Select **Configure** from the Gen6 Home screen.
- 2 Select **Dispensers** under Instrument Setup and select the **Dispenser 1** tab.
- 3 Select **Get Volumes**.
- 4 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 5.
  - If there is a mismatch:
    - a Press **CTRL+SHIFT+M** to enter maintenance mode for the Dispenser 1 window.
    - b Enter the syringe calibration values from the corresponding label on the rear of the dispense module.

### 3 Installation

#### Run a System Test

- c Select **Send Volumes** and then select **Get Volumes** to verify that the entered values were sent to the reader.
- 5 Select the **Dispenser 2** tab and repeat steps 3 and 4 for Dispenser 2.

## Run a System Test

---

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

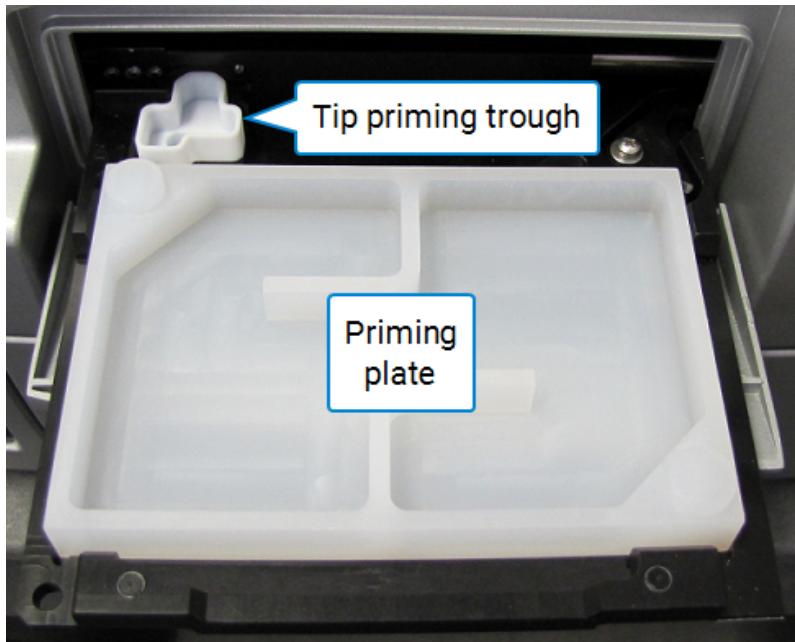
Instructions for performing the test using Gen5/Gen6 are provided in [System Test](#).

## Test the Injection System

---

*Applies only to models equipped with injectors*

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



**Figure 3-11:** Priming trough and plate installed on the carrier.

- 4 Fill the two reagent bottles with distilled or deionized water. Place the bottles in their holders, and place the holders directly in front of the syringes. Insert the inlet tubes into the bottles.
- 5 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5 and select the **Prime** tab.  
**Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen and select the **Prime** tab.

### 3 Installation

#### Operational/Performance Qualification

- 6 With Dispenser set to **1**, set the Volume to **5000  $\mu$ L** and click **Prime**. The syringe should move down and up repeatedly, drawing fluid from the bottle and pumping it through the tubing and into the priming plate. Examine the fittings; no leaks should be detected. If leaks are detected, tighten all fittings and repeat the prime. If leaks are still detected, contact Technical Support.
- 7 When finished, set the Volume to **2000  $\mu$ L** and select **Purge** to clear the fluid lines.
- 8 Set the Dispenser to **2** and repeat steps 6 and 7.
- 9 Remove and empty the priming plate.

## Operational/Performance Qualification

Your Synergy HTX was fully tested prior to shipment and should operate properly following the successful completion of the installation and setup procedures described in this chapter.

If you suspect that problems occurred during shipment, if you received the reader following service or repair, or if regulatory requirements dictate that Operational/Performance Qualification is necessary, turn to [Chapter 7 Instrument Qualification](#).

**NOTE**

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

## Repackaging and Shipping Instructions



**IMPORTANT**

Please read all of the information provided below before preparing the Synergy HTX for shipment.

**WARNING**

Decontamination prior to shipping is required by the U.S Department of Transportation regulations. If the instrument 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. See [Decontamination](#) for decontamination instructions.

**NOTICE**

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

**NOTICE**

Ensure the microplate carrier and tip prime trough are empty before shipment. Spilled fluids can contaminate the optics and damage the instrument.

- Contact Technical Support before returning equipment for service.
- 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.
- Replace the shipping hardware before repackaging the instrument. Please contact Technical Support if you need to replace any of these items.
- If you need to ship the instrument for service or repair, be sure to use the supplied packaging materials. Other forms of commercially available packaging are not recommended and can void the warranty.

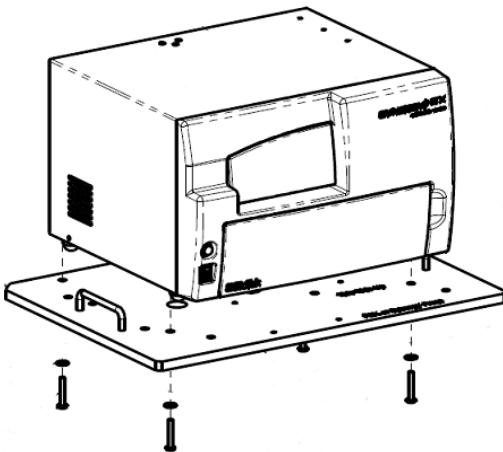
### 3 Installation

#### Repackaging and Shipping Instructions

- If the packaging materials have been damaged or lost, or if the same set has been used more than four times, contact Agilent to order a replacement set.

### Repackage the Instrument

- 1 Contact Technical Support for instructions.
- 2 Decontaminate the reader and, if attached, the dispense module, according to the instructions provided in [Decontamination](#).
- 3 If you will also be shipping the dispense module, perform the steps described in [Prepare the Dispense Module for Shipment](#). If you are not shipping the dispense module, disconnect it from the reader now.
- 4 If applicable, remove the tip priming trough from the microplate carrier.
- 5 Retract the microplate carrier. Turn off and unplug the reader.
- 6 Remove the lamp assembly and pack it in bubble wrap (reverse the instructions in [Install the Fluorescence Lamp Assembly](#)).
- 7 Replace the microplate carrier shipping bolt (reverse the instructions in [Remove the Shipping Hardware](#)).
- 8 Tip the reader onto its back. Attach the shipping panel to the bottom of the reader using the four screws and washers.

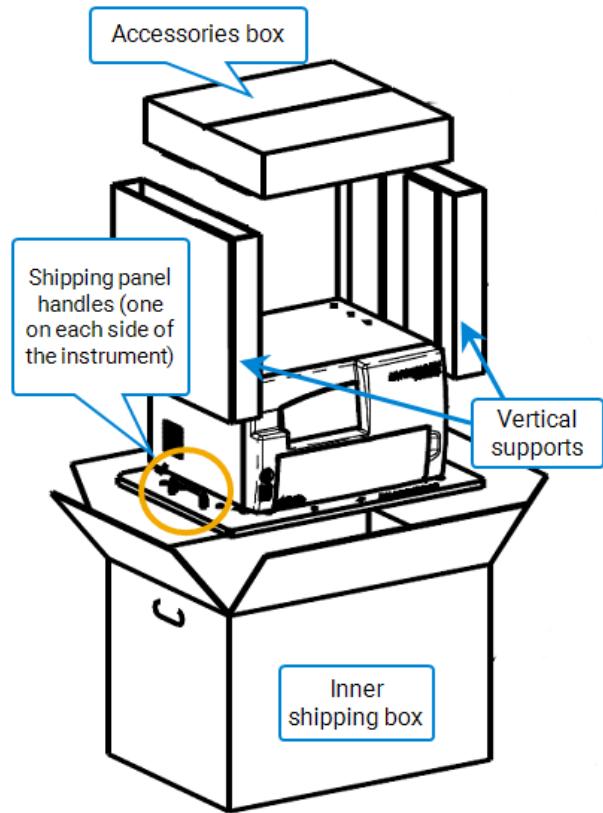


**Figure 3-12:** Reattach the shipping panel.

- 9 Wrap the plastic bag around the reader and shipping panel.
- 10 Locate the original outer shipping box. Place four foam blocks in the four bottom corners of the box. Place the inner shipping box inside the outer box.
- 11 Grasp the handles on the shipping panel and carefully lower the reader into the inner shipping box.

### 3 Installation

#### Repackaging and Shipping Instructions

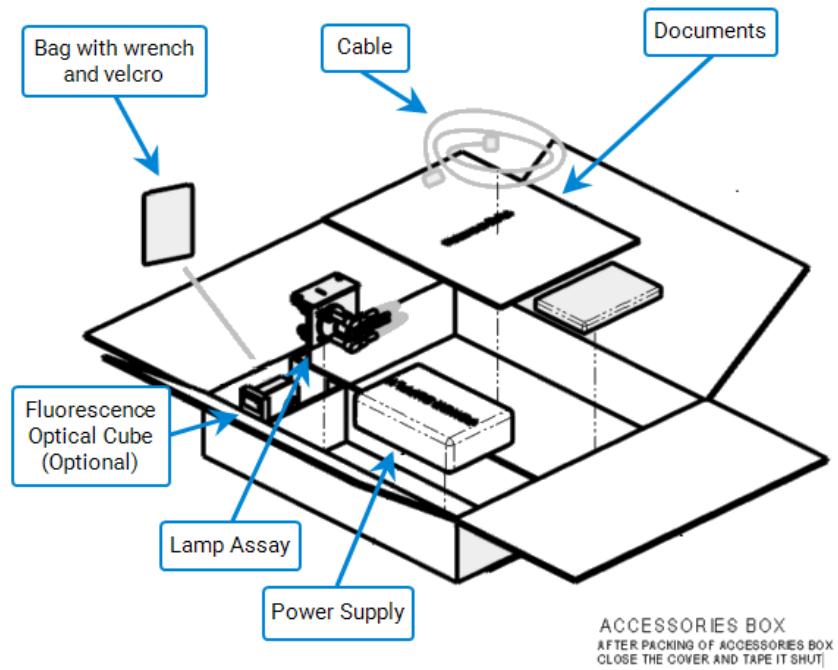


**Figure 3-13:** Lowering the reader into the inner shipping box.

- 12 Slide the foam vertical supports into place around the reader.
- 13 Repack the accessories box and place it on top of the reader.

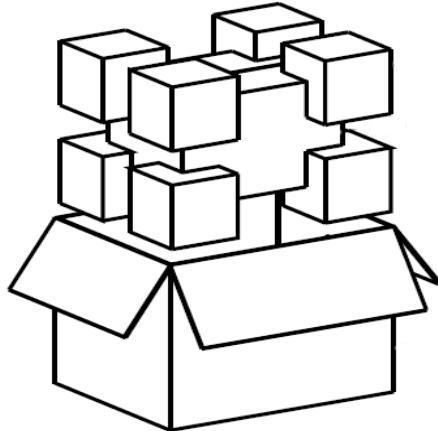
### 3 Installation

#### Repackaging and Shipping Instructions



**Figure 3-14:** Repacking the accessories box.

- 14 Close and seal the inner box with tape.
- 15 Place four foam corner blocks around the inner shipping box. Close and seal the outer box with tape.



**Figure 3-15:** Foam corner blocks around inner shipping box.

### 3 Installation

#### Repackaging and Shipping Instructions

## Prepare the Dispense Module for Shipment

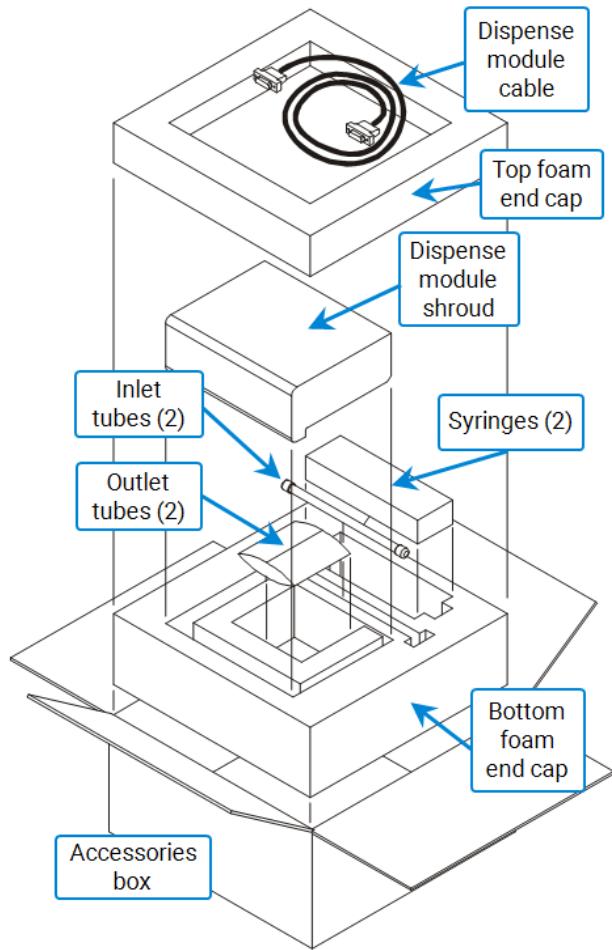
### NOTE

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

- 1 If you have not already done so, contact Technical Support for shipping instructions before returning equipment for service.
- 2 Decontaminate the module according to the instructions in [Decontamination](#). Be sure to purge the dispense module of all fluid when finished.
- 3 **Gen5 users:** With the reader on, start Gen5 and select **System > Instrument Control > Synergy HTX**.  
**Gen6 users:** With the reader on, start Gen6 and select **Prime/Dispense** from the Instrument Control area of the Home screen.
- 4 Perform this step twice, once per dispenser: Click the **Prime** tab and set the Dispenser number (**1** or **2**). Click **Maintenance**. The syringe bracket lowers. Remove the thumbscrew from underneath the bracket. Carefully unscrew the top of the syringe from the syringe valve. Lift out the syringe and store it in its original box.
- 5 Fully detach the dispense module from the reader. Set the module aside for the moment.
- 6 Remove the tip priming trough and store it in the dispenser accessories bag.
- 7 Remove the two inlet tubes from the syringe valves and store them in their plastic canisters.
- 8 Remove the two outlet tubes from the syringe valves. Attach the clear plastic shrouds to the fittings of the outlet tubes. Place the tubes in a plastic bag.
- 9 Remove the front cover from the dispenser.
- 10 Insert the bottom foam end cap in the dispenser module accessories shipping box and place the accessories in the insert.
- 11 Insert the bottom foam end cap in the shipping box, and place the dispense module inside the end cap.
- 12 Insert the foam insert that holds the reagent bottle holders and injector tubing into the shipping box and place the bottle holders and tubing in it.
- 13 Slide the dispenser accessories box into the shipping box.
- 14 Insert the top foam end cap. Close and seal the outer box with tape.

### 3 Installation

#### Repackaging and Shipping Instructions



**Figure 3-16:** Packing the dispense module accessories.

### 3 Installation

#### Repackaging and Shipping Instructions

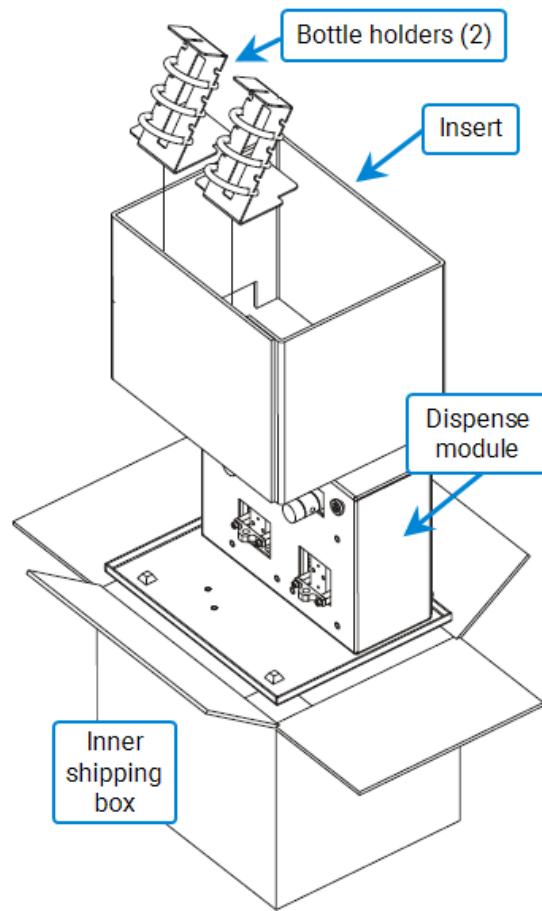


Figure 3-17: Packing the dispense module inner box.

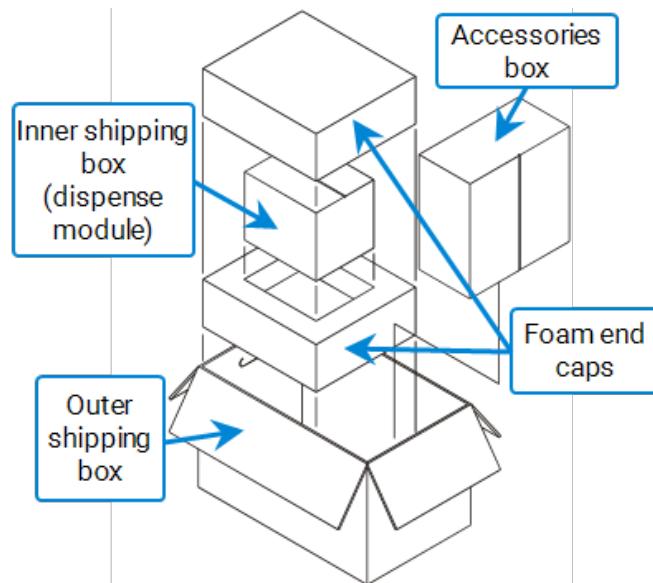


Figure 3-18: Packing the dispense module outer box.

## 4 Getting Started

This chapter provides an introduction to using the Synergy HTX. It also contains recommendations for optimum performance.

---

External Components .....	42
Internal Components .....	44
Getting Started with Gen5 Software .....	51
Getting Started with Gen6 Software .....	53
Dispense Module Control .....	55
Plate Shaking Options .....	56
Recommendations for Optimum Performance .....	57

## External Components

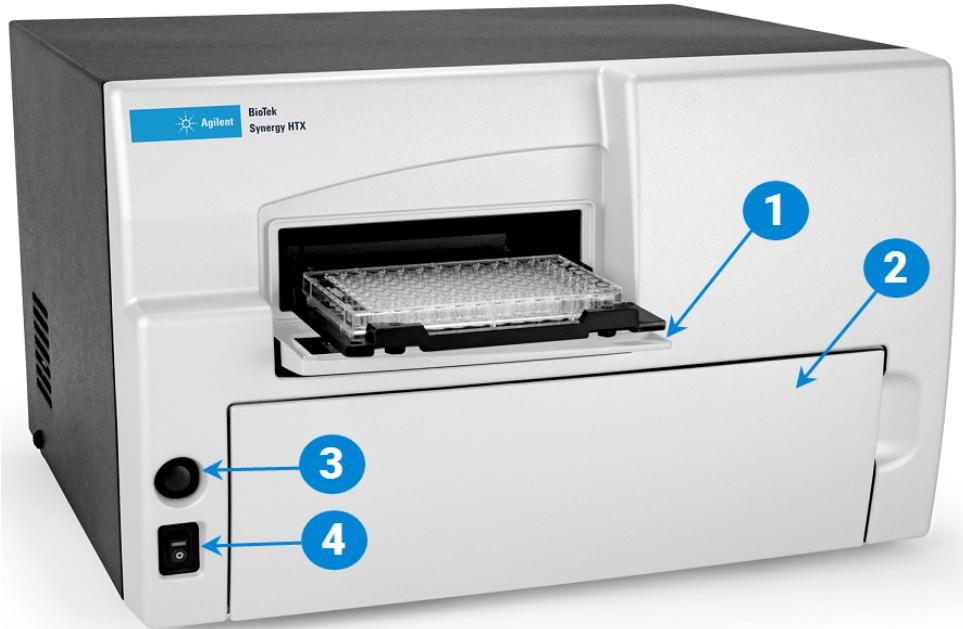


Figure 4-1: Synergy HTX, front view.

#	Component
1	Microplate carrier access door
2	Filter wheel and lamp access door
3	Carrier eject button
4	Power switch

- The power switch contains an LED, which is illuminated green when the power is on.
- The carrier eject button can be used to move the microplate carrier into or out of the measurement chamber and also to stop the instrument from beeping when it encounters an error.
- The microplate carrier supports microplates and adapter plates as described in [Appendix A Specifications](#) appendix. The plate is positioned so that well A1 is in the left rear corner of the carrier. A spring clip holds the plate securely in place. The microplate loading door helps to ensure a light-impermeable measurement chamber. When a plate read is initiated, the carrier slides into the measurement chamber and then moves on the X and Y axes to align each microwell with the top or bottom fluorescence probe, or bottom absorbance probe, as specified in the Gen5 procedure/Gen6 experiment. When the read is complete, the plate carrier slides to its full-out position.



TIP

For fluorescence and luminescence reading modes, the height of the top optic probe can be adjusted. Use the Read Height option to define how far the top probe shall be offset from the top surface of the plate during the read. In Gen5/Gen6, this option is found in the read step of a procedure/experiment. Refer to the Gen5/Gen6 Help for further instructions.

## 4 Getting Started

### External Components



Figure 4-2: Synergy HTX, top right of rear panel.

#	Component
1	Port for dispenser syringe 1 (with nylon screw installed)
2	Port for dispenser syringe 2 (with nylon screw installed)



Figure 4-3: Synergy HTX, bottom right of rear panel.

#	Component
1	Serial communication port
2	USB communication port
3	Dispenser communication port
4	Power inlet

## Internal Components

### Lamp Assembly and Filter Wheel Access

Applies only to models with fluorescence and/or luminescence capabilities

#### WARNING

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

#### NOTICE

**Fluorescence Lamp Assembly.** Do not touch the glass lenses. Fingerprints on the condenser lens or heat absorber may negatively affect performance.

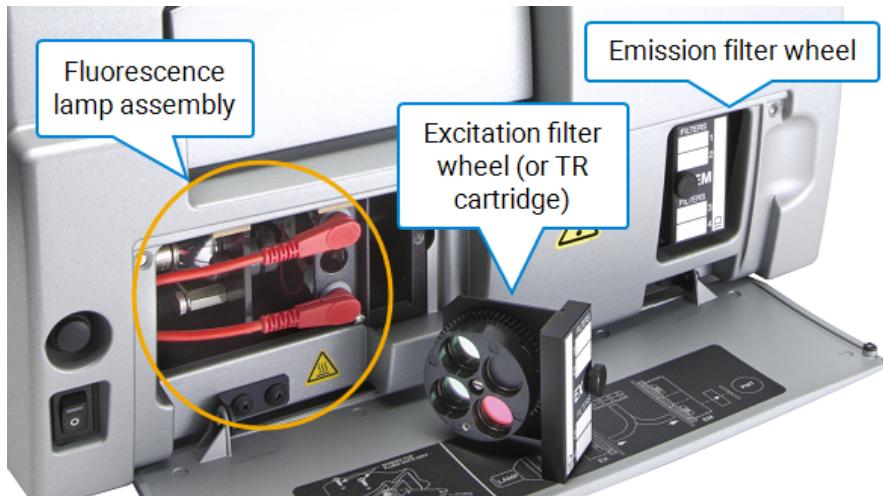


Figure 4-4: Fluorescence lamp assembly and filter wheels.

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

The Synergy HTX has two lamps:

- **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/Gen6 displays an error message. The lamp (part number 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 instru-

## 4 Getting Started

### Internal Components

ment will require service. Contact Technical Support for assistance (this lamp is not user-replaceable).

## Excitation and Emission Filter Wheels

### CAUTION

**Filters.** The reader is shipped with a set of excitation and emission filters installed. The reader's onboard software is preconfigured with the filter values and their locations. If you change the contents of a filter wheel, you must update Gen5/Gen6's filter table and then download the information to the reader. The reader does not automatically detect which filters are installed.

### NOTICE

When changing, cleaning, or replacing filters, it is critical that the filters be placed in the filter wheel in the correct orientation. 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. Do not touch the filters with your bare fingers.

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 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 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 show the proper direction of light through the filter.

Agilent recommends 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.

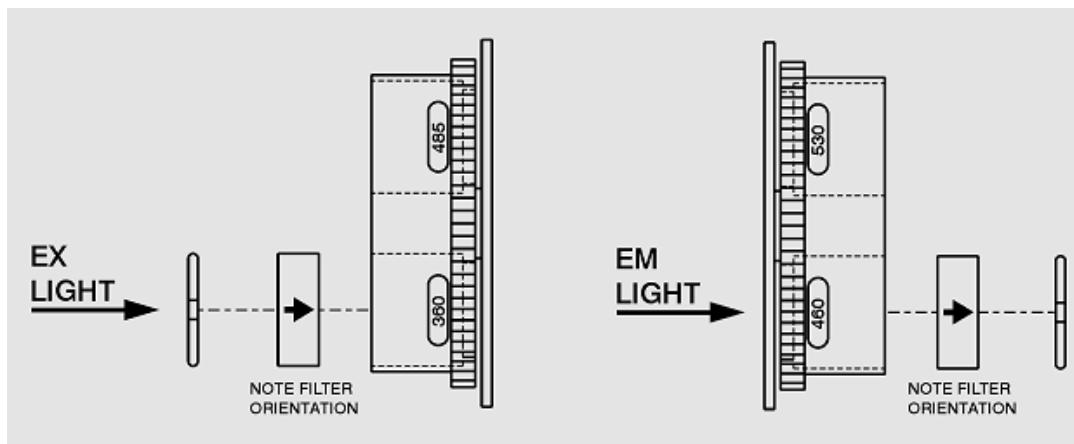


Figure 4-5: Diagram showing the proper orientation of the filters in their wheels.

## 4 Getting Started

### Internal Components

## Removing the Filter Wheels, Changing Filters

- The excitation and emission filter wheels are not interchangeable and are labeled as follows: EX = Excitation, EM = Emission. (TR = Time-Resolved Cartridge; see [Installing the Time-Resolved Cartridge](#).)
- Filter direction within a filter wheel is important, and the direction differs depending on the filter wheel. See the diagram on the inside of the instrument's front door.
- Each filter is marked with an arrow showing the proper direction of light. Refer to the figure on page [45](#) for proper filter orientation.

### To remove a filter wheel:

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

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

### To remove a filter or plug:

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

### To replace a filter or plug:

- 1 Hold the metal bracket with the filter wheel facing up.
- 2 Properly orient the filter or plug and drop it into the desired filter wheel location.
- 3 Using your fingers, squeeze the sides of the C-clip filter retainer, and then insert it into the top of the hole containing the new filter. Cover your finger with several layers of lens paper, and then push down on all sides of the C-clip until it sits flush against the filter.
- 4 Clean both sides of the filter with lens paper.

### To reinstall a filter wheel:

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

## Installing the Time-Resolved Cartridge

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

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

The Synergy HTX automatically detects the presence of the TR cartridge. At the start of a time-resolved fluorescence assay, Gen5/Gen6 will prompt you to install the TR cartridge if it is missing.

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



**Figure 4-6:** The TR cartridge for time-resolved fluorescence.

## Configuring the System for Luminescence Measurements

**CAUTION**

**Filters.** The reader is shipped with a set of excitation and emission filters installed. The reader's onboard software is preconfigured with the filter values and their locations. If you change the contents of a filter wheel, you must update Gen5/Gen6's filter table and then download the information to the reader. The reader does not automatically detect which filters are installed.

**NOTICE**

When changing, cleaning, or replacing filters, it is critical that the filters be placed in the filter wheel in the correct orientation. 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. Do not touch the filters with your bare fingers.

- For best results when taking luminescence measurements, the excitation filter wheel should have no empty locations, and it should have at least one "plug" (also referred to as a "dummy filter") installed to prevent light from reaching the samples. Remove the excitation filter wheel and examine its contents. Ensure that there are no empty locations and that 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 to ensure that there is an empty location.

## Injection System

**NOTICE**

Avoid continuous contact with harsh chemicals. Rinse the fluid path with deionized water after contact with any strong acid, base, or solvent.

**NOTE**

For information on the materials used in the injection system, see the *Agilent BioTek Dual-Reagent Injector Module Chemical Compatibility* technical note available on [Agilent.com](http://Agilent.com).

- The tubing and injectors should be cleaned at least every three months. See [Clean the Dispense Tubes and Injector Heads](#) for instructions.
- Inspect the injection 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. See [Dispense Module, Syringe Replacement](#) for instructions.

## Dispense Module

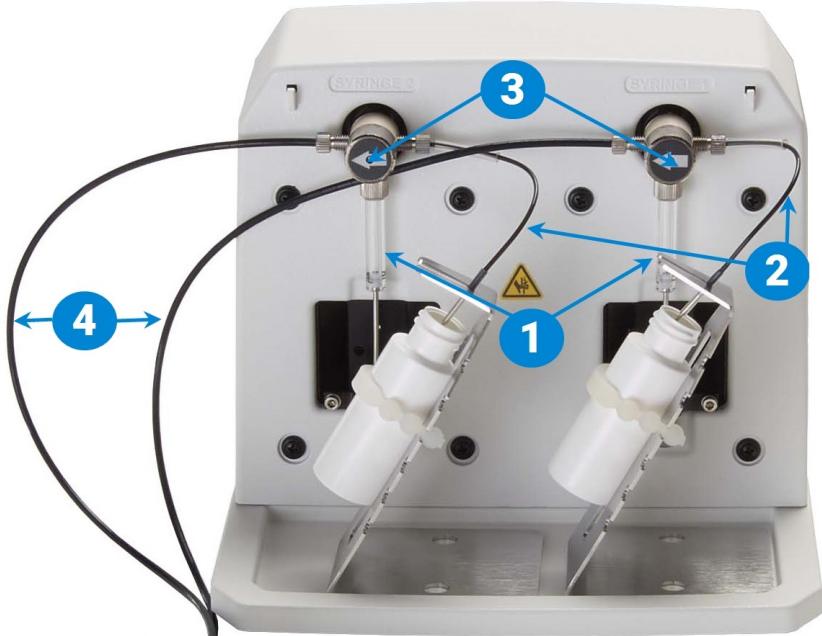
**NOTICE**

**Dispenser Tubing.** Do not bend the dispenser inlet or outlet tubes. Bending may cause the tubes to become permanently warped or pinched, which leads to obstructed liquid flow.

The dispense module 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.

## 4 Getting Started

### Internal Components



**Figure 4-7:** Dispense module components.

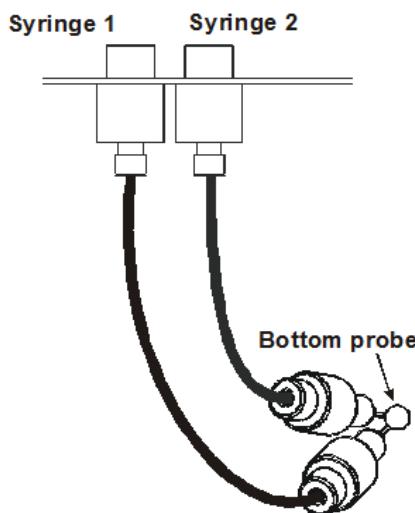
#	
1	Two 250 $\mu$ L syringes draw fluid from the supply bottles.
2	Inlet tubes transport fluid from the supply bottles to the syringes. These tubes are short pieces of opaque PTFE tubing connected to stainless-steel probes on one end and threaded fittings on the other end.
3	Valves switch the syringe flow from the inlet tubes to the outlet tubes.
4	Outlet tubes transport fluid from the syringes into the instrument, through the tubing ports on the reader's rear panel. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

## 4 Getting Started

### Internal Components

#### Internal Tubing

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



**Figure 4-8:** Injectors inside the reader.

#### Priming the Injection System

Before running a Dispense assay, use Gen5/Gen6 to prime the system with the reagent or dispensing fluid. An additional tip prime 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 using Gen5/Gen6 (see [Dispense Module Control](#)).

##### NOTICE

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.

Both types of primes require a fluid reservoir to be present on the microplate carrier. See [Test the Injection System](#).

- The priming plate is placed on the microplate carrier for a Prime operation, to prime the dispense system with fluid.
- The tip priming trough is placed in the rear pocket of the carrier, and is used for performing the Tip Prime before dispensing. The trough holds up to 1.5 mL of liquid and must be periodically emptied and cleaned by the user.
- Do not perform tip priming when using tall plates. Generally, plates with fewer than 96 wells are too tall for error-free tip priming and tip priming is rarely required for these larger-volume plates.
- The priming plate should be empty before priming and it should contain fluid after priming.

## 4 Getting Started

### Getting Started with Gen5 Software

## Getting Started with Gen5 Software

Gen5 software supports all Synergy HTX reader models. Use Gen5 to control the reader, the dispense module (if equipped), and the BioStack (if equipped); perform data reduction and analysis on the measurement values; print or export results; and more. This section provides brief instructions for working with Gen5 to create protocols and experiments and to read plates. For more information, refer to publications provided with Gen5 and the Gen5 help system ([Help > Help Topics](#)).

#### NOTE

The instructions in this section refer to **Standard** mode, which is accessible in Gen5 v. 3.06 and higher. **Simple** mode can be used after instrument installation is completed. Simple mode is a basic workflow designed for faster set up and execution of experiments; it limits user choices and guides users toward success using interactive dialogs.

## Viewing and Modifying Filter Wheel Information in Gen5

If configured with fluorescence or luminescence capability, the Synergy HTX ships with a set of excitation and/or emission filters installed, and the reader's onboard software is preconfigured with the filter values and their locations. When Gen5 establishes communication with the reader, it uploads and stores this information.

#### To view filter wheel information:

- 1 In Gen5, select **System > Instrument Configuration**.
- 2 Highlight the **Synergy HTX**, and select **View/Modify**.
- 3 Select **Setup** and the **Fluorescence/Luminescence** tab.

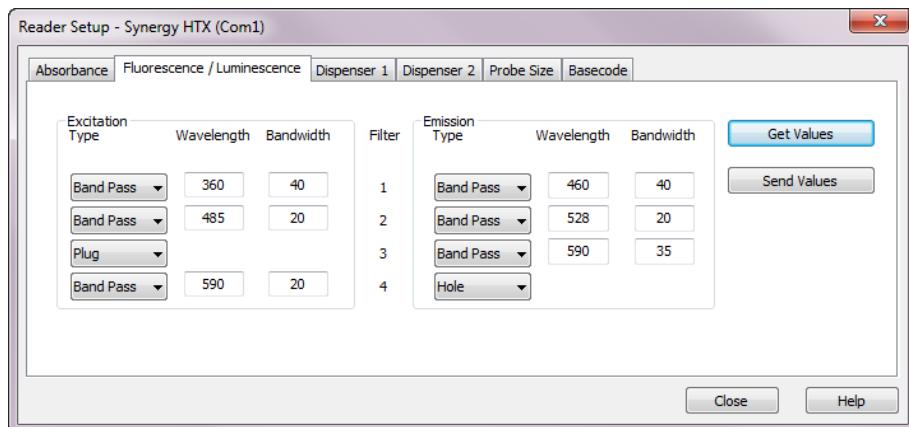


Figure 4-9: Viewing EX/EM filter wheel information in Gen5.

#### To modify the settings and download them to the instrument:

- 1 Select **Band Pass**, **Plug**, or **Hole** from the drop down lists for Excitation Type and Emission Type.
- 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, select **Send Values** to download the information to the reader. (Selecting **Get Values** uploads information from the reader.)

## 4 Getting Started

### Getting Started with Gen5 Software

- 4 Select **OK** to save the settings and close this dialog. The settings are now available for selection in the Read step dialog in a Procedure.

## Protocols and Experiments

In Gen5, a protocol contains instructions for controlling the reader and (optionally) 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. Run the experiment to read plates and analyze the data.

### To create a protocol:

- 1 Optional, but recommended: Create the protocol with the reader connected to the computer and turned on.
- 2 In the Gen5 Task Manager, select **Protocol > Create New**.
- 3 Open the Procedure dialog. If prompted to select a reader, select **Synergy HTX** and click **OK**.
- 4 Select a **Plate Type** to match your assay plate.



**Gen5 stores measurements and other characteristics for individual plate types in a database. It is essential that you select the plate type to match the assay plate. Otherwise, results may be invalid.**

**IMPORTANT**

- 5 Add a **Read** step to the Procedure.
  - a Choose a **Detection Method**. You will only be able to select options that are applicable to your instrument configuration.
  - b Choose a **Read Type**: Endpoint/Kinetic, Spectral Scanning, or Area Scanning.
  - c Choose an **Optics Type**: Filters, Monochromators, or Luminescence fiber. Click **OK**.
  - d The Read Step dialog contains parameters specific to the Detection Method. Click the **Help** button for guidance.
  - e If the experiment will be run on a partial plate, click the **Full Plate** button and highlight the wells to read.
  - f Click **OK** to return to the Procedure dialog.
- 6 If this is a kinetic protocol, click **Start Kinetic** to add a kinetic loop. Drag the **Read** step and drop it between the **Start** and **End Kinetic** steps.
- 7 Click **Validate** to confirm that the reader supports the defined steps. Follow any instructions provided by Gen5 to adjust the protocol parameters.
  - If the reader is connected and turned on, Gen5 will communicate with the reader during this step to ensure that the protocol is valid.
  - If the reader is not connected, Gen5 will validate the syntax of the protocol (for example, the Read step is set between the Start and End Kinetic steps). Later, when you run the experiment, Gen5 conducts another check and will present a message if any Gen5 protocol settings require changing before the experiment can be run.
- 8 Optionally, perform any of these steps to analyze and report the results:
  - Open the **Plate Layout** dialog and assign blanks, samples, controls, and/or standards to the plate.
  - Open the **Data Reduction** dialog to add analysis steps. Categories include Transformation,

## 4 Getting Started

### Getting Started with Gen6 Software

Well Analysis, Curve Analysis, and Qualitative Analysis.

- Create a report or export template via the **Report/Export Builders**.
- 9 Select **File > Save** and give the file an identifying name.

**To create an experiment and read a plate:**

- 1 In the Gen5 Task Manager, select **Experiment > Create using an existing protocol**.
- 2 Select the desired protocol and click **OK**.
- 3 Select a plate in the menu tree and click the **Read Now** button.
- 4 When the read is complete, measurement values appear in Gen5. Select the desired data set from the Data list.
- 5 Select **File > Save** and give the file an identifying name.

## Getting Started with Gen6 Software

Gen6 software supports all Synergy HTX reader models. Use Gen6 to control the reader, the dispense module (if equipped), and the BioStack (if equipped); perform data reduction and analysis on the measurement values; print or export results; and more. This section provides brief instructions for working with Gen6 to create experiments and read plates. For more information, refer to publications provided with Gen6 and the Gen6 help system (**Help > Help Topics**).

### Viewing and Modifying Filter Wheel Information in Gen6

If configured with fluorescence or luminescence capability, the Synergy HTX ships with a set of excitation and/or emission filters installed, and the reader's onboard software is preconfigured with the filter values and their locations. When Gen6 establishes communication with the reader, it uploads and stores this information.

**To view filter wheel information:**

- 1 Select **Configure**.
- 2 Highlight the **Synergy HTX**.
- 3 Select **Filter Wheels** under Instrument Setup. The **Fluorescence/Luminescence** tab opens.

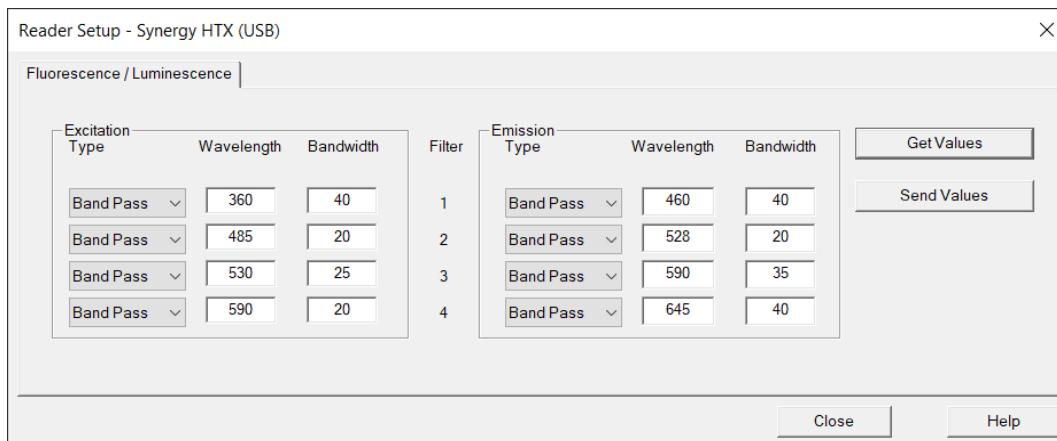


Figure 4-10: Viewing EX/EM filter wheel information in Gen6.

## 4 Getting Started

### Getting Started with Gen6 Software

#### To modify 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 when formatting a Read step in an experiment Method.

## Experiments

#### To create and run an experiment:

- 1 Optional, but recommended: Create an experiment with the reader connected to the computer and turned on.
- 2 Select **File > New** or click the **New Experiment** button.
- 3 Highlight a plate type that matches your assay plate, and click **Select Plate**.



#### IMPORTANT

Gen6 stores measurements and other characteristics for individual plate types in a database. It is essential that you select the plate type to match the assay plate. Otherwise, results may be invalid.

- 4 Add a read step:
  - a Select the **METHOD** box on the left side of the screen.
  - b Select **+Add Steps** and select **Read**.
  - c Choose a **Read Type**: Endpoint, Area Scan, Kinetic, or Spectral Scan. If you choose Kinetic, the Read step will be placed between the Start and End Kinetic steps.
  - d Select the desired **Read Mode**. You will only be able to select options that are applicable to your instrument configuration.
  - e If applicable, a list of optics types will appear under the selected Read Mode. Choose the desired optics type, then select **OK**.
  - f Parameters specific to the selected Read Mode are displayed. Select the **Help** button for guidance.
  - g If the experiment will be run on a partial plate, select **Full Plate** in the Wells area and highlight the wells to read. Selected wells are represented by a dark blue circle.
  - h Select **Apply** when you are finished setting the parameters for your read step.
- 5 Select **Validate** to confirm that the reader supports the defined steps. Follow any instructions provided by Gen6 to adjust the parameters.
  - If the reader is connected and turned on, Gen6 will communicate with the reader during this step to ensure that the method is valid.
  - If the reader is not connected, Gen6 will validate the syntax (for example, the Read step is set between the Start and End Kinetic steps). Later, when you run the experiment, Gen6 conducts another check and will present a message if any method parameters require changing before the experiment can be run.
- 6 Select **OK** to return to the method.

## 4 Getting Started

### Dispense Module Control

- 7 Optionally, add steps to the experiment to analyze and report the results. The following options are found on the bottom left of the screen, next to the Validate button.
  - Select **PLATE LAYOUT** to assign blanks, samples, controls, and standards to the plate.
  - Select **ANALYZE DATA** to add data analysis steps. You are only able to select options that are applicable to the method you defined. Hover the mouse over an option to learn more about it.
  - Select **EXPORTS** to create a template for exporting (reporting) results to Excel.
- 8 When you are ready to run the experiment, place a plate on the carrier, select **Read Plate**, and select **OK**.
- 9 When the read is complete, measurement values appear in Gen6.
- 10 Select the desired Data View from the list on the Matrix or Stats tab.
- 11 Select the Excel icon to export results.
- 12 Select **File > Save As** or **Save As** to save the experiment with the results.

## Dispense Module Control

---

*Applies only to models equipped with injectors*

Gen5/Gen6 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 information provided with Gen5/Gen6 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 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5, and then select the **Prime** tab.  
**Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen, and then select 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  $\mu$ L**.
- 6 Enter a prime **Rate**, in  $\mu$ L/second.
- 7 Select **Prime** to start the process. When finished, carefully remove the priming plate from the carrier and empty it.

#### NOTE

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

## 4 Getting Started

### Plate Shaking Options

## Purge

To conserve reagent, Gen5/Gen6 provides the option to purge fluid from the system back into the supply bottle.

- 1 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5, and then select the **Prime** tab.
- 2 **Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen, and then select the **Prime** tab.
- 3 Select the **Dispenser** number (1 or 2) associated with the supply bottle.
- 4 Enter the desired purge **Volume** in  $\mu\text{L}$  (e.g., 2000).
- 5 Enter a prime **Rate** in  $\mu\text{L}/\text{second}$ .
- 6 Select **Purge** to start the process.

## Plate Shaking Options

The Synergy HTX supports multiple plate shaking options, as described in the table below. Shaking is controlled using Gen5 by adding a Shake Step to a protocol's procedure, or using Gen6 by adding a Shake Step to an experiment's method.

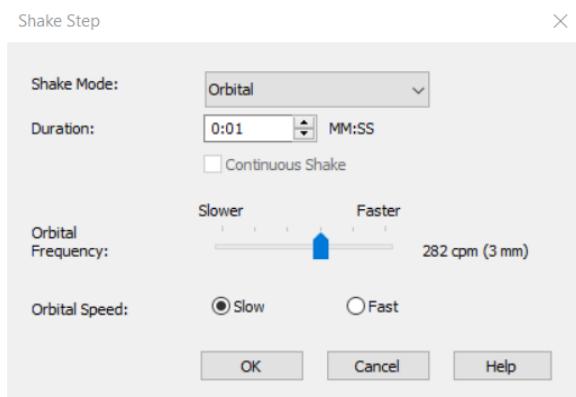


Figure 4-11: Gen5 Shake Step options.

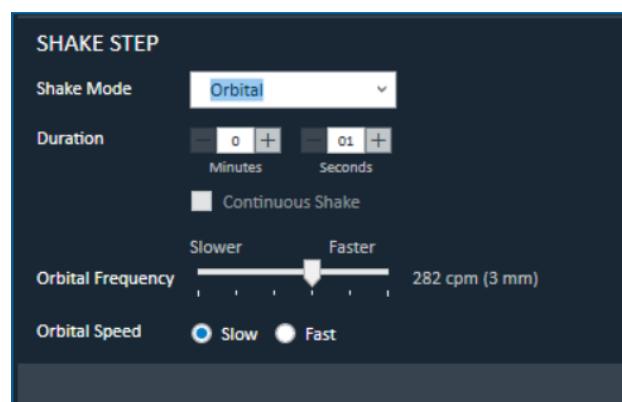


Figure 4-12: Gen6 Shake Step options.

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

\* Frequency is based on the Amplitude selected

## Recommendations for Optimum Performance

---

### General

- Microplates should be clean and free from dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.
- Before preparing your microplates, make sure the reader is on and communicating with Gen5/Gen6. You may want to run a System Test if the reader has not been turned off/on in a few days. Design your Gen5 protocol/Gen6 experiment 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, when using absorbance, the reader achieves optimum performance with flat-bottomed wells. See [Appendix A Specifications](#) for more information on the supported plates.
- When using absorbance, nonuniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results in most cases, use at least 100  $\mu$ L per well in a 96-well plate, and 25  $\mu$ L in a 384-well plate.
- Pipetting solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies. For best results, however, remove the air bubbles by degassing the plate in a vacuum chamber or spinning the plate in a centrifuge before reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Shake the microplate before reading to help bring it within acceptable limits. Use Tween 20 if possible (or some other wetting agent) to normalize the meniscus for absorbance measurements. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change. The meniscus shape will stabilize over time.
- It is the user's responsibility to understand the volumetric limits of the plate type in use as it applies to the assay being run.
- Use of liquids with concentrations of acids, corrosives, or solvents of 3% and greater can begin attacking the materials inside the instrument's chamber. Running multiple plates with concentrations < 3% in long kinetic experiments may also have a destructive effect. If the experiment is incubated, it will accelerate the deterioration of chamber components. When in doubt about the use of acids, corrosives, or solvents, please contact Technical Support.

### Using 384-Well Microplates

When using a 384-well microplate, you can use the Auto Map feature to ensure you are using an accurate plate map for your reads. See the Gen5/Gen6 Help for more information.

## 4 Getting Started

### Recommendations for Optimum Performance

## Luminescence Measurements

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

## Models with Injectors

- To keep the dispense system in top condition, flush and purge the fluid lines with deionized (DI) water every day or upon completion of an assay run, whichever is more frequent. Some reagents may crystallize or harden after use, clogging the fluid passageways. Flushing the tubing at the end of each day, letting the DI water soak, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. See [Chapter 5 Periodic Maintenance](#) for more information.
- When dispensing volumes less than or equal to 20  $\mu$ L/well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20  $\mu$ L/well, we recommend a tip prime volume of 20  $\mu$ L.
- To avoid spillage and possible contamination of the instrument, empty the tip prime trough frequently and do not exceed the total fluid volume of the plate well when dispensing.

## Incubation and Partial Plates

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

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

# 5 Periodic Maintenance

This chapter provides instructions for cleaning and maintaining the Synergy HTX and the external dispense module.

---

Periodic Maintenance Overview .....	60
Daily Cleaning for the Dispense Module .....	60
Recommended Maintenance Schedule .....	61
Maintenance Hazards .....	62
Clean Exposed Surfaces .....	63
Inspect/Clean Excitation and Emission Filters .....	64
Flush/Purge Fluid Path .....	65
Run a Dispense Protocol (Optional) .....	66
Empty/Clean the Tip Priming Trough .....	67
Clean the Priming Plate .....	67
Clean the Internal Components (As Applicable) .....	67

## 5 Periodic Maintenance

### Periodic Maintenance Overview

## Periodic Maintenance Overview

---

A general maintenance regimen for the Synergy HTX includes periodically cleaning all exposed surfaces and inspecting and cleaning the excitation and emission filters, if applicable.

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.



**IMPORTANT** Review the [Maintenance Hazards](#) before performing any maintenance procedure.

## Daily Cleaning for the Dispense Module

---

To ensure accurate performance and a long life for the dispense module and injectors, flush and purge the fluid lines with deionized (DI) water every day or after completing an assay run, whichever is more frequent. Some reagents may crystallize or harden and then clog the fluid passageways. Take special care when using molecules that are active at very low concentrations (e.g., enzymes, inhibitors). Remove any residual reagent in the dispense lines using a suitable cleaning solution. Review the reagent's package insert for specific recommendations.

Flushing the tubing at the end of each day, letting the DI water soak overnight, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. Perform a visual inspection of the dispense accuracy before running an assay protocol that includes dispense steps.

It is also recommended to flush the module with DI water before conducting the decontamination procedure described in [Decontamination](#).

**Models with injectors:** Accumulated algae, fungi, or mold may also require decontamination.

## 5 Periodic Maintenance

### Recommended Maintenance Schedule

## Recommended Maintenance Schedule

This table recommends maintenance tasks for the Synergy HTX and the frequency to perform each task.

#### NOTE

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

Task	Daily	Quarterly	As Needed
<b>All models:</b>			
Clean exposed surfaces			✓
Decontaminate the instrument		before shipment or storage	
<b>Models with fluorescence and/or luminescence capability:</b>			
Inspect/clean excitation and emission filters		✓	
<b>Models with injectors and an external dispense module:</b>			
Flush/purge the fluid path	✓		
(Optional) Run a Dispense protocol			✓
Empty/clean tip prime trough	✓		
Clean priming plate			✓
Clean internal components (as applicable)		✓	✓
• Tubing and injector heads			
• Optical probes			
• Internal surfaces			

## 5 Periodic Maintenance

### Maintenance Hazards

## Maintenance Hazards

---

Read the following before performing any maintenance procedures:

#### ⚠️ **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.

#### ⚠️ **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.

#### **NOTICE**

**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.

#### **NOTICE**

**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%.

#### **NOTICE**

**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.

#### **NOTICE**

**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.

## 5 Periodic Maintenance

### Clean Exposed Surfaces

## Clean Exposed Surfaces

---

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

### Materials

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

### Procedure

- 1 Turn off and unplug the instrument.
- 2 Wet a cloth or paper towel with water, or with water and mild detergent, and then thoroughly wring it out so that liquid does not drip from it. *Do not soak the cloth.*
- 3 Wipe the plate carrier, the inside of the plate carrier door and front access door, and all exposed surfaces of the instrument.
- 4 Wipe all exposed surfaces of the dispense module (if used).
- 5 If detergent was used, wipe all surfaces with a cloth moistened (not soaked) with water.
- 6 Use a clean, dry cloth to dry all wet surfaces.

**NOTE**

If liquid is spilled inside the reader, contact Technical Support.

**NOTE**

**Models with injectors:** If the tip priming trough overflows or other spills occur inside the instrument, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. If overflow is significant, you may need to remove the reader's shroud to better access the interior; see [Clean the Internal Components \(As Applicable\)](#).

## 5 Periodic Maintenance

### Inspect/Clean Excitation and Emission Filters

# Inspect/Clean Excitation and Emission Filters

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

Agilent recommends inspecting the filters for dust and other debris every three months.

## Required Materials

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

## Procedure

### NOTICE

Do not touch the filters with your bare fingers.

- 1 Turn off and unplug the instrument.
- 2 Pull down the hinged door on the front of the instrument. Locate the two thumbscrews within the compartment. The left thumbscrew holds the excitation (EX) filter wheel in place; the right secures the emission (EM) filter wheel. Remove each thumbscrew and pull the filter wheel out of the compartment.

### NOTE

[Chapter 4 Getting Started](#) contains illustrations for identifying the filter wheels and their unique characteristics. It also contains instructions for replacing filters, if necessary.

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

## 5 Periodic Maintenance

### Flush/Purge Fluid Path

## Flush/Purge Fluid Path

---

*Applies only to models equipped with injectors*

At the end of each day that the dispense module is in use, flush the fluid path using the Gen5/Gen6 priming utility. Leave the fluid to soak overnight or over a weekend, and then purge the fluid before using the instrument again.

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

#### To flush the fluid path:

- 1 Fill two supply bottles with deionized or distilled water. Insert the supply (inlet) tubes into the bottles.
- 2 Place the priming plate on the carrier.
- 3 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5.  
**Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen.
- 4 Select the **Prime** tab and then select Dispenser **1**.
- 5 Set the Volume to **5000  $\mu$ L**. Keep the default prime rate.
- 6 Select **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 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5.  
**Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Home screen.
- 3 Select the **Prime** tab and then select Dispenser **1**.
- 4 Set the Volume to **2000  $\mu$ L**.
- 5 Select **Purge** to start the process.
- 6 When the purge is complete, repeat the process for Dispenser **2**.

## 5 Periodic Maintenance

### Run a Dispense Protocol (Optional)

*Applies only to models equipped with injectors*

After flushing/purging the system (see [Flush/Purge Fluid Path](#)) and before running an assay that requires dispense, take a moment to visually inspect the dispense accuracy.

Use a DI H<sub>2</sub>O–Tween solution to visually inspect the dispense accuracy following maintenance: e.g., add 1 mL Tween 20 to 1000 mL of deionized water.

- 1 Create a new protocol in Gen5 or a new experiment in Gen6. Select a **Plate Type** that matches the plate you are using.
- 2 Add a **Dispense** step with the following parameters:
  - a Select Dispenser **1**.
  - b Set Tip Priming to **Before this dispense step** and Volume to **10 µL**.
  - c Set the Dispense Volume to **100 µL** (or an amount to match your assay protocol).
  - d Adjust the Rate to support the dispensing volume.
- 3 Add another **Dispense** step with the same parameters, selecting Dispenser **2**.
- 4 Add a quick **Read** step with parameters relevant to your reader model. This is necessary because Gen5/Gen6 requires that a Read step follow the Dispense step.
- 5 Save the protocol/experiment with an identifying name, such as “Dispense Observation.”
- 6 Fill the supply bottles with the DI H<sub>2</sub>O–Tween solution mentioned above.
- 7 **Gen5 users:** Create an experiment in Gen5 based on the Dispense Observation protocol and select **Read**.  
**Gen6 users:** Select **Read Plate** to run the experiment in Gen6.
- 8 When the experiment is complete, visually assess the fluid level in the wells. Well volumes should appear evenly distributed across the plate.

If the well volume appears to be unevenly distributed, clean the internal dispense tubes and injectors as described in [Clean the Internal Components \(As Applicable\)](#) and run the protocol again.

## 5 Periodic Maintenance

### Empty/Clean the Tip Priming Trough

*Applies only to models equipped with injectors*

The tip priming trough is a removable cup located in the rear pocket of the microplate carrier, used for performing the tip prime. The trough holds about 1.5 mL of liquid and must be periodically emptied and cleaned. Gen5/Gen6 instructs you to do this at the start of an experiment that requires dispensing.

- 1 Extend the microplate carrier and carefully remove the tip priming trough from its pocket in the 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

*Applies only to models equipped with injectors*

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

## Clean the Internal Components (As Applicable)

### NOTICE

**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.

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

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

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

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

## Required Materials

For all tasks:

- Protective gloves
- Safety glasses

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

For removing the shroud and some of the internal components:

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

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, part number 2872304)

For cleaning the optical probes:

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

## Remove the Reader's Shroud

- 1 If applicable, purge the injection system of all fluid.
- 2 Disconnect power and all cables. If applicable, set the external dispense module aside.
- 3 Remove four mounting screws: one at the bottom rear corner on each side, and two at the top center of the rear panel.

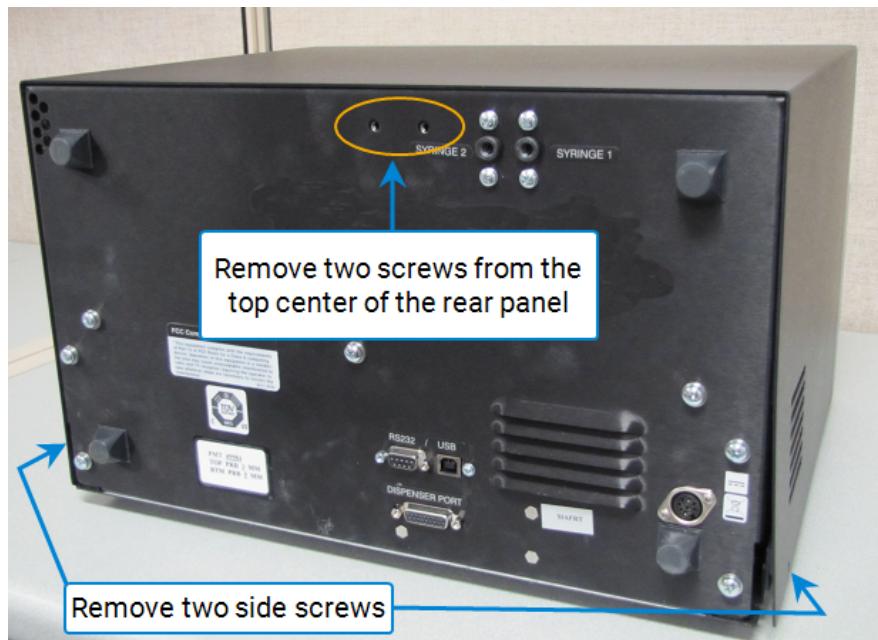


Figure 5-1: Removing the screws that secure the shroud.

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

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

**NOTE**

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

## Remove the Internal Dispense Tubes and Injector Heads

*Applies only to models equipped with injectors*

Review the next figure to identify the components described in this section:

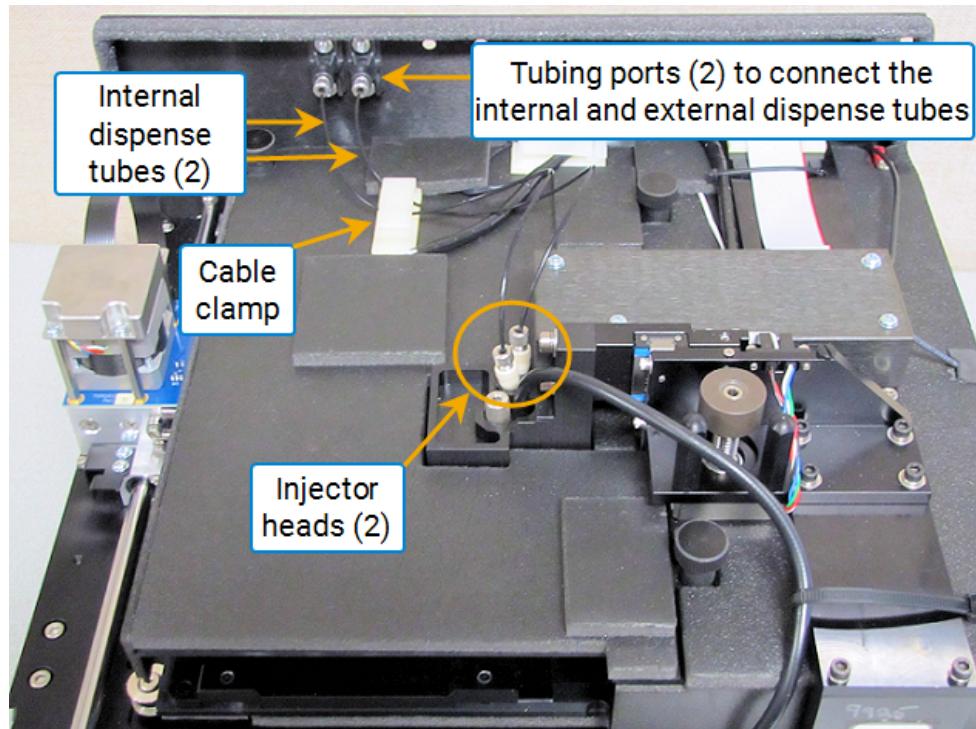
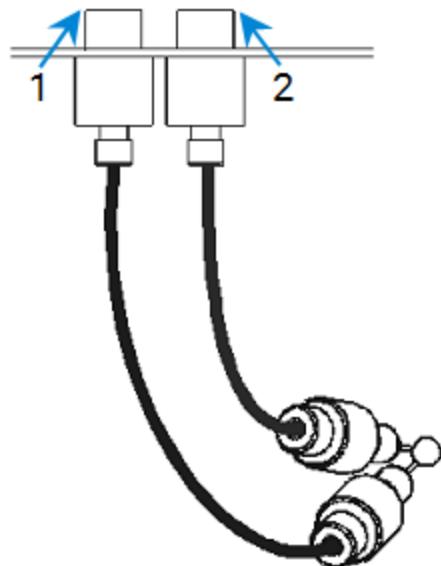


Figure 5-2: Internal components for the injection system.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

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



**Figure 5-3:** Identifying the numbered syringe ports on the back of the reader

#### To remove both sets of internal dispense tubes and injector heads:

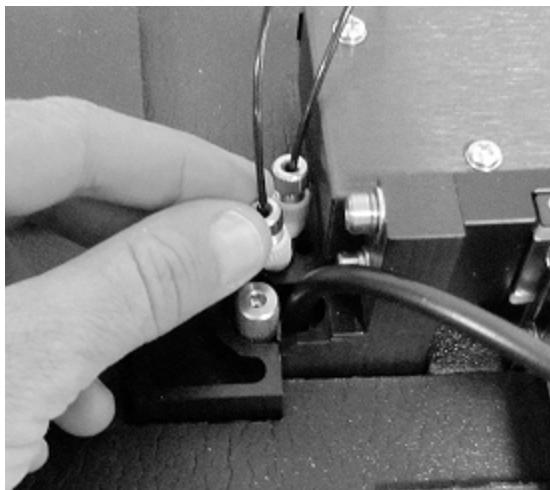
- 1 Open the cable clamp to release the tubes.
- 2 Locate the tubing ports on the reader's rear wall. Turn each tube's thumbscrew counterclockwise and gently pull the tube from the port.
- 3 Locate the injector heads. Turn each tube's thumbscrew counterclockwise to disconnect the tube from the injector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

## 5 Periodic Maintenance

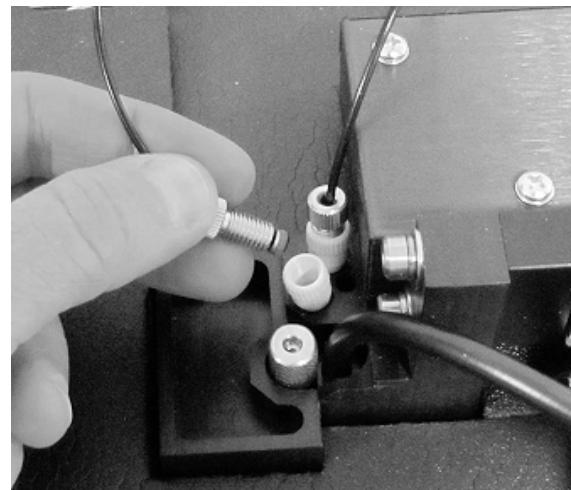
Clean the Internal Components (As Applicable)



**Figure 5-4:** Locating the dispense tube thumbscrew.



**Figure 5-5:** Turning the thumbscrew counterclockwise.



**Figure 5-6:** The dispense tube disconnected from the injector head.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

- 4 Turn the injector heads counterclockwise and gently pull them out of their sockets. Refer to the next two figures.

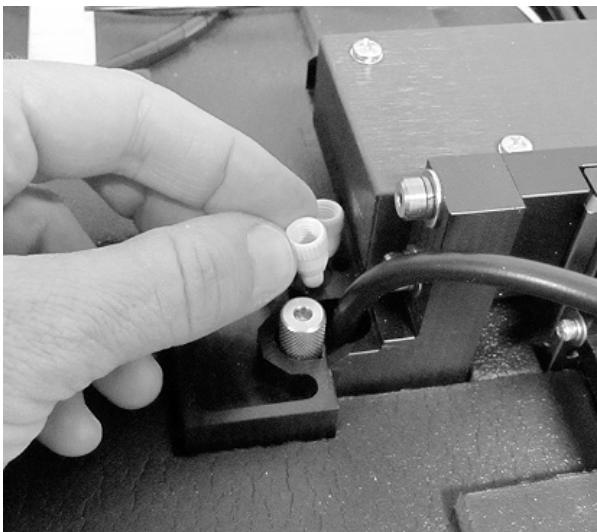


Figure 5-7: Turning the injector head counterclockwise.



Figure 5-8: Removing the injector head from its socket.



Be sure to seat the injector tips securely when reinstalling. See [Reassemble the Components](#).

#### IMPORTANT

## Clean the Dispense Tubes and Injector Heads

*Applies only to models equipped with injectors*

As discussed in [Daily Cleaning for the Dispense Module](#), 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 or injectors.



Figure 5-9: Injector heads and internal dispense tubes.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

#### To clean the internal tubes:

- 1 Soak the 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.

#### To clean the injector tips:

- 1 Gently insert the stylus into each injector tip to clear any blockages. The stylus (BTI #2872304) is stored in a cylinder affixed to the back of the dispense module or reader.
- 2 Stream water through the pipe to be sure it is clean. If the water does not stream out, try soaking in hot, soapy water and then reinserting the stylus.

#### NOTICE

Do not bend the injector tips. A bent tip may not dispense accurately.

Do not remove the O-rings (if equipped).

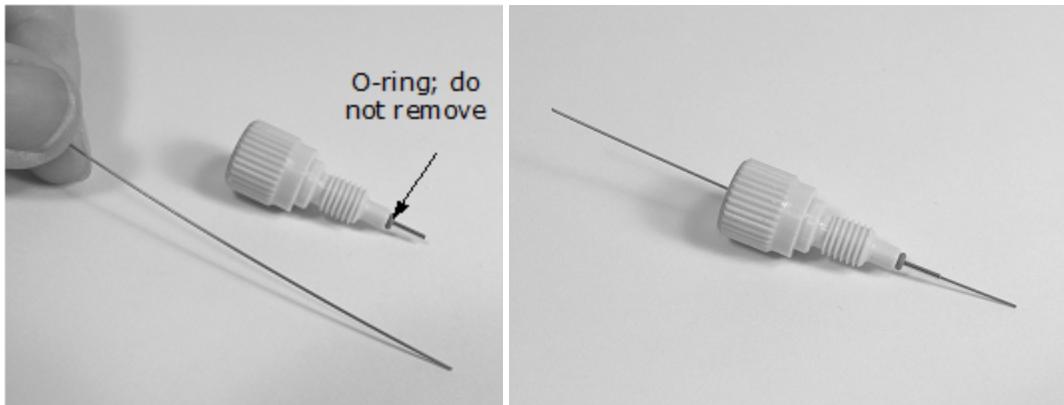


Figure 5-10: Using the stylus to clean an injector head.

## Clean the Optical Probes



#### IMPORTANT

Steps 1 through 6 of this procedure must be performed before you can progress to [Clean the Reader's Internal Surface](#)

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

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

- To access the optical probes, the first step is to unplug the reader and remove its shroud (cover). If you haven't already done this, see [Remove the Reader's Shroud](#) 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 in [Clean the Dispense Tubes and Injector Heads](#).

## 5 Periodic Maintenance

Clean the Internal Components (As Applicable)

### Required Materials

- Small container of isopropyl alcohol
- Small container of deionized or distilled water
- Lens-cleaning tissue
- Cotton swabs

See the next figure to identify the components described in this section.

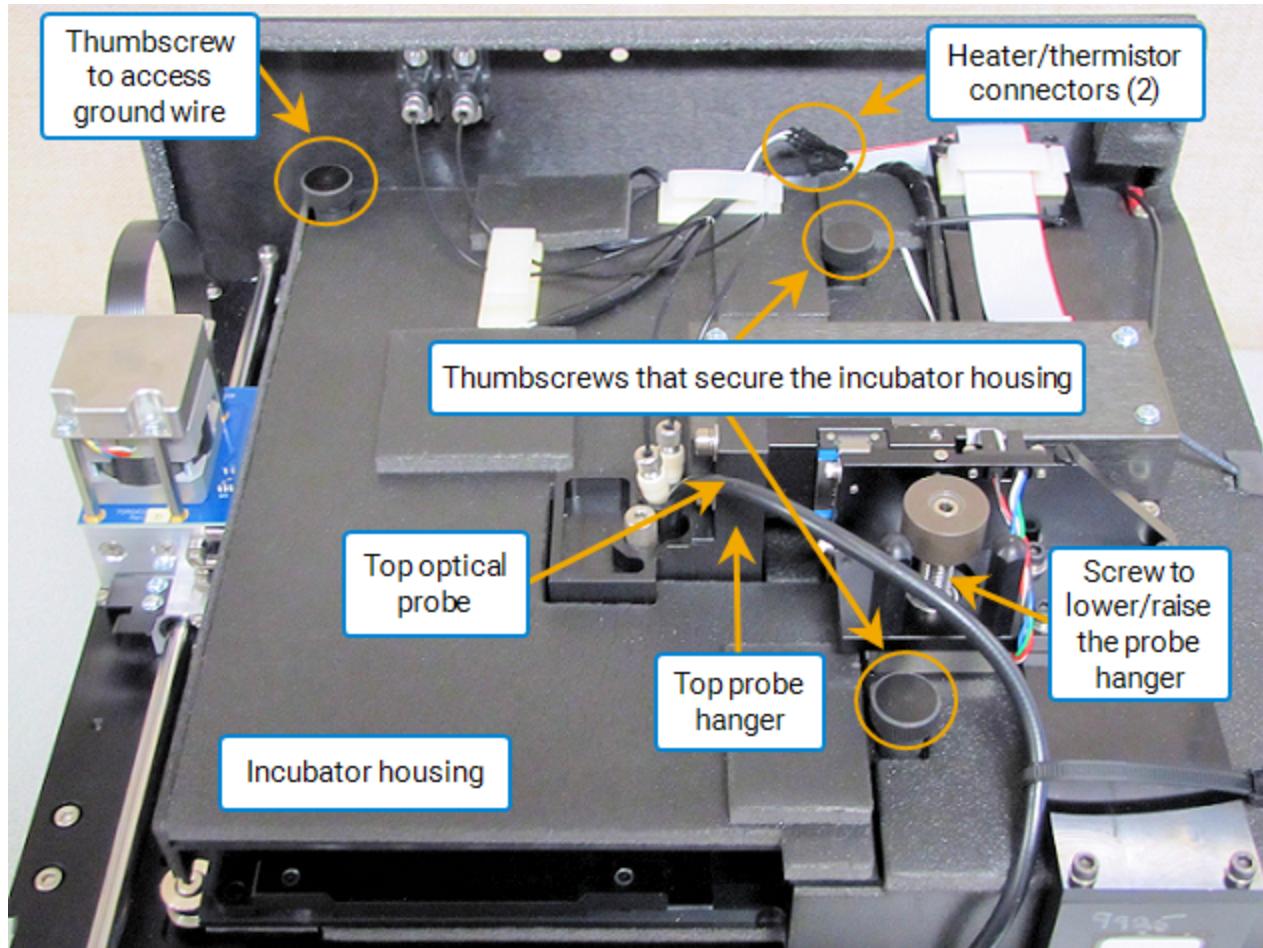


Figure 5-11: Internal components discussed in this section.

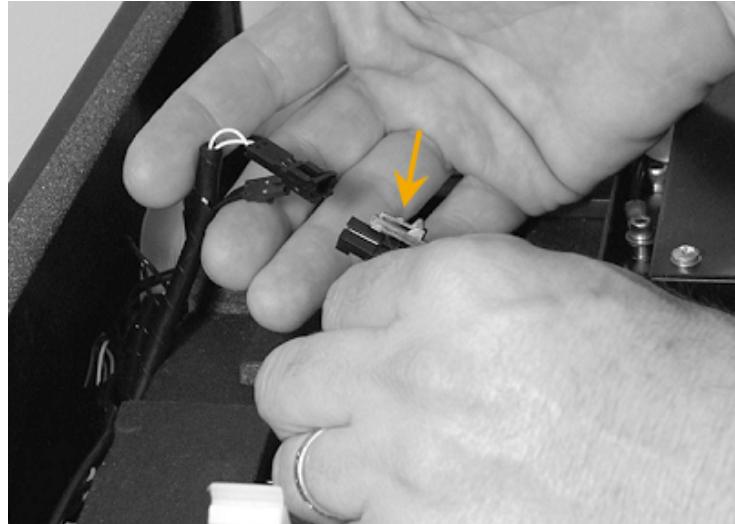
## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

#### Procedure

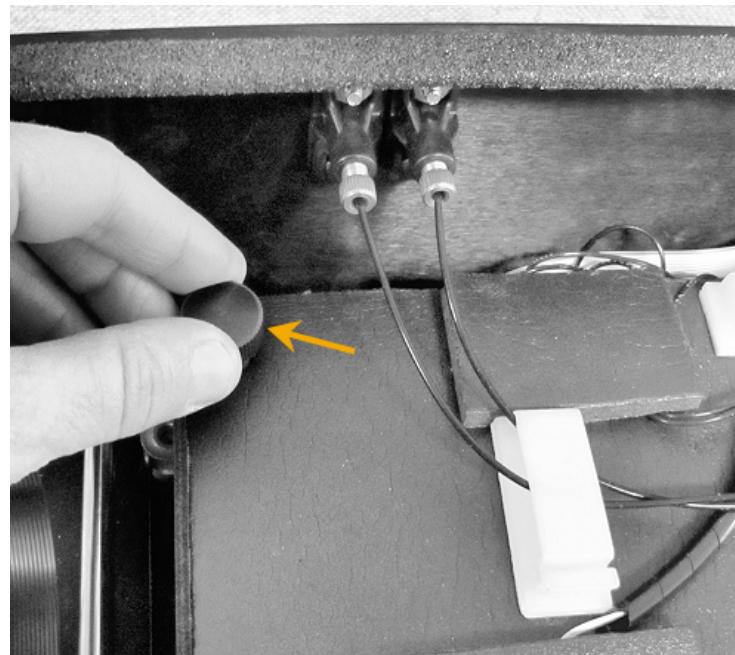
Once the shroud has been removed and the internal tubes and injector heads have been removed and cleaned, perform the following steps to remove a few more components and then clean the optical probes.

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



**Figure 5-12:** Disconnecting the heater and thermistor wires.

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



**Figure 5-13:** Removing a thumbscrew to expose the ground wire.

## 5 Periodic Maintenance

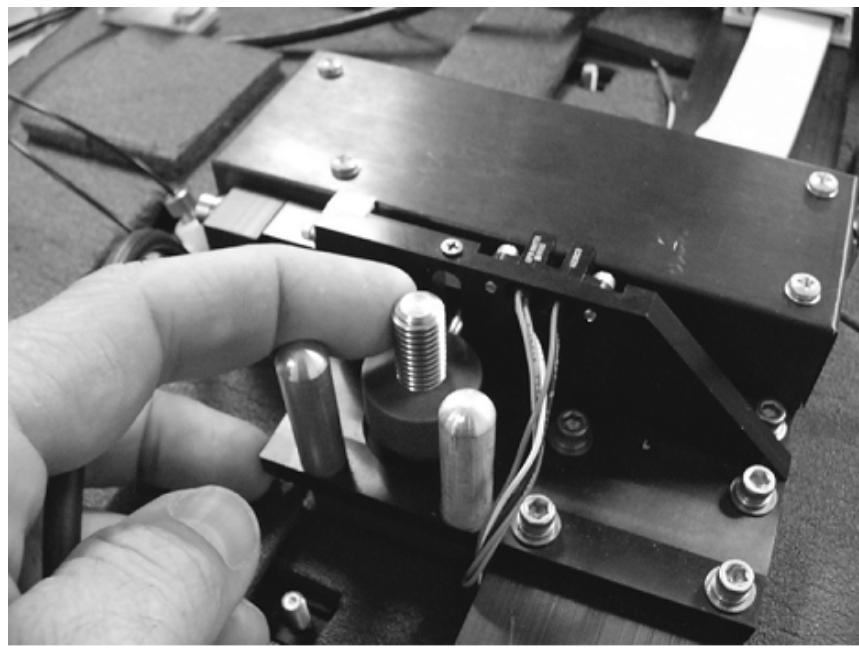
### Clean the Internal Components (As Applicable)

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



**Figure 5-14:** Moving the ground wire.

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



**Figure 5-15:** Lowering the probe hanger.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

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



**TIP** When replacing the incubator housing, the two "forks" on its right side should wrap around the holding screws. The forks should not slide under the fixed foam.

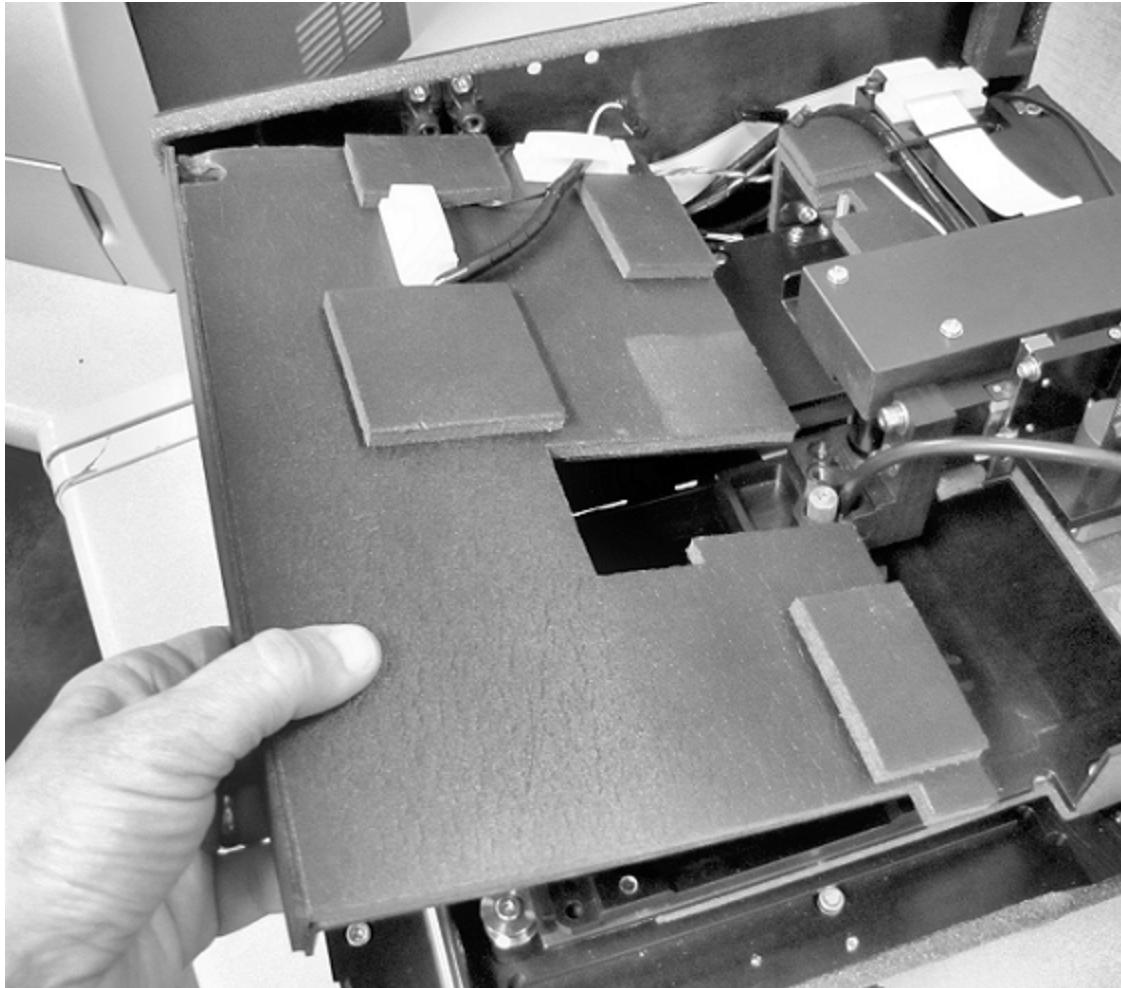


Figure 5-16: Removing the incubator housing.



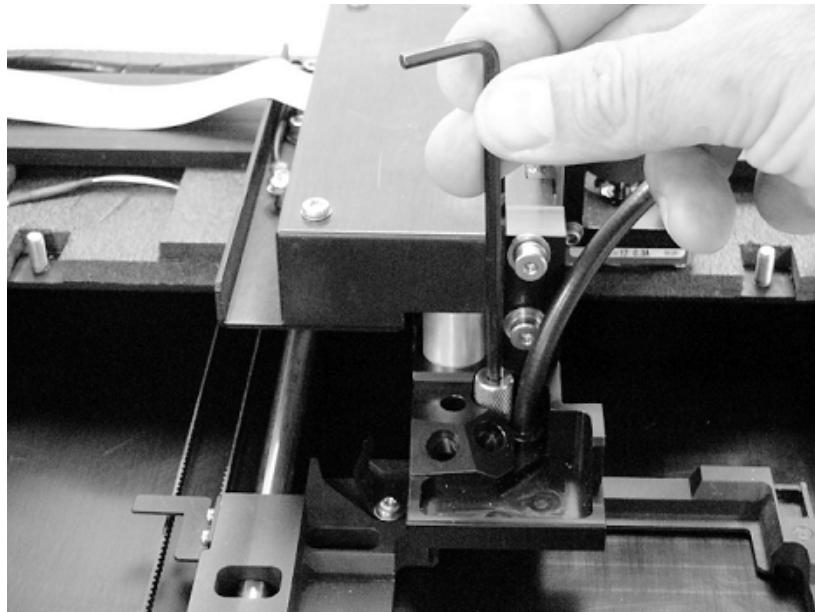
**IMPORTANT**

For the remainder of this procedure, perform only the steps which are applicable to your reader model.

## 5 Periodic Maintenance

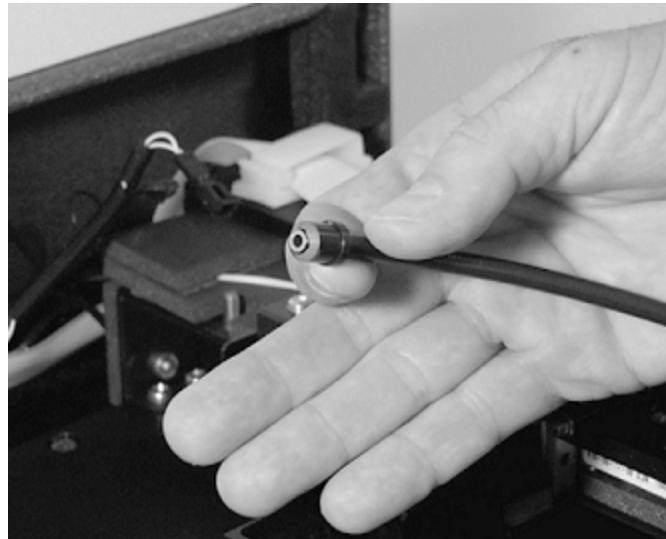
### Clean the Internal Components (As Applicable)

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



**Figure 5-17:** Removing the top probe's holding screw.

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



**Figure 5-18:** Optical probe, ready for cleaning.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

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

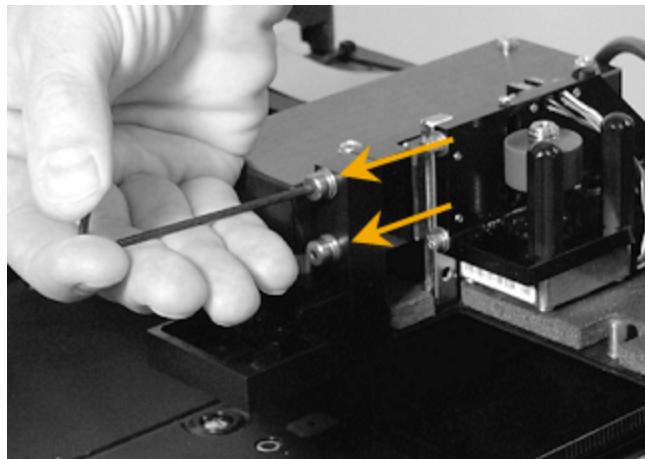


Figure 5-19: Removing two screws on the top probe hanger.

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

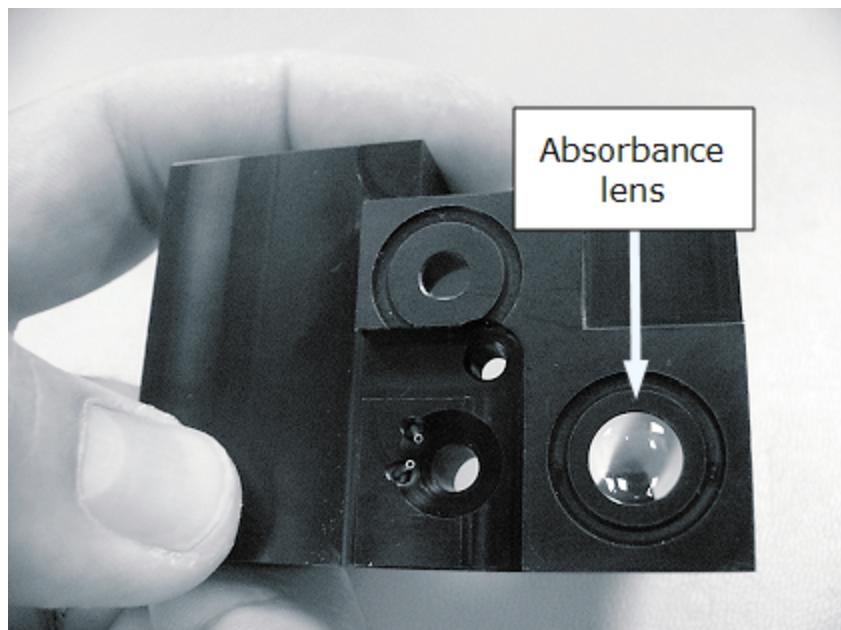


Figure 5-20: Viewing the underside of the probe hanger.

**NOTICE**

When cleaning the absorbance lens with the swab, *use very gentle pressure to the lens.* Applying too much pressure can push the lens out of its holder. Reinstallation must be performed by Agilent service personnel. If the lens does fall out, contact Technical Support.

## 5 Periodic Maintenance

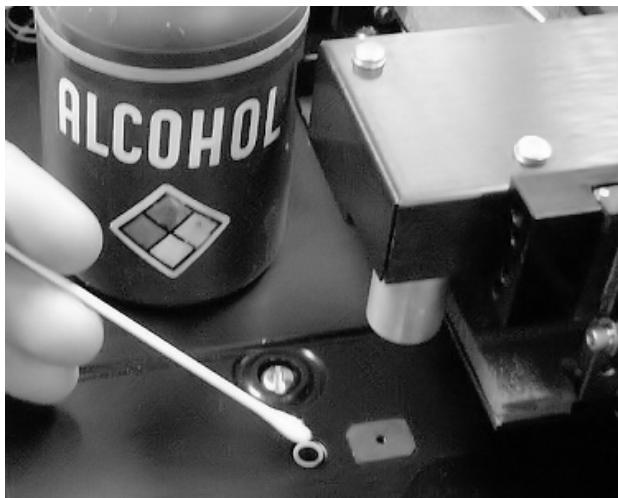
### Clean the Internal Components (As Applicable)

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



**Figure 5-21:** Cleaning the absorbance lens.

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



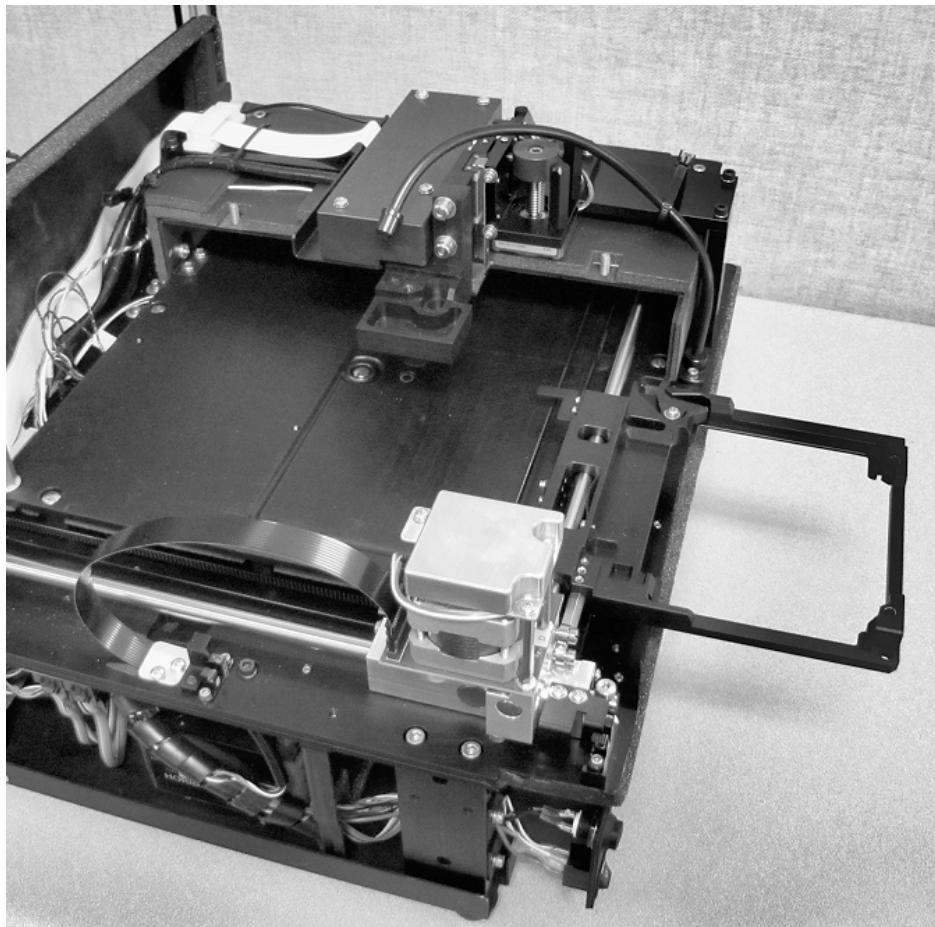
**Figure 5-22:** Cleaning the lower optical probe on the instrument surface.

## Clean the Reader's Internal Surface

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

## 5 Periodic Maintenance

Clean the Internal Components (As Applicable)

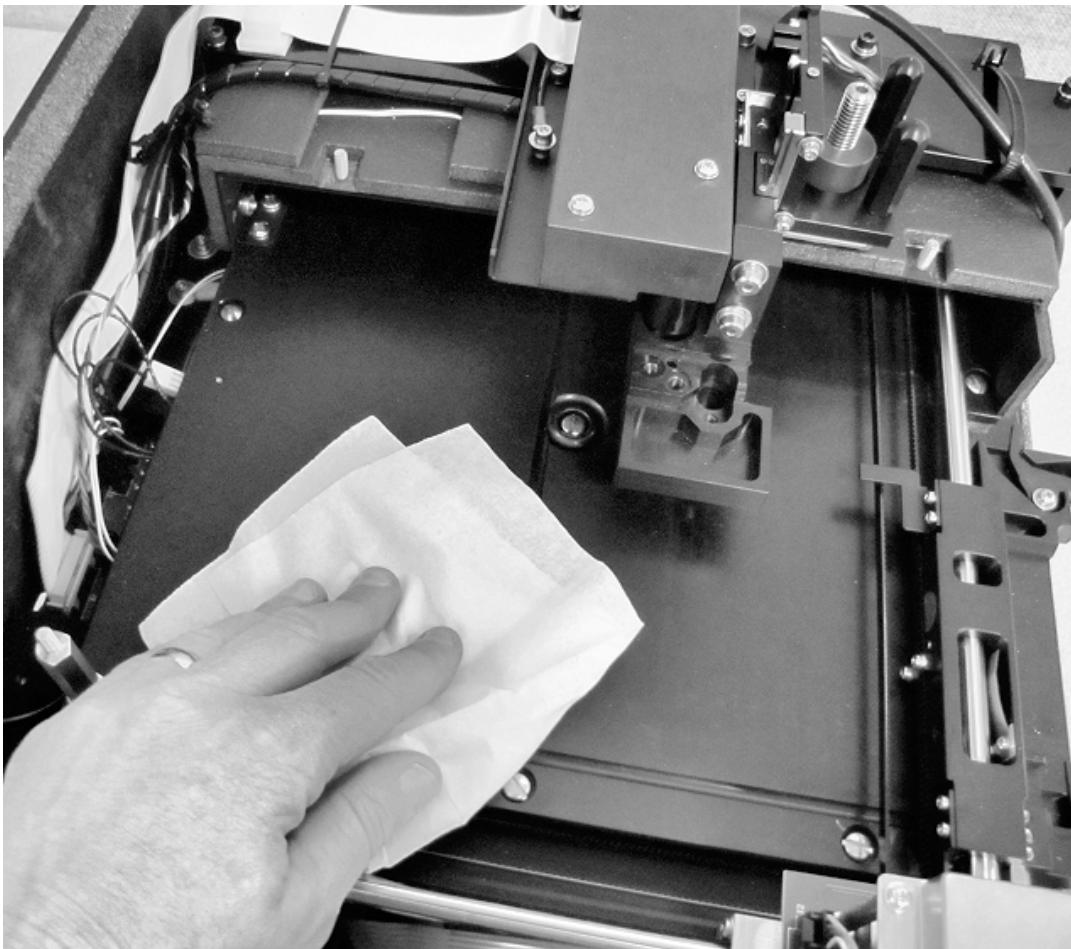


**Figure 5-23:** Microplate carrier fully extended.

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

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



**Figure 5-24:** Wiping the reader's internal surface.

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

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

## Reassemble the Components

### NOTE

Perform the steps in the order listed to reassemble the components. See the referenced page numbers for further instructions and photos demonstrating the steps.

- 1 Slide the microplate carrier back into the instrument.
- 2 If applicable, 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.

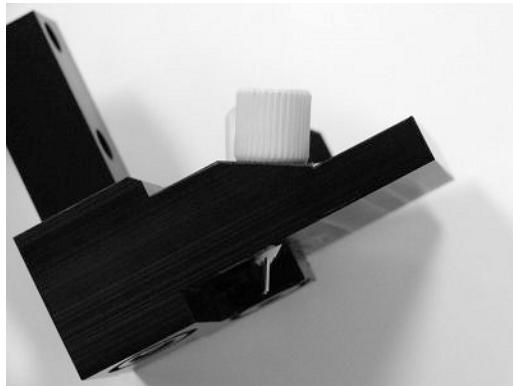


Figure 5-25: Reinstalling the injector head.

- 3 If applicable, attach the two internal dispense tubes to the injector heads, as shown below. *Do not overtighten the thumbscrews.*

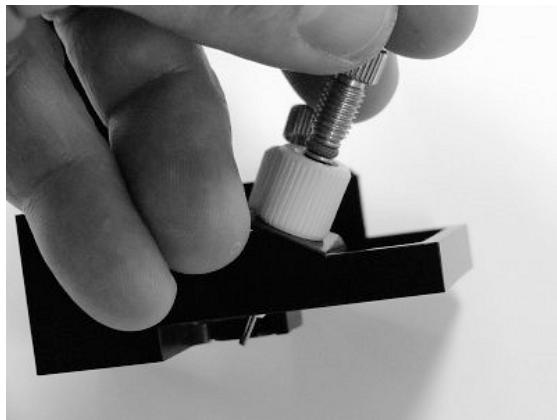


Figure 5-26: Reinstalling the dispense tubes.

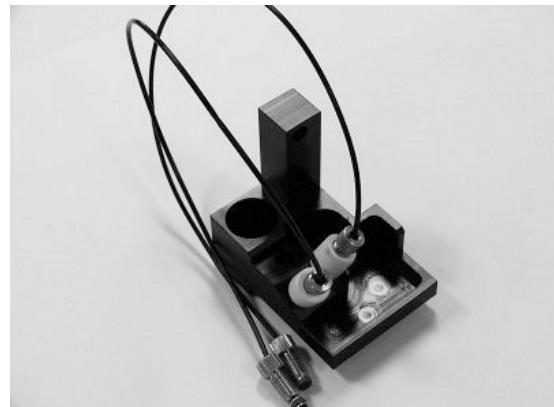


Figure 5-27: Top probe hanger, ready for reinstallation.

- 4 Replace the top probe hanger and shoulder screws using the 3/32" hex wrench. See the figure on page [79](#).
- 5 Insert the top optic probe into its socket and replace its holding screw (using the 1/8" hex wrench). See the figure on page [78](#).
- 6 Replace the incubator housing and two thumbscrews. Do not slide the two "forks" on the housing's right side under the fixed foam housing. See the figure on page [77](#).

## 5 Periodic Maintenance

### Clean the Internal Components (As Applicable)

- 7 Replace the ground wire and its thumbscrew. See the figures on page [76](#).
- 8 Reconnect the heater and thermistor wires. Be sure to connect wires of the same color. See the figure on page [75](#).
- 9 If applicable, attach the two internal dispense tubes to the tubing ports, taking care to align the correct port with the correct injector head. See the figures on page [70](#).
- 10 If applicable, slide the two internal dispense tubes into the cable clamp and close the clamp.
- 11 Review the steps you just performed to make sure the components have been properly reassembled.
- 12 Slide the shroud onto the instrument.
- 13 Replace the four screws to securely attach the shroud to the base. See the figure on page [68](#).

## Performance Check

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

- Plug the instrument in, turn it on, and allow its run-time system test to complete. Run a [System Test](#) through Gen5/Gen6.
- Run any required OQ/PQ tests.

# 6 As Needed Maintenance

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

---

Decontamination .....	86
Dispense Module, Syringe Replacement .....	90

## 6 As Needed Maintenance

### Decontamination

## Decontamination

---

#### ⚠️ **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.

#### ⚠️ **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.

#### ⚠️ **WARNING**

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

#### **NOTICE**

**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.

#### **NOTICE**

**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 HTX 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.

## 6 As Needed Maintenance

### Decontamination

## Materials

- Sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as an alternative to bleach)
- Deionized or distilled water
- Safety glasses
- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags
- 125 mL beakers
- Clean, lint-free cotton cloths

Additional materials for models with the dispense module:

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

## Procedure for Models without the Dispense Module

- 1 Turn off and unplug the instrument.
- 2 Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

### NOTE

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

- 3 Moisten a clean, lint-free cloth with the bleach solution, then thoroughly wring it out so that liquid does not drip from it. *Do not soak the cloth.*
- 4 Wipe the plate carrier and all exposed surfaces of the instrument, including in the inside of the microplate carrier access door.
- 5 Allow the instrument to dry for 20 minutes for thorough decontamination by the bleach.
- 6 Moisten a cloth with deionized or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution.
- 7 Use a clean, dry, lint-free cloth to dry all wet surfaces.
- 8 Reassemble the instrument as necessary.
- 9 Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

## Procedure for Models with the Dispense Module

Perform the **Routine Procedure**, described next, when the equipment is functioning normally. If you are unable to perform a prime due to a system failure, perform the [Alternate Procedure](#).

## 6 As Needed Maintenance

### Decontamination

#### Routine Procedure

- If disinfecting with sodium hypochlorite (bleach), flush repeatedly with deionized water to remove the bleach. Perform the rinse procedure provided in [Rinse the Fluid Lines](#).
- If disinfecting with alcohol, do not immediately prime with deionized water. The drying effect of the alcohol is an important aspect of its disinfectant properties.

#### Clean Exposed Surfaces

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

#### NOTE

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

- 3 Open the plate carrier door and slide out the plate carrier.
- 4 Wet a cloth or paper towel with the bleach solution or alcohol, and then thoroughly wring it out so that liquid does not drip from it. *Do not soak the cloth.*
- 5 Wipe the plate carrier and the exposed surfaces of the external dispense module.
- 6 Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
- 7 Use a clean, dry cloth to dry all wet surfaces.
- 8 Reassemble the instrument as necessary.
- 9 If the dispense module is installed, purge any fluid (see [Flush/Purge Fluid Path](#)) and detach the outlet tubes from the instrument. If it is not installed, attach only the dispense module's communication cable to the instrument. Remove the supply bottles and their holders.
- 10 Perform the decontamination procedures described below.

#### Decontaminate the Fluid Lines

- 1 Place a beaker with 20 mL of 0.5% sodium hypochlorite solution or 70% isopropyl alcohol near **SYRINGE 1** on the dispense module.
- 2 Place the **SYRINGE 1** inlet tube in the beaker.
- 3 If you have not already done so, detach the dispense module's outlet tubes from the instrument. Place the ends of the outlet tubes in an empty beaker and set the beaker next to the dispense module.
- 4 **Gen5 users:** Launch Gen5 and select **System > Instrument Control > Synergy HTX**.  
**Gen6 users:** Launch Gen6 and select **Prime/Dispense** from the Instrument Control area of the Home screen.
- 5 Select the **Prime** tab.
- 6 Select Dispenser **1**, enter a Volume of **5000  $\mu$ L**, and keep the default dispense Rate.
- 7 Place the priming plate on the carrier.

## 6 As Needed Maintenance

### Decontamination

- 8 Run two prime cycles, for a total of 10,000  $\mu$ L.
- 9 Wait at least 20 minutes to allow the solution to disinfect the tubing.
- 10 Remove the inlet tube from the beaker of disinfectant solution.
- 11 In the Prime tab, change the Volume to **1000  $\mu$ L**.
- 12 Run one prime cycle, to flush the disinfectant out of the fluid lines.
- 13 Empty the beaker containing the outlet tubes. Put the tubes back in the empty beaker.
- 14 If sodium hypochlorite (bleach) was used, perform the next procedure, **Rinse the Fluid Lines**. Otherwise, or after performing the Rinse procedure, repeat steps 1–13 for **SYRINGE 2**/Dispenser 2.

### Rinse the Fluid Lines

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

- 1 Place a beaker containing at least 30 mL of deionized water on the dispense module.
- 2 Place the **SYRINGE 1** or **2** inlet tube in the beaker.
- 3 If you have not already done so, place the outlet tubes in an empty beaker.
- 4 From the Prime tab, select Dispenser **1** or **2**, set the Volume to **5000  $\mu$ L**, and keep the default dispense Rate.
- 5 Run five prime cycles, for a total of 25,000  $\mu$ L.
- 6 Pause for 10 minutes and then run one prime cycle with **5000  $\mu$ L**. This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
- 7 Empty the beaker containing the outlet tubes.
- 8 Wipe all surfaces with deionized water.
- 9 Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

### Clean the Dispense Tubes and Injectors

Turn to [Clean the Internal Components \(As Applicable\)](#) for instructions on removing the reader's shroud and removing/cleaning the internal dispense tubes and injector heads.

### Decontaminate the Tip Priming Trough and Priming Plate

- 1 Remove the tip priming trough from the instrument's microplate carrier.
- 2 Wash the tip priming trough and priming plate in hot, soapy water. Use a small brush or cloth to clean the corners of the trough and plate.
- 3 To decontaminate, soak the trough and plate in a container of 0.5% sodium hypochlorite or 70% isopropyl alcohol for at least 20 minutes.
  - If decontaminating in a bleach solution, thoroughly rinse the trough and plate with DI water.
  - If decontaminating with alcohol, let the trough and plate air dry.
- 4 Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

## 6 As Needed Maintenance

### Dispense Module, Syringe Replacement

## Alternate Procedure

If you are unable to prime the system due to an equipment failure, decontaminate the instrument and the dispense module as follows:

- 1 Turn to [Clean the Internal Components \(As Applicable\)](#) for instructions on removing the reader's shroud and removing/cleaning the internal dispense tubes and injector heads.
- 2 Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

#### NOTE

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

- 3 Slide the microplate carrier out of the instrument.
- 4 Wet a cloth or paper towel with the bleach solution or alcohol, and then thoroughly wring it out so that liquid does not drip from it. *Do not soak the cloth.*
- 5 Use the cloth to wipe:
  - All exterior surfaces of the instrument
  - All surfaces of the plate carrier
  - The exposed surfaces of the dispense module, including the syringe valves
- 6 Remove the tubing and the syringes from the dispense module and soak them in the bleach or alcohol solution. Wait for 20 minutes. See [Syringe Maintenance Position](#) for the procedure to remove the syringes.
- 7 Moisten a cloth with DI or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
- 8 Rinse all tubing and the syringes with DI water.
- 9 Use a clean, dry cloth to dry all surfaces on the instrument and the dispense module.
- 10 Reassemble the dispense module as necessary.
- 11 Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

## Dispense Module, Syringe Replacement

Refer to [Chapter 5 Periodic Maintenance](#) for cleaning procedures you must perform regularly and in cases of poor performance (for example, when the Dispense Accuracy and Precision tests fail). If cleaning the injection system does not eliminate performance problems, or if a syringe is leaking, perform these instructions to replace a faulty syringe. Contact Technical Support to order replacement syringes.

To change a syringe, first use Gen5/Gen6 to put the syringe in its maintenance position.

## Syringe Maintenance Position

#### NOTE

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

## 6 As Needed Maintenance

### Dispense Module, Syringe Replacement

Gen5/Gen6 provides access to syringe setup functions for maintenance and calibration purposes. When a syringe needs to be installed or replaced, it must first be moved to its maintenance position.

- 1 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5.
- Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen.
- 2 Select the **Prime** tab.
- 3 Select the appropriate Dispenser, **1** or **2**, associated with the syringe.
- 4 Select **Maintenance**. The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve.

## Replace the Syringe

Refer to the figures in [Install the Dispense Module](#).

### After using Gen5/Gen6 to put the syringe in its maintenance position:

- 1 Using your fingers, unscrew the bottom thumbscrew that secures the syringe, underneath the bracket. Retain this bottom thumbscrew; it is needed for the replacement syringe.
- 2 Unscrew the top thumbscrew to disengage the syringe from the valve.
- 3 Remove the new syringe from its protective box.
- 4 Hold the syringe vertically with the threaded end at the top. Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
- 5 Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
- 6 Pass the thumbscrew (used to hold the old syringe) up through this hole and thread it into the bottom of the syringe. Hold the syringe from rotating while tightening the thumbscrew. Finger-tighten only.
- 7 **Gen5 users:** Select **System > Instrument Control > Synergy HTX** in Gen5.
- Gen6 users:** Select **Prime/Dispense** from the Instrument Control area of the Gen6 Home screen.
- 8 Select the **Prime** tab, and then select **Initialize**.

# 7 Instrument Qualification

This chapter describes the tests for qualifying all models of the Synergy HTX. It introduces the various test methods, describes the materials and parameters used to execute the tests, details the step-by-step test procedures, explains how to analyze test results, and provides troubleshooting tips in the event of a failure.

---

Instrument Qualification Overview .....	93
IQ/OQ/PQ Description .....	93
Recommended Qualification Schedule .....	94
System Test .....	95
Plate Shaker Test .....	97
Absorbance Testing Overview .....	98
Absorbance Plate Tests .....	98
Absorbance Liquid Tests .....	102
Fluorescence Testing Overview .....	112
Fluorescence Plate Test .....	112
Fluorescence Liquid Tests .....	114
Luminescence Test .....	132
Dispense Module Tests .....	138

## Instrument Qualification Overview

---

This chapter contains the recommended qualification tests for all Synergy HTX models.

Every Synergy HTX reader is fully tested prior to shipment and should operate properly upon initial setup. If you suspect that a problem occurred during shipment, if you have received the equipment after returning it to the factory for service, and/or if regulatory requirements dictate that you qualify the equipment on a routine basis, perform the procedures outlined in this chapter.

See the [Recommended Qualification Schedule](#) to determine which qualification tests shall be conducted for your reader and to meet your site's regulatory requirements.

A Product Qualification Package for the Synergy HTX is available for purchase (part number 1340508). The package contains complete procedures, Gen5 protocols<sup>1</sup>, checklists, and logbooks for performing Installation Qualification, Operational Qualification, Performance Qualification, and Maintenance. Contact your local Agilent representative for more information.

## IQ/OQ/PQ Description

---

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

- The recommended IQ procedure consists of setting up the instrument and its components as described in [Chapter 3 Installation](#), and performing the System Test. For models with injectors, a quick test with fluid is also performed, to ensure that the dispense module is properly installed and there are no leaks.
- The IQ procedure should be performed initially (before the reader is used for the first time).
- The successful completion of the IQ procedure verifies that the instrument is installed correctly. The Operational Qualification procedure should be performed immediately following the successful IQ.

**Operational Qualification** confirms that the equipment operates according to specification initially and over time.

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

---

<sup>1</sup>The supplied Gen5 protocols can be opened in Gen6 as experiments using Gen6 version 1.04 or higher.

## 7 Instrument Qualification

### Recommended Qualification Schedule

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

- The recommended PQ procedure consists of performing the system test and a series of tests for the absorbance, fluorescence, and luminescence systems, as applicable for your instrument model. If the external dispense module is used, Dispense Accuracy and Precision Tests are also included in the PQ.
- Your facility's operating policies may also require that you routinely perform an actual assay to confirm that the reader will consistently give adequate results for the assays to be run on it.
- These tests should be performed routinely; the recommended interval is monthly or quarterly, depending on the test. This frequency may be adjusted depending on the trends observed over time.
- The successful completion of the PQ procedure confirms that the equipment is performing consistently under normal operating conditions.

## Recommended Qualification Schedule

This table defines the recommended intervals for qualifying a Synergy HTX that is used two to five days a week. The actual frequency, however, may be adjusted depending on your usage of the instrument and its various models. The schedule assumes that the instrument is properly maintained as outlined in [Chapter 5 Periodic Maintenance](#).

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
<b>All models:</b>				
Unpacking, installation, setup, and configuration of the reader, host computer, and Gen5/Gen6 software.		✓		
System Test	✓		✓	✓
Plate Shaker Test		✓		
<b>Models with absorbance capability:</b>				
Absorbance Plate Test		✓	✓	
Absorbance Liquid Test 1 or Liquid Test 2*		✓		✓
(Optional) Absorbance Liquid Test 3 or 340 nm Absorbance Plate Test (using part number 7260551)		✓		✓
<b>Models with fluorescence capability:</b>				
Corners, Sensitivity, Linearity (Fl) Tests		✓	✓	
<b>Models with luminescence capability:</b>				
Luminescence Test		✓	✓	
<b>Models with injectors and an external dispense module:</b>				

## 7 Instrument Qualification

### System Test

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
Installation and setup of external dispense module		✓		
Injection System Test		✓		
Dispense Accuracy and Precision Test			✓	✓

\* If you have Absorbance Test Plate part number 7260522, perform Liquid Test 1. Otherwise, perform Liquid Test 2.

**NOTE**

Perform the FI tests using a Fluorescence Test Plate (part number 1400501) or the Fluorescence Liquid Test procedures described in [Fluorescence Liquid Tests](#).

## System Test

Each time the Synergy HTX is turned on, it automatically performs a series of tests on the reader's motors, lamp(s), the PMT, and various sub-systems. The duration of this system test depends on the reader model and can take a few minutes to complete. If all tests pass, the microplate carrier moves to its forward position and the LED on the power switch will remain on and constant. The reader is then ready for use.

You can also initiate a system test through Gen5/Gen6.

If any test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria, the reader beeps repeatedly and the LED on the power switch will flash. If this occurs, press the carrier eject button to stop the beeping.

**NOTE**

If the system test fails, an error code is returned. Look up the error code in [Appendix B Error Conditions](#). If the problem is something you can fix, turn off the reader, fix the problem, turn the reader back on, and retry the test. If the test continues to fail, or if the cause is not something you can fix, contact Technical Support.

## Test Setup

If your assays use incubation, Agilent recommends enabling temperature control for at least 37°C and allowing the incubator to reach its set point before running the System Test. To access this feature in Gen5, select **System > Instrument Control** and click the **Pre-Heating** tab. To access this feature in Gen6, select **Incubate** from the Instrument Control area.

If applicable, adjust the Gen5/Gen6 Absorbance table for the Synergy HTX to wavelength values that you use most frequently. To access this feature in Gen5, select **System > Instrument Configuration > Synergy HTX > View/Modify > Setup > Absorbance**. To access this feature in Gen6, select **Configure > Synergy HTX**, then select **Absorbance Filter Table** in the Instrument Setup area.

## 7 Instrument Qualification

### System Test

#### Gen5

- 1 From the Gen5 main screen, select **System > Diagnostics > Run System Test**. If prompted to select a reader, select **Synergy HTX** and click **OK**. The duration of the test depends on the reader model; it can take a few minutes to complete.
- 2 If a message appears stating that the reader has a pending system test report, click **OK**, then click **Close**. Repeat step 1.

#### NOTE

If the test fails during execution, a message box appears in the software. Close the box; the test report contains the error code generated by the failure.

- 3 When the test is complete, a dialog appears, requesting additional information. Enter any required information and then click **OK**.
- 4 The results report appears. It shows either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL \*\*\* ERROR (error code) DETECTED."
  - The Gen5 software stores system test information in its database; you can retrieve it at any time.
  - You can save the system test report as a text file: click **Save As** in the System Test Results dialog.
- 5 If required, print, sign, and date the report, and store it with your test documentation.
- 6 Turn off the incubator, if applicable.

#### Gen6

- 1 From the Gen6 Home screen, select **Diagnostics**, navigate to the **System Test** tab, and select **Start System Test**. The duration of the test depends on the reader model; it can take a few minutes to complete.
- 2 If a message appears stating that the reader has a pending system test report, click **OK**, then click **Close**. Repeat step 1.

#### NOTE

If the test fails during execution, a message box appears in the software. Close the box; the test report contains the error code generated by the failure.

- 3 While the test is running, a dialog appears requesting additional information; enter any required information. Click **Save Test Results** when the test is complete.
- 4 The results report appears. It shows either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL \*\*\* ERROR (error code) DETECTED."
  - The Gen6 software stores system test information in its database; you can retrieve it at any time.
  - You can save the system test report as a text file: click **Save As** in the System Test Report dialog.
- 5 If required, print, sign, and date the report, and store it with your test documentation.
- 6 Turn off the incubator, if applicable.

## Plate Shaker Test

---

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

### Gen5

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

- 1 Create a protocol with two steps in its procedure.
  - In the Shake step, set the Mode to **Linear** and the Duration to **30 seconds**. You can further define the frequency, if you wish.
  - Add a simple Read step.
- 2 Create an experiment based on this protocol and then read a plate. If you can hear the plate shaker, the shaker is operating properly.

### Gen6

**NOTE** Refer to the Gen6 Help system for complete instructions for defining an experiment and setting the shake parameters.

- 1 Create an experiment with two steps in its method.
  - In the Shake step, set the Shake Mode to **Linear** and the Duration to **30 seconds**. You can further define the frequency, if you wish.
  - Add a simple Read step.
- 2 Click **Read Plate**. If you can hear the plate shaker, the shaker is operating properly.

**NOTE** If you do not hear the plate shaker, contact Agilent.

# Absorbance Testing Overview

---

*Applies only to models with absorbance capability*

Agilent developed a series of tests for the absorbance system using a combination of solid state Absorbance Test Plates and liquid plates. An advantage of running liquid tests is the liquid in the wells has a meniscus, whereas the test plate's neutral density glass filters do not. The test plates and the materials used for creating the liquid plates are available for purchase from Agilent. See [Optional Accessories](#).

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

- Absorbance Liquid Test 1 and Absorbance Plate Test (using part number 7260522) or
- Absorbance Liquid Test 2

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

- Absorbance Liquid Test 3 or Absorbance Plate Test at 340 nm (using part number 7260551)

## Absorbance Plate Tests

---

Agilent 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, and a glass filter in position C6 to test the wavelength accuracy of the monochromator-based absorbance system. Agilent Absorbance Test Plate part number 7260551 uses NIST-traceable neutral density filters to confirm absorbance specifications in the UV range (340 nm).

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

Before the Absorbance Plate Test can be performed, the OD Standard values and Expected Peak/Test Range combinations must be entered into Gen5/Gen6. Enter and save these values once initially and then update them annually when the test plate is recertified by Agilent.

Check the calibration due date on the test plate's label. If the test plate is overdue for recalibration, contact Agilent to schedule service.

## Test Method

The Absorbance Plate Test is conducted using Gen5/Gen6 software. The test plate is accessed via Gen5 by selecting **System > Diagnostics > Test Plates**, and via Gen6 by selecting **Diagnostics > Test Plates**. The test confirms wavelength accuracy, mechanical alignment and optical density accuracy, linearity, and repeatability. When complete, a results report displays Pass or Fail for each individual test.

- **Peak Absorbance:** The part number 7260522 test plate contains a glass filter in position C6 that is used to check the wavelength accuracy of the absorbance monochromator. The filter is scanned across a specified wavelength range in 1 nm increments. The wavelength(s) of

## 7 Instrument Qualification

### Absorbance Plate Tests

maximum absorbance are compared to the expected peak wavelength(s) supplied on the test plate's data sheet. The accuracy of the wavelength should be  $\pm 3$  nm ( $\pm 2$  nm instrument,  $\pm 1$  nm filter allowance).

- **Alignment:** The test plate has precisely machined holes to confirm mechanical alignment. The amount of light that shines through these holes is an indication of whether the microplate carrier is properly aligned with the absorbance optical path. A reading of more than 0.015 OD for any of the designated alignment holes indicates that the light is being "clipped" and the reader may be out of alignment.
- **Accuracy:** The test plate contains NIST-traceable neutral-density glass filters of known OD values at one or more wavelengths. Actual measurements are compared against the expected values provided in the test plate's data sheet. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is proven that the reader is accurate at these OD values, the reader is also considered to be linear. To further verify this, you can perform a linear regression analysis on the test plate OD values in a program such as Microsoft Excel; an  $R^2$  value of at least 0.9900 is expected.
- **Repeatability:** This test ensures the instrument meets its repeatability specification by conducting repeated reads of each neutral-density filter on the test plate and comparing the results.

## Requirements

To perform this test, you will need:

- Absorbance Test Plate, part number 7260522
- (Optional) 340 nm Absorbance Test Plate, part number 7260551
- Current Absorbance Test Plate Calibration Certificate(s)
- Gen5 or Gen6 software

## Setup

Before an Absorbance Test Plate can be used for qualification, you must enter information from its Calibration Certificate into Gen5/Gen6. Perform these steps initially, and then repeat them annually after the test plate is recertified by Agilent.

- 1 Obtain the current Test Plate Calibration Certificate.
- 2 **Gen5 users:** Select **System > Diagnostics > Test Plates > Add/Modify Plates > Add** in Gen5.  
**Gen6 users:** Select **Diagnostics > Test Plates > Add** in Gen6.  
The Absorbance Test Plate dialog appears.
- 3 Select the appropriate **Plate Type**, and then enter the plate's serial number.
- 4 Enter the **Last Certification** and **Next Certification** dates from the calibration label on the Test Plate.
- 5 If the wavelength values in the top row of the grid are appropriate for your tests, enter the OD Standard values from the Calibration Certificate into the grid. Make sure you enter the correct value for each well/wavelength combination.

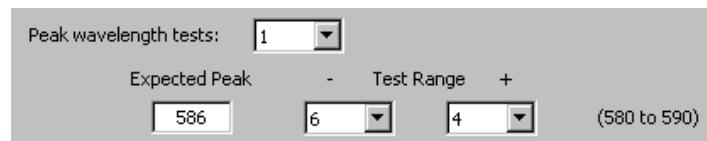
### NOTE

If you need to change the wavelength values, click **Wavelength List**. Add, change, or delete the values as needed and click **OK**.

## 7 Instrument Qualification

### Absorbance Plate Tests

- 6 Select the number of Peak Wavelength tests (up to four) based on the number of expected peak wavelength values provided on the certificate.
- 7 Enter the Expected Peak values from the Certificate and set the Test Range – and + values.
  - If the C6 filter is Erbium or Holmium glass, the certificate contains two Spectral Bandpass tables:
    - We recommend using the peak values in the 2.4 nm table for the Synergy HTX.
    - Holmium glass (i.e., the lot number contains "H"): We recommend that you use the expected peak values *closest* to 242, 279, 362, 417, and 538 nm.
    - Erbium glass: Any peak value in the table can be used.
  - If your C6 filter is Didymium glass, a single peak wavelength value is provided. Enter this value and set the Test Range – and + values so the range displayed in parentheses is 580 to 590, as shown in the figure.



**Figure 7-1:** Setting Didymium peak value.

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

## Test Procedure

- 1 **Gen5 users:** From the Gen5 main screen, click **System > Diagnostics > Test Plates > Run**.  
**Gen6 users:** From the Gen6 Home screen, click **Diagnostics > Test Plates > Run**.
- 2 If prompted, select the desired Test Plate and click **OK**.
- 3 When the Absorbance Test Plate Options dialog appears, select **Perform Peak Wavelength Test**, if not already selected, and enter any required information.
- 4 Highlight the wavelength(s) to be included in this test.

#### NOTE

Select only those wavelengths most appropriate for your use of the reader.

- 5 (Optional) Enter a comment.
- 6 Click **Start Test**.
- 7 Place the Absorbance Test Plate on the microplate carrier, with well A1 in the proper location.
- 8 Click **OK** to run the test.
- 9 When the test is complete, the results report appears. Scroll through the report; every result should show "PASS".

## Troubleshooting

If a test fails, try the troubleshooting tips below. If the test continues to fail, contact Technical Support.

### NOTICE

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

### NOTE

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

## Peak Absorbance Test

- Check the filter in the C6 position to ensure it is clean. If needed, clean the filter with lens paper. Do not remove the filter, and do not use alcohol or other cleaning agents.
- Verify that the Peak wavelength information entered for the plate in Gen5/Gen6 matches the information provided on the test plate's data sheet.
- Check the calibration due date on the test plate's label. If the test plate is overdue for recalibration, contact Agilent to schedule service.
- Check the microplate carrier to ensure it is clear of debris.

## Alignment Test

- Ensure that the test plate is properly seated in the microplate carrier.
- Check the four alignment holes (A1, A12, H1, H12) to ensure they are clear of debris.
- Check the microplate carrier to ensure it is clear of debris.

## Accuracy Test

- Check the neutral-density filters to ensure they are clean (positions C1, D4, E2, F5, G3, H6). If needed, clean the filters with lens paper. Do not remove any filters, and do not use alcohol or other cleaning agents.
- Verify that the wavelength/expected OD values entered for the plate in Gen5/Gen6 match the information provided on the test plate's data sheet.

## Repeatability Test

- Check the neutral-density filters to ensure there is no debris that may have shifted between readings and caused changes.
- Check the microplate carrier to ensure it is clear of debris.

## Absorbance Liquid Tests

---

Agilent developed a series of liquid tests for testing your reader's absorbance system.

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

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

### Test Methods

**Absorbance Liquid Test 1** confirms repeatability and alignment when a solution is used in the microplate. If these tests pass, then the lens placement and optical system cleanliness are proven. For the Repeatability portion of this test, two columns containing a color-absorbing solution are read five times at 405 nm. For each well, an "allowed deviation" is determined based on its Mean OD and the reader's repeatability specification. Each well's Standard Deviation must be less than its Allowed Deviation to pass. To confirm the reader's mechanical alignment, the plate is rotated 180 degrees in the carrier (e.g., A1 is now in the H12 position), and the same two columns are read. The initial and new OD readings are compared using the reader's accuracy specification. If the two readings in the same well do not meet specification, the reader may be out of alignment.

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

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

## 7 Instrument Qualification

### Absorbance Liquid Tests

## Gen5 Protocol Parameters

If using Gen5 to conduct the absorbance liquid tests, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen5 protocols. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

#### NOTE

The Plate Type setting in each Gen5 protocol should match the actual 96-well plate in use.

### Synergy HTX Abs Test 1.prt

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

### Synergy HTX Abs Test 2.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Linear, 4 minutes, default frequency
<b>Two Read Steps</b>	
Kinetic loop (one per Read step)	Set a Run time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromators
Step labels	First Read step: "Normal" Second Read step: "Turnaround"

## 7 Instrument Qualification

### Absorbance Liquid Tests

Parameter	Setting
Read wells	Full plate
Wavelengths	2 (450 nm, 630 nm)
Read Speed	Normal
Delay after plate movement	100 msec
<i>Data Reduction</i>	Define two Delta OD transformations (450–630 nm), one per Read data set

### Synergy HTX Abs Test 3.prt

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

### Gen6 Experiment Parameters

If using Gen6 to conduct the absorbance liquid tests, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen6 experiments. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

#### NOTE

The Plate Type setting in each Gen6 experiment should match the actual 96-well plate in use.

### Synergy HTX Abs Test 1.xpt

Parameter	Setting
Plate Type	96 WELL PLATE
<b>Two Read Steps</b>	
Read Mode	Absorbance
Read Type	Kinetic

## 7 Instrument Qualification

### Absorbance Liquid Tests

Parameter	Setting
Kinetic loop (one per Read step)	Set a Run time/Interval combination to read the plate five times with minimal delay
Wells	First Read step: A1–H2 Second Read step: A11–H12
Wavelength	405 nm
Read Speed	Normal
Delay after plate movement	100 msec
Plate Out/In step between loops	Text "rotate the plate 180 degrees"

### Synergy HTX Abs Test 2.xpt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Linear, 4 minutes, default frequency
<b>Two Read Steps</b>	
Read Mode	Absorbance
Read Type	Kinetic
Kinetic loop (one per Read step)	Set a Run time/Interval combination to read the plate five times with minimal delay
Step Label	First Read step: "Normal" Second Read step: "Turnaround"
Wells	Full plate
Wavelengths	2 (450 nm, 630 nm)
Read Speed	Normal
Delay after plate movement	100 msec
Analyze Data	Define two Delta OD transformations (450–630 nm), one per Read data set

### Synergy HTX Abs Test 3.xpt

Parameter	Setting
Plate Type	96 WELL PLATE
Read Mode	Absorbance
Read Type	Kinetic
Kinetic loop	Set a Run time/Interval combination to read the plate five times with minimal delay
Wells	A1–H6
Wavelength	340 nm

## 7 Instrument Qualification

### Absorbance Liquid Tests

Parameter	Setting
Read Speed	Normal
Delay after plate movement	100 msec

## Absorbance Liquid Test 1

### Materials

**NOTE** Manufacturer part numbers are subject to change.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended)
- Stock Solution A or B, which may be formulated by diluting a dye solution available from Agilent (A) or from the materials listed below (B)
- Gen5 protocol described in [Gen5 Protocol Parameters](#):
  - [Synergy HTX Abs Test 1.prt](#)
- or Gen6 experiment described in [Gen6 Experiment Parameters](#):
  - [Synergy HTX Abs Test 1.xpt](#)

### Solution A

- Agilent BioTek QC Check Solution No. 1 (part number 7120779, 25 mL; or 7120782, 125 mL)
  - Deionized water
  - 5 mL Class A volumetric pipette
  - 100 mL volumetric flask
- 1 Pipette a 5 mL aliquot of Agilent QC Check Solution No. 1 into a 100 mL volumetric flask.
  - 2 Add 95 mL of DI water; cap and shake well. The solution should measure approximately 2.000 OD when using 200  $\mu$ L in a flat-bottom microwell.

### Solution B

- Deionized water
  - FD&C Yellow No. 5 dye powder (typically 90% pure)
  - Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or Agilent wetting agent, part number 7773002 (a 10% Tween solution)
  - Precision balance with capacity of 100 g minimum and readability of 0.001 g
  - 1 liter volumetric flask
  - Weigh boat
- 1 Weigh out 0.092 gram of FD&C No. 5 yellow dye powder into a weigh boat.
  - 2 Rinse the contents into a 1 liter volumetric flask.
  - 3 Add 0.5 mL of Tween 20 or 5 mL of Agilent's wetting agent.
  - 4 Make up to 1 liter with DI water; cap and shake well.

## 7 Instrument Qualification

### Absorbance Liquid Tests

#### Test Procedure

##### NOTE

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

- 1 Using freshly prepared stock solution (Solution A or B), prepare a 1:2 dilution using deionized water (one part stock, one part deionized water; the resulting solution is a 1:2 dilution). The concentrated stock solution should have an optical density of approximately 2.000 OD or lower.
- 2 Pipette 200  $\mu$ L of the stock solution into column 1.
- 3 Pipette 200  $\mu$ L of the *diluted* solution into column 2.

##### NOTE

After pipetting the diluted test solution into the microplate and before reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. Alternatively, wait 20 minutes after pipetting the test solution before reading the plate.

- 4 Create a Gen5/Gen6 experiment based on [Synergy HTX Abs Test 1](#) and read the plate. When prompted, rotate the plate 180 degrees and continue.
- 5 Save the experiment when it is finished.
- 6 Refer to the instructions in [Results Analysis](#) to perform calculations and determine pass/fail. See [Troubleshooting](#) for troubleshooting tips.

#### Absorbance Liquid Test 2

##### NOTE

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

#### Materials

##### NOTE

Manufacturer part numbers are subject to change.

- A new 96-well, clear, flat-bottom microplate (Corning Costar #3590 is recommended)
- Ten test tubes, numbered consecutively, set up in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Solution A or B (see the instructions for Liquid Test 1)
- A 0.05% solution of deionized water and Tween 20
- Gen5 protocol described in [Gen5 Protocol Parameters](#):
  - [Synergy HTX Abs Test 2.prt](#)
- Or Gen6 experiment described in [Gen6 Experiment Parameters](#):
  - [Synergy HTX Abs Test 2.xpt](#)

## 7 Instrument Qualification

### Absorbance Liquid Tests

#### Test Procedure

- 1 Create a percentage dilution series, beginning with 100% of the original concentrated stock solution (A or B) in the first tube, 90% of the original solution in the second tube, 80% in the third tube, all the way to 10% in the tenth tube. Dilute using the 0.05% solution of deionized water and Tween 20. This solution can also be made by diluting the Agilent wetting agent 200:1.

Tube Number	1	2	3	4	5	6	7	8	9	10
Volume of Original Concentrated Solution (mL)	20	18	16	14	12	10	8	6	4	2
Volume of 0.05% Tween Solution (mL)	0	2	4	6	8	10	12	14	16	18
Absorbance expected if original solution is 2.0 at 200 $\mu$ L	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

#### NOTE

The choice of dilutions and the absorbance of the original solution can be varied. Use this table as a model for calculating the expected absorbances of a series of dilutions, given a different absorbance of the original solution.

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

#### NOTE

After pipetting the diluted test solution into the microplate and before reading the plate, Agilent strongly recommends shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. Alternatively, wait 20 minutes after pipetting the test solution before reading the plate.

- 4 Create a Gen5/Gen6 experiment based on [Synergy HTX Abs Test 2](#) and read the plate. When prompted, rotate the plate 180 degrees.
- 5 Save the experiment when it is finished.
- 6 Refer to the instructions in [Results Analysis](#) to perform calculations and determine pass/fail. See [Troubleshooting](#) for troubleshooting tips.

#### Absorbance Liquid Test 3 (optional)

#### NOTE

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

#### Materials

#### NOTE

Manufacturer part numbers are subject to change.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended)
- Calibrated hand pipette(s)

## 7 Instrument Qualification

### Absorbance Liquid Tests

- Beakers and graduated cylinder
- Precision balance with readability to 0.01 g
- Buffer solution described below
- Gen5 protocol described in [Gen5 Protocol Parameters](#):
  - **Synergy HTX Abs Test 3.prt**
- Or Gen6 experiment described in [Gen6 Experiment Parameters](#):
  - **Synergy HTX Abs Test 3.xpt**

### Buffer Solution

- Deionized water
  - Phosphate-Buffered Saline (PBS), pH 7.2–7.6, Sigma tablets, #P4417 (or equivalent)
  - $\beta$ -NADH Powder ( $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma bulk catalog number N 8129, or preweighed 10 mg vials, Sigma number N6785-10VL (or Agilent part number 98233). Store the powder according to the guidelines on its packaging.
- 1 Prepare a PBS solution from the Sigma tablets.
  - 2 In a beaker, mix 50 mL of the PBS solution with 10 mg of the  $\beta$ -NADH powder and mix thoroughly. This is the *100% Test Solution*.

### Test Procedure

- 1 Prepare the *75% Test Solution* by mixing 15 mL of the 100% Test Solution with 5 mL of the PBS Solution.
- 2 Prepare the *50% Test Solution* by mixing 10 mL of the 100% Test Solution with 10 mL of the PBS Solution.
- 3 Carefully pipette the three solutions into a new 96-well microplate:
  - 150  $\mu$ L of the 100% Test Solution into all wells of columns 1 and 2
  - 150  $\mu$ L of the 75% Test Solution into all wells of columns 3 and 4
  - 150  $\mu$ L of the 50% Test Solution into all wells of column 5 and 6

#### NOTE

After pipetting the diluted test solution into the microplate and before reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. Alternatively, wait 20 minutes after pipetting the test solution before reading the plate.

- 4 Create a Gen5/Gen6 experiment based on **Synergy HTX Abs Test 3** and read the plate.
- 5 Save the experiment when it is finished.
- 6 Refer to the instructions in [Results Analysis](#) to perform calculations and determine pass/fail. See [Troubleshooting](#) for troubleshooting tips.

## 7 Instrument Qualification

### Absorbance Liquid Tests

## Results Analysis

All tests are conducted using the Normal read speed.

### Absorbance Liquid Test 1

#### Accuracy Specification:

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

#### Repeatability Specification:

$\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD  
 $\pm 3.0\% \pm 0.005$  OD from 2.000 to 3.000 OD

- 1 The plate is read five times in the "Normal" position at 405 nm. Calculate the Mean OD and Standard Deviation of those five reads for each well in columns 1 and 2.
- 2 For each well in columns 1 and 2, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate ( $\text{Mean OD} \times 0.01 + 0.005$ ). For each well, its Standard Deviation should be less than its Allowed Deviation.
  - Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a Mean of 0.8004 and a Standard Deviation of 0.0018. The Mean multiplied by 1.0% ( $0.8004 \times 0.01$ ) equals 0.008, and when added to 0.005 equals 0.013; this is the Allowed Deviation for well A1. Since the Standard Deviation for well A1 is less than this value, the well meets the test criteria.
- 3 The plate is read five times in the "Turnaround" position at 405 nm. Calculate the Mean OD of those five reads for each well in columns 11 and 12.
- 4 Perform a mathematical comparison of the Mean values for each well in its Normal and Turnaround positions (that is, compare A1 to H12, A2 to H11, B1 to G12,... H2 to A11). To pass the test, the differences in the compared Mean values must be within the Accuracy specification for a 96-well microplate.
  - Example: If the Mean value for well A1 in the Normal position is 1.902 with a specified accuracy of  $\pm 1.0\% \pm 0.010$  OD, then the expected range for the Mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD.
  - $1.902 \times 0.010 + 0.010 = 0.029; 1.902 - 0.029 = 1.873; 1.902 + 0.029 = 1.931.$

### Absorbance Liquid Test 2

#### Accuracy Specification:

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

#### Repeatability Specification:

$\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD  
 $\pm 3.0\% \pm 0.005$  OD from 2.000 to 3.000 OD

- 1 The plate is read five times at 450/630 nm ("Normal" position), resulting in five sets of Delta OD data. Calculate results for Linearity:

## 7 Instrument Qualification

### Absorbance Liquid Tests

- a Calculate the mean absorbance for each well, and average the means for each concentration.
    - b Perform a regression analysis on the data to determine if there is adequate linearity. Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an  $R^2$  value of at least 0.9900 is considered adequate.
  - 2 Calculate the results for Repeatability:

    - a Calculate the Mean and Standard Deviation for the five readings taken at each concentration. Only one row of data needs to be analyzed.
    - b For each Mean below 2.000 OD, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate of  $\pm 1.0\% \pm 0.005$  OD. (If above 2.000 OD, apply the  $\pm 3.0\% \pm 0.005$  specification.)
    - c The Standard Deviation for each set of readings should be less than the Allowed Deviation.
    - Example: Readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a Mean of 1.951, and a Standard Deviation of 0.0026. The Mean (1.951) multiplied by 1.0%:  $(1.951 \times 0.01) = 0.01951$ , when added to the 0.005 ( $0.01951 + 0.005 = 0.02451$  OD, which is the Allowed Deviation. Since the Standard Deviation is less than this value, the reader meets the test criteria.
  - 3 After gathering data for the Linearity Test, the plate is read five more times with the A1 well in the H12 position ("Turnaround" position). This results in values for the four corner wells that can be used to assess alignment. Calculate results for the Alignment Test:

    - a Calculate the means of the wells A1 and H1 in the Normal plate position (data from Linearity Test) and in the Turnaround position.
    - b Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the Accuracy specification for 96-well plates. If the four corner wells are within the accuracy range, the reader is in alignment.
    - Example: If the mean of well A1 in the normal position is 1.902, where the specified accuracy is  $\pm 1.0\% \pm 0.010$  OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. ( $1.902 \times 1.0\% = 0.019 + 0.010 = 0.029$ , which is added to and subtracted from 1.902 for the range.)

### Absorbance Liquid Test 3

#### Repeatability Specification:

$\pm 1.0\% \pm 0.005$  OD from 0.000 to 2.000 OD  
 $\pm 3.0\% \pm 0.005$  OD from 2.000 to 3.000 OD

- 1 The plate is read five times at 340 nm. For each well, calculate the Mean OD and Standard Deviation of the five readings.
- 2 For each Mean calculated in step 1, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate ( $\text{Mean OD} \times 0.010 + 0.005$ ). For each well, its Standard Deviation should be less than its Allowed Deviation.

  - Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a Mean of 0.8004 and a Standard Deviation of 0.0018. The Mean multiplied by 1.0% ( $0.8004 \times 0.01$ ) equals 0.008, and when added to 0.005 equals 0.013; this is the Allowed Deviation for well

## 7 Instrument Qualification

### Fluorescence Testing Overview

A1. Since the Standard Deviation for well A1 is less than this value, the well meets the test criteria.

#### 3 Calculate results for Linearity:

- a For each of the three test solutions, calculate the average Mean OD for the wells containing that solution (mean of wells A1 to H2, A3 to H4, and A5 to H6).
- b Perform a regression analysis on the data to determine if there is adequate linearity. The three average Mean OD values are the "Y" values. The solution concentrations are the "X" values (1.00, 0.75, 0.50). Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an  $R^2$  value of at least 0.9900 is considered adequate.

## Troubleshooting

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

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

## Fluorescence Testing Overview

---

For models with fluorescence capability, Agilent provides two options for testing the fluorescence system. One uses a solid state Fluorescence Test Plate (package part number 1400501).<sup>1</sup> The other uses liquid plates, the materials for which are available for purchase from Agilent. See [Materials for Conducting Liquid Tests](#).

## Fluorescence Plate Test

---

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

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

---

<sup>1</sup>Fluorescence Test Plate part number 7092092 cannot be used for these tests.

## 7 Instrument Qualification

### Fluorescence Plate Test

#### NOTE

Gen6 experiments can be created from the Gen5 protocols supplied with the Fluorescence Test Plate.<sup>1</sup> In Gen6, select **File > Open**. A file explorer will appear with the default file type set to Experiment files (\*.xpt). Change the file type to **Protocol files (\*.prt)**, browse to the desired Gen5 protocol, and select **Open**. The Gen5 protocol will open as a Gen6 experiment.

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

## Requirements

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

## Test Procedure

The **Qualification Tests** section of the *Fluorescence Test Plate User Guide* contains a procedure for cleaning the plate and then creating and running experiments in either Gen5 or Gen6 based on supplied Gen5 protocols.

As described in the user guide, when each experiment is finished, Gen5/Gen6 exports the measurement data to a prepared Microsoft Excel .xls file. The worksheet(s) within that file calculate results and determine pass or fail. Identify the reader-specific Gen5 protocols on the USB flash drive that came with the test plate. Use only those protocols that apply to your reader model and your organization's qualification requirements.

## Results Analysis

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

Fluorescence Intensity (FI) Tests	
Corners	%CV < 3.0
Linearity	$R^2 \geq 0.9500$
<b>Sensitivity, filter-based system:</b>	
Sodium Fluorescein analogue, Top optics	Detection Limit $\leq 53 \text{ pM}$
Sodium Fluorescein analogue, Bottom optics	Detection Limit $\leq 30 \text{ pM}$
Methylumbellifereone analogue, Top optics	Detection Limit $\leq 160 \text{ pg/mL}$
Methylumbellifereone analogue, Bottom optics	Detection Limit $\leq 160 \text{ pg/mL}$

<sup>1</sup>Using Gen6 version 1.04 or higher.

## Fluorescence Liquid Tests

---

Agilent has developed a series of liquid tests for verifying fluorescence performance. The tests are conducted using Sodium Fluorescein (SF) and/or Methylumbelliferone (MUB), as required by your assays.

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

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

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

## Test Methods

- The **Corners Test** uses fluorescence compounds to verify that the plate carrier is properly aligned in relation to the fluorescence optics. This test may be conducted using either the top or bottom optics.
- The **Sensitivity Test** uses a fluorescence compound and buffer solution to test the fluorescence reading capability of the instrument. The ability to detect specific compounds at the required limit of detection ensures that the filters, optical path, and PMT are all in working order. This test verifies that the difference between the concentration well under investigation and the mean of the median buffer well is statistically distinguishable. This test may be conducted using either the top or bottom optics.
- The **Linearity Test** verifies that the system is linear; that is, the signal changes proportionally with changes in concentration ( $R^2$  value). Proving that the system is linear allows the Sensitivity Test to be run on two points instead of using serial dilutions. This test may be conducted using either the top or bottom optics.

## Gen5 Protocol Parameters

If using Gen5 to conduct the fluorescence liquid tests, follow these instructions. The information in the following tables represents the recommended reading parameters for the referenced Gen5 protocols. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

**NOTE**

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

## 7 Instrument Qualification

### Fluorescence Liquid Tests

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

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915) (FI_T) "Greiner SensoPlate" (#655892) (FI_B)
<b>Read Step 1</b>	
Kinetic loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read"
Read well	D7
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Scale to High Wells, D7, 50000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)
<b>Read Step 2</b>	
Kinetic loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read Buffer"
Read wells	C9–E9
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Use first filter set gain from FIRST read step
Read Speed	Normal

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FL_T)
<b>Read Step 3</b>	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Corners Read"
Read wells	A1–A3, A10–A12, H1–H3, H10–H12
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FL_T) or Bottom (FL_B)
Gain	Auto, Scale to high wells, A3, 50000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FL_T)
<b>Read Step 4</b>	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Linearity Read"
Read wells	C1–F5
Filter Set	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FL_T) or Bottom (FL_B)
Gain	Auto, Scale to high wells, C1, 50000
Read Speed	Normal

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm (FI_T)

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

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915)
<b>Read Step 1</b>	
Kinetic loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read"
Read well	D7
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to High Wells, D7, 80000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm
<b>Read Step 2</b>	
Kinetic loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Sensitivity Read Buffer"
Read wells	C9-E9

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Use first filter set gain from FIRST step
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm
<b>Read Step 3</b>	
Detection Method	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Linearity Read"
Read wells	C1-F5
Filter Set	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to high wells, C1, 80000
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Light Source	Tungsten
Read Height	1.00 mm

## 7 Instrument Qualification

### Fluorescence Liquid Tests

## Gen6 Experiment Parameters

If using Gen6 to conduct the fluorescence liquid tests, follow these instructions. The information in the following tables represents the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

### NOTE

The Plate Type setting in each Gen6 experiment should match the plate you are actually using.

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

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915) (FI_T) "Greiner SensoPlate" (#655892) (FI_B)
<b>Read Step 1</b>	
Read Mode	Fluorescence intensity
Read Type	Kinetic
Kinetic Loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Optics Type	Filters
Step Label	"Sensitivity Read"
Wells	D7
Filter Sets	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FI_T) or Bottom (FI_B)
Gain	Auto, Scale to High Wells, D7, Scale % 50
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm (FI_T)
<b>Read Step 2</b>	
Read Mode	Fluorescence intensity
Read Type	Kinetic
Kinetic Loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Optics Type	Filters
Step Label	"Sensitivity Read Buffer"
Wells	C9-E9

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Filter Sets	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FL_T) or Bottom (FL_B)
Gain	Auto, Scale to High Wells, D7, Scale % 50
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm (FL_T)
<b>Read Step 3</b>	
Read Mode	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Corners Read"
Wells	A1–A3, A10–A12, H1–H3, H10–H12
Filter Sets	1
Excitation	485/20 nm
Emission	528/20 nm
Optics Position	Top (FL_T) or Bottom (FL_B)
Gain	Auto, Scale to high wells, A3, Scale % 50
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm (FL_T)
<b>Read Step 4</b>	
Read Mode	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Linearity Read"
Wells	C1–F5
Filter Sets	1
Excitation	485/20 nm

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Emission	528/20 nm
Optics Position	Top (FL_T) or Bottom (FL_B)
Gain	Auto, Scale to high wells, C1, Scale % 50
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm (FL_T)

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

Parameter	Setting
Plate Type	"Costar 96 black opaque" (#3915)
<b>Read Step 1</b>	
Read Mode	Fluorescence intensity
Read Type	Kinetic
Kinetic Loop	Run time 0:00:45, Interval 0:00:03 (16 reads)
Optics Type	Filters
Step Label	"Sensitivity Read"
Wells	D7
Filter Sets	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to High Wells, D7, Scale % 80
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm
<b>Read Step 2</b>	
Read Mode	Fluorescence intensity
Read Type	Kinetic
Kinetic Loop	Run time 0:01:35, Interval 0:00:06 (16 reads)
Optics Type	Filters

## 7 Instrument Qualification

### Fluorescence Liquid Tests

Parameter	Setting
Step Label	"Sensitivity Read Buffer"
Wells	C9–E9
Filter Sets	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to High Wells, D7, Scale % 80
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm
<b>Read Step 3</b>	
Read Mode	Fluorescence intensity
Read Type	Endpoint
Optics Type	Filters
Step Label	"Linearity Read"
Wells	C1–F5
Filter Sets	1
Excitation	360/40 nm
Emission	460/40 nm
Optics Position	Top
Gain	Auto, Scale to high wells, C1, Scale % 80
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	50
Dynamic Range	Standard
Read Height	1.00 mm

## 7 Instrument Qualification

### Fluorescence Liquid Tests

## Required Materials



#### IMPORTANT

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

#### NOTE

Kits containing the microplates and solutions required by the Liquid Tests are available for purchase. If using test kit part number 7160013, the buffer and SF are pre-diluted. See [Materials for Conducting Liquid Tests](#).

#### NOTE

Manufacturer part numbers are subject to change.

## All Tests

- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- Aluminum foil
- (Optional, but recommended) 0.45 micron filter
- (Optional) Black polyethylene bags to temporarily store plates
- 95% ethanol (for cleaning clear-bottom plates)
- Gen5 protocols described in [Gen5 Protocol Parameters](#) or Gen6 experiments described in [Gen6 Experiment Parameters](#).

### For the filter-based fluorescence system:

Synergy HTX FI_T_SF.prt/.xpt	Corners, Sensitivity, Linearity tests, Top optics, Sodium Fluorescein (SF)
Synergy HTX FI_B_SF.prt/.xpt	Corners, Sensitivity, Linearity tests, Bottom optics, Sodium Fluorescein (SF)
Synergy HTX FI_T_MUB.prt/.xpt	Sensitivity and Linearity tests, Top optics, Methylumbelliferon (alternate/ supplemental test for Top optics)

## Corners, Sensitivity, Linearity (FI) Tests

#### NOTE

The materials listed here are for use with Sodium Fluorescein. Methylumbelliferon can be used as an alternate or supplemental method for performing these tests. See [Alternate/Supplemental Tests Using Methylumbelliferon \(MUB\)](#) for the materials, procedures, and pipette map used to perform these tests with MUB.

- Buffer:

- NIST-traceable Sodium Borate Reference Standard (pH 9.18) (e.g., Fisher-Scientific 1 L Sodium Borate Mfr. #159532, or equivalent), or
- Phosphate-Buffered Saline (PBS), pH 7.2–7.6 (e.g., Sigma tablets, Mfr. #P4417, or equivalent) and pH meter or pH indicator strips with pH range 4 to 10

## 7 Instrument Qualification

### Fluorescence Liquid Tests

- Sodium Fluorescein Powder (1 mg vial, Agilent BioTek part number 98155)
- Bottom optics: A new, clean 96-well glass-bottom Greiner SensoPlate (Mfr. #655892); or a clean Hellma Quartz 96-well titration plate (Mfr. #730.009.QG); or equivalent
- Top optics: A new, clean 96-well solid black microplate, such as Corning Costar #3915, or equivalent. The Greiner SensoPlate mentioned above can also be used
- Installed and configured in Gen5/Gen6:
  - Excitation filter 485/20 nm
  - Emission filter 528/20 nm

## Test Solutions

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

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

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

- 1 The Sodium Borate solution does not require further preparation; proceed to step 2. If you are using PBS, prepare the solution:
  - a (Optional, but recommended) Using a 0.45 micron filter, filter 200 mL of deionized or distilled water.
  - b Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
  - c Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
  - d Check the pH; it should be between 7.2 and 7.6 at 25°C.
- 2 Prepare the sodium fluorescein stock solution:
  - a Add 2.0 mL of the buffer solution to the 1 mg Sodium Fluorescein (SF) vial. This yields a 1.3288 mM stock solution.
  - b Ensure that the dye has completely dissolved and is well mixed.
- 3 Carefully prepare the dilutions. Label each with "SF" and the concentration:

Mix This SF Solution:	With Buffer:	To Make:
0.53 mL of 1.3288 mM stock solution	13.47 mL	50.2 $\mu$ M
110 $\mu$ L of 50.2 $\mu$ M SF	13.89 mL	400 nM
3.5 mL of 400 nM SF	10.5 mL	100 nM
0.46 mL of 100 nM SF	13.54 mL	3.3 nM; For use in Corners Test
4.24 mL of 3.3 nM SF	9.76 mL	1 nM; For use in Sensitivity/Linearity Tests

## Pipette Map

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

## Corners, Sensitivity, and Linearity (FI) Tests

1 For the **Corners** test:

- Use a single-channel pipette.
- Pipette 200  $\mu$ L of the 3.3 nM SF solution into the "corner" wells: A1-A3, A10-A12, H1-H3, and H10-H12.
- Pipette 200  $\mu$ L of buffer into the wells surrounding the 3.3 nM SF wells (labeled CBUF in the plate map). (Omit if using a solid black plate or Greiner SensoPlate.)

2 For the **Sensitivity** test:

- Use a single-channel pipette.
- Pipette 200  $\mu$ L of the 1 nM SF solution into well D7.
- Pipette 200  $\mu$ L of the buffer solution into wells C9, D9, and E9.

3 For the **Linearity** test:

- Use a multichannel pipette with just four tips installed.
- Pipette 150  $\mu$ L of buffer solution into wells C2-F5 (not column 1). Discard the tips.
- Pipette 150  $\mu$ L of the 1 nM SF solution into wells C1-F1.
- Pipette 150  $\mu$ L of the 1 nM SF solution into wells C2-F2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from wells C2-F2, and dispense into wells C3-F3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from wells C3-F3, and dispense into wells C4-F4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from wells C4-F4, and dispense into wells C5-F5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from wells C5-F5, and discard the tips.

## 7 Instrument Qualification

### Fluorescence Liquid Tests

	1	2	3	4	5	6	7	8	9	10	11	12
A	3300pM_200	3300pM_200	3300pM_200	CBUF					CBUF	3300pM_200	3300pM_200	3300pM_200
B	CBUF	CBUF	CBUF	CBUF					CBUF	CBUF	CBUF	CBUF
C	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150				BUF_200			
D	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150		1000pM_200		BUF_200			
E	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150				BUF_200			
F	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150							
G	CBUF	CBUF	CBUF	CBUF					CBUF	CBUF	CBUF	CBUF
H	3300pM_200	3300pM_200	3300pM_200	CBUF					CBUF	3300pM_200	3300pM_200	3300pM_200

Figure 7-2: FI test plate map.

## Test Procedure

- 1 If you have not already done so, create the Gen5 protocols described in [Gen5 Protocol Parameters](#) or the Gen6 experiments described in [Gen6 Experiment Parameters](#).
- 2 If you have not already done so, prepare the solutions for the tests you plan to perform. See [Test Solutions](#).

**NOTE**

Refer to the [Pipette Map](#) for the remaining steps.

- 3 Perform the Corners, Sensitivity, and Linearity tests using the Bottom optics of the filter-based fluorescence system:
  - a Pipette the solutions for the FI tests into a clean 96-well glass-bottom or quartz plate.
  - b Create a Gen5/Gen6 experiment based on [Synergy HTX FI\\_B\\_SF](#) and read the plate.
- 4 Perform the Corners, Sensitivity, and Linearity tests using the Top optics of the filter-based fluorescence system:
  - a Pipette the solutions for the FI tests into a clean, new, 96-well solid black or glass-bottom plate.
  - b Create a Gen5/Gen6 experiment based on [Synergy HTX FI\\_T\\_SF](#) and read the plate.
- 5 Save the experiments.
- 6 See [Results Analysis](#) to calculate results and determine pass/fail. See [Troubleshooting](#) for troubleshooting tips.

**NOTE**

The following **Results Analysis** and **Troubleshooting** sections are applicable to fluorescence tests performed with either Sodium Fluorescein (SF) or Methylumbelliflone (MUB).

## 7 Instrument Qualification

### Fluorescence Liquid Tests

## Results Analysis

### Corners Test

- 1 Calculate the Mean of the 12 "corner" wells, which contain the 3.3 nM SF test solution (A1–A3, A10–A12, H1–H3, and H10–H12).
- 2 Calculate the Standard Deviation for the same 12 wells.
- 3 Calculate the %CV:  
$$(\text{Standard Deviation} / \text{Mean}) * 100$$

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

### Sensitivity Test

- 1 Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (C9, D9, E9).
- 2 Among the three buffer wells, find the Median, Standard Deviation, and corresponding Mean.
- 3 Calculate the Mean for the 16 reads of the SF (or MUB) Concentration well (D7).
- 4 Calculate the Signal-to-Noise Ratio (SNR) using the Mean SF (or MUB) Concentration, Buffer Media STD with its corresponding Buffer Mean:  
$$(<\text{SF or MUB}> \text{ Mean} - \text{Buffer Mean}) / 3 * \text{Buffer STD}$$
- 5 Calculate the Detection Limit:
  - **If using SF:** Calculate the Detection Limit in pM using the known concentration value of SF and the Calculated SNR:  $1000 / \text{SNR}$ .

Test	To pass, the Detection Limit must be:
Bottom 5 mm probe	$\leq 30 \text{ pM (10 pg/mL)}$
Top 3 mm probe	$\leq 53 \text{ pM (20 pg/mL)}$

- **If using MUB:** Calculate the Detection Limit in ng/mL using the known concentration value of MUB and the calculated SNR:  $17.6 / \text{SNR}$ .

Test	To pass, the Detection Limit must be:
Top 3 mm probe	$\leq 0.16 \text{ ng/mL (0.91 nM)}$

### Linearity Test

- 1 Calculate the Mean of the four wells for each concentration in columns 1–5.
- 2 Perform linear regression using these values as inputs:

Sodium Fluorescein	
X	Y
1000	Mean of the 1000 pM wells
500	Mean of the 500 pM wells
250	Mean of the 250 pM wells

## 7 Instrument Qualification

### Fluorescence Liquid Tests

#### Sodium Fluorescein

X	Y
125	Mean of the 125 pM wells
62.5	Mean of the 62.5 pM wells

#### Methylumbellifерone

X	Y
100	Mean of the 100 nM wells
50	Mean of the 50 nM wells
25	Mean of the 25 nM wells
12.5	Mean of the 12.5 nM wells
6.25	Mean of the 6.25 nM wells

- 3 Calculate the  $R^2$  value; it must be  $\geq 0.9500$  to pass.

## Troubleshooting

If any tests fail, please consider the following suggestions. If the tests continue to fail, print the results and contact Technical Support.

- Are the solutions fresh? Discard the plate and any opened, unused test solutions after seven days.
- Are the excitation/emission filters clean? Are they in the proper locations and in the proper orientation in the filterwheel?
- If the Corners Test continues to fail, the hardware may be misaligned. Contact Technical Support.
- Are you using new/clean plates? If the base of a clear-bottom plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster. If the test fails again, the optical probe(s) may need to be cleaned. Contact Technical Support for instructions.
- Review the pipetting instructions to verify the plate was correctly prepared.
- Does the Plate Type setting in the Gen5 protocol/Gen6 experiment match the plate you used?
- For injector models, spilled fluid inside the reader may be fluorescing, which can corrupt your test results. If you suspect this is a problem, contact Technical Support for assistance.
- The Read steps in the protocols use the Automatic Gain Adjustment feature to determine optimum sensitivity values for the plate. If an Auto Gain Result value is outside the range of 30–200, this may indicate a problem.

If the value is less than 30:

- The stock solution/dilution concentrations may be too high. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
- If all of the tests pass but the Gain value is low, a PMT in your reader may just be very sensitive. Contact Technical Support to confirm that this may be the case.

If the value is greater than 200:

## 7 Instrument Qualification

### Fluorescence Liquid Tests

- The stock solution/dilution concentrations may be too low. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
- The PMTs or optical path(s) may be deteriorating, or the optics or other hardware may be misaligned. Contact Technical Support.

## Alternate/Supplemental Tests Using Methylumbelliferone (MUB)

As an alternative to using Sodium Fluorescein, Methylumbelliferone (MUB) can be used to perform the sensitivity and linearity tests for the top optics.

### Required Materials



#### IMPORTANT

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

#### NOTE

Agilent offers a liquid test kit (part number 7160012) containing the microplates and solutions used in the MUB fluorescence liquid tests. See [Materials for Conducting Liquid Tests](#).

#### NOTE

Manufacturer part numbers are subject to change.

- Methylumbelliferone (MUB) (10 mg vial, Agilent part number 98156)
- Carbonate-Bicarbonate buffer (CBB) capsules (Agilent part number 98158)
- 100% methanol (Agilent part number 98161)
- A new, clean 96-well solid black plate microplate, such as Corning Costar #3915 or equivalent. A Greiner SensoPlate (Mfr. #655892) may also be used
- Excitation filter 360/40 nm and Emission filter 460/40 nm installed in the reader and configured in Gen5/Gen6
- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- 95% ethanol (for cleaning clear-bottom plates)
- Aluminum foil
- (Optional, but recommended) 0.45 micron filter
- (Optional) Black polyethylene bags to temporarily store plates
- Gen5 protocols described in [Gen5 Protocol Parameters](#) or Gen6 experiments described in [Gen6 Experiment Parameters](#).

#### For the filter-based fluorescence system:

Synergy HTX FI\_T\_MUB.prt/.xpt

Sensitivity and Linearity tests, Top optics, Methylumbelliferone (alternate/ supplemental test for Top optics)

## 7 Instrument Qualification

### Fluorescence Liquid Tests

#### Test Solutions

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

Wrap the vial containing the MUB stock solution in foil to prevent exposure to light.

Discard any open, unused solutions after seven days.

**1** Prepare the buffer (CBB) solution:

- a** (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
- b** Open and dissolve the contents of two CBB capsules (do not dissolve the outer gelatin capsule) into 200 mL of the water.
- c** Stir the solution (preferably using a stir table) until the CBB is completely dissolved.

**2** Prepare the MUB stock solution:

- a** Add 1 mL of 100% methanol to the 10 mg vial of MUB.
- b** Make sure all of the dye has completely dissolved and is well mixed. This yields a 10 mg/mL stock solution.
- c** Wrap the solution in aluminum foil to prevent exposure to light.

**3** Prepare the dilutions. Label each with "MUB" and the concentration.

Mix This MUB Solution:	With:	To Make:
0.5 mL of 10 mg/mL stock solution	4.5 mL of 100% methanol	1 mg/mL
0.88 mL of 1 mg/mL solution	4.12 mL of CBB	176 µg/mL
0.1 mL of 176 µg /mL solution	9.9 mL of CBB	1.76 µg /mL
0.5 mL of 1.76 µg /mL solution	4.5 mL of CBB	176 ng/mL
1 mL of 176 ng/mL solution	9 mL of CBB	17.6 ng/mL (100 nM)

#### Pipette Map

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

**Using a single-channel pipette:**

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

## 7 Instrument Qualification

### Fluorescence Liquid Tests

#### Using a multichannel pipette with just four tips installed:

- Pipette 150  $\mu$ L of buffer into wells C2-F5 (not column 1). Discard the tips.
- Pipette 150  $\mu$ L of the 17.6 ng/mL (100 nM) solution into column 1. Discard the tips.
- Pipette 150  $\mu$ L of the 17.6 ng/mL (100 nM) solution into column 2. Do not discard the tips.
- Aspirate 150  $\mu$ L from column 2 and dispense it into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from column 3 and dispense it into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from column 4 and dispense it into column 5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150  $\mu$ L from column 5. Discard the tips.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	100nM_150	50nM_150	25nM_150	12_5nM_150	6_25nM_150				BUF_200			
D	100nM_150	50nM_150	25nM_150	12_5nM_150	6_25nM_150		100nM_200		BUF_200			
E	100nM_150	50nM_150	25nM_150	12_5nM_150	6_25nM_150				BUF_200			
F	100nM_150	50nM_150	25nM_150	12_5nM_150	6_25nM_150							
G		▲▼										
H												

Figure 7-3: Alternative FI tests plate map.

## Test Procedure

- 1 If you have not already done so, create the Gen5 protocols described in [Gen5 Protocol Parameters](#) or the Gen6 experiments described in [Gen6 Experiment Parameters](#).
- 2 If you have not already done so, prepare the test solutions. See [Test Solutions](#).
- 3 Perform the Sensitivity and Linearity tests using the Top optics of the filter-based fluorescence system:
  - a Pipette the solutions into a clean, 96-well solid black plate. Refer to the [Pipette Map](#).
  - b Create a Gen5/Gen6 experiment based on [Synergy HTX FI\\_T\\_MUB](#) and read the plate.
- 4 Save the experiment.
- 5 Refer to [Results Analysis](#) to calculate results and determine pass/fail. See [Troubleshooting](#) for troubleshooting tips.

## Luminescence Test

---

For models with luminescence capability, the Harta Luminometer Reference Microplate is used to test the luminescence system. The test plate is LED-based and NIST-traceable. Contact Agilent to purchase a plate (part number 8030015; includes microplate carrier adapters).

### Test Method

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

### Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A		REF					LED7	LED8				
B												
C												
D	Buffer	Buffer	Buffer	Buffer								
E	Buffer	Buffer	Buffer	Buffer								
F	Buffer	Buffer	Buffer	Buffer								
G	Buffer	Buffer	Buffer	Buffer								
H												

Figure 7-4: Plate layout for [Synergy HTX LumTest\\_Harta](#).

## 7 Instrument Qualification

### Luminescence Test

## Gen5 Protocol Parameters

If you are using Gen5 to test luminescence, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen5 protocol. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

**NOTE**

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

### Synergy HTX LumTest\_Harta.prt

Parameter	Setting
Plate Type	"8030015 Harta - with 8032028 adapter"
Delay Step	3 minutes
<b>Read Step 1:</b>	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Reference well A2
Read well	A2
Filter Set	1
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	150
Integration Time	0:10.00 MM:SS.ss
Delay After Plate Movement	100 msec
Dynamic Range	Standard
Read Height	1.00 mm
<b>Read Step 2:</b>	
Detection Method	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Background
Read wells	F1-G12
Filter Set	1
Excitation	<Plug>

## 7 Instrument Qualification

### Luminescence Test

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

## 7 Instrument Qualification

### Luminescence Test

## Gen6 Experiment Parameters

If you are using Gen6 to test luminescence, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen6 experiment. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

#### NOTE

The Plate Type setting in each Gen6 experiment should match the plate you are actually using.

### Synergy HTX LumTest\_Harta.xpt

Parameter	Setting
Plate Type	"8030015 Harta - with 8032028 adapter"
Delay Step	3 minutes
<b>Read Step 1</b>	
Read Mode	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Reference well A2
Wells	A2
Filter Sets	1
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	150
Luminescence Integration	Integration Time: 0:10.00 MM:SS.ss Delay After Plate Movement: 100 msec
Dynamic Range	Standard
Read Height	1.00 mm
<b>Read Step 2</b>	
Read Mode	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Background
Wells	F1-G12
Filter Sets	1
Excitation	<Plug>
Emission	Hole

## 7 Instrument Qualification

### Luminescence Test

Parameter	Setting
Optics Position	Top
Gain	150
Luminescence Integration	Integration Time: 0:10.00 MM:SS.ss Delay After Plate Movement: 100 msec
Dynamic Range	Standard
Read Height	1.00 mm
<b>Read Step 3</b>	
Read Mode	Luminescence
Read Type	Endpoint
Optics Type	Filters
Step Label	Battery check
Wells	A7–A8
Filter Sets	1
Excitation	<Plug>
Emission	Hole
Optics Position	Top
Gain	50
Luminescence Integration	Integration Time: 0:01.00 MM:SS.ss Delay After Plate Movement: 100 msec
Dynamic Range	Extended
Read Height	1.00 mm

## Required Materials

- Harta Luminometer Reference Microplate, part number 8030015 (which includes microplate carrier adapter part number 8032028)
- A Plug in the Excitation filter wheel
- An open position (Hole) in the Emission filter wheel
- Gen5 protocol described in [Gen5 Protocol Parameters](#):
  - [Synergy HTX LumTest\\_Harta.prt](#)
- or Gen6 experiment described in [Gen6 Experiment Parameters](#):
  - [Synergy HTX LumTest\\_Harta.xpt](#)

## 7 Instrument Qualification

### Luminescence Test

#### Test 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.

##### NOTE

The test light may be difficult to see in bright light. Change your angle of view or move to a darker environment if you cannot see it.

- 3 Place the Harta plate adapter on the reader's carrier, then place the test plate on top of the adapter.
- 4 Create a Gen5/Gen6 experiment based on [Synergy HTX LumTest\\_Harta](#). The experiment starts with a three-minute Delay step.
- 5 When the experiment is complete, calculate and evaluate results as described under [Results Analysis](#). See [Troubleshooting](#) for troubleshooting tips.
- 6 When finished, turn off the Harta reference plate to preserve battery life.

#### Results Analysis

##### NOTE

Through a manual correlation process, it was found that the system requires approximately 35 photons per attomole of ATP, thus a conversion factor of 0.02884 attomole/photon was applied to determine ATP concentration from the NIST data in photons/s.

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

##### NOTE

A replacement battery is included with each Harta plate. A new spare battery will be supplied when the plate is recertified.

- 2 On the Harta plate's Calibration Certificate, locate the NIST measurement for the A2 position and convert it to attomoles:  
$$(A2 \text{ NIST measurement} * 0.02884)$$
- 3 Calculate the signal-to-noise ratio:  
$$(A2 - \text{Mean of the buffer cells}) / (3 * \text{Standard deviation of buffer cells})$$
- 4 Calculate the detection limit:  
$$A2 \text{ NIST measurement in attomoles} / \text{signal-to-noise ratio}$$

#### Pass/Fail Criteria

- If the reader is equipped with the low-noise PMT, the detection limit must be  $\leq 60$  amol to pass.
- If the reader is equipped with the red-shifted PMT, the detection limit must be  $\leq 500$  amol to pass.

##### NOTE

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

## Troubleshooting

If the luminescence test fails, try the following suggestions. If it continues to fail, print the results and contact Technical Support.

- Ensure that the reading is performed through a hole in the EM filter wheel, not through a glass filter.
- Verify that the filter wheel settings in Gen5/Gen6 match the physical item installed in the reader.
- If the test continues to fail, the optics block may need to be cleaned. Contact Technical Support for instructions.

## Dispense Module Tests

---

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

### Test Method

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

The test uses a green dye test solution (available for purchase, see [Materials for Conducting Liquid Tests](#)) and one 96-well microplate per injector to test the three different volumes. The balance is tared with the empty plate and the 80  $\mu$ L dispense is performed for columns 1–4. The fluid is weighed and the balance is tared again with the plate. This process is repeated for the 20  $\mu$ L and 5  $\mu$ L dispenses.

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

The **Precision Test** is a measure of the variation among volumes dispensed to multiple wells, and uses the green test dye solution. For each volume dispensed (80  $\mu$ L, 20  $\mu$ L, and 5  $\mu$ L) 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  $\mu$ L, 7.0% for 20  $\mu$ L, and 10.0% for 5  $\mu$ L. Columns 1–4 are read at 405/750 nm and columns 5–12 at 630/750 nm.

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

## 7 Instrument Qualification

### Dispense Module Tests

## Gen5 Protocol Parameters

If using Gen5 to perform the dispense module tests, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen5 protocols. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

#### NOTE

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

### Synergy HTX Disp 1 Test.prt and Synergy HTX Disp 2 Test.prt

For use with models with Absorbance capability.

Parameter	Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Wells A1–H4 Tip prime before this dispense step, 20 $\mu$ L Dispense 80 $\mu$ L at 275 $\mu$ L/sec
Plate Out,In	Comment: Weigh the plate (80 $\mu$ L 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 $\mu$ L Dispense 20 $\mu$ L at 250 $\mu$ L/sec
Plate Out,In	Comment: Weigh the plate (20 $\mu$ L 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 $\mu$ L Dispense 5 $\mu$ L at 225 $\mu$ L/sec
Plate Out,In	Comment: Weigh the plate (5 $\mu$ L test). RECORD the weight, TARE the balance. PIPETTE 150 $\mu$ L/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 $\mu$ l Read_Disp <1 or 2> Wells: A1–H4 Wavelengths, 2: 405 nm, 750 nm Speed: Normal

## 7 Instrument Qualification

### Dispense Module Tests

Parameter	Setting
Read Step	Detection Method: Absorbance Read Type: Endpoint Optics Type: Monochromator Step label: 20 and 5 $\mu$ L 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 $\mu$ L Read step, columns 1–4 630–750 nm for the 20 and 5 $\mu$ L Read step, columns 5–12

### Synergy HTX Disp 1 Test No Read.prt and Synergy HTX Disp 2 Test No Read.prt

For use with models without Absorbance capability.

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

## 7 Instrument Qualification

### Dispense Module Tests

#### Synergy HTX Disp Test Other Reader.prt

For use with an Agilent BioTek absorbance-capable reader other than Synergy HTX.

Parameter	Setting
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
<i>Data Reduction</i>	Define two Delta OD transformations: 405-750 nm for the 80 ul Read step, columns 1-4 630-750 nm for the 20 and 5 ul Read step, columns 5-12

## 7 Instrument Qualification

### Dispense Module Tests

## Gen6 Experiment Parameters

If using Gen6 to perform the dispense module tests, follow these instructions. The information in this section represents the recommended reading parameters for the referenced Gen6 experiment (s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

#### NOTE

The Plate Type setting in each Gen6 experiment should match the actual plate in use.

### Synergy HTX Disp 1 Test.xpt and Synergy HTX Disp 2 Test.xpt

For use with models with Absorbance capability.

Parameter	Setting
Plate Type	96 WELL PLATE
Dispense Step	Dispenser <1 or 2> Tip prime before this dispense step, 20 $\mu$ L Dispense 80 $\mu$ L at 275 $\mu$ L/sec Wells A1–H4
Plate Out/In	Comment: Weigh the plate (80 $\mu$ L test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Tip prime before this dispense step, 20 $\mu$ L Dispense 20 $\mu$ L at 250 $\mu$ L/sec Wells A5–H8
Plate Out/In	Comment: Weigh the plate (20 $\mu$ L test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click OK to continue.
Dispense Step	Dispenser <1 or 2> Tip prime before this dispense step, 5 $\mu$ L Dispense 5 $\mu$ L at 225 $\mu$ L/sec Wells A9–H12
Plate Out/In	Comment: Weigh the plate (5 $\mu$ L test). RECORD the weight, TARE the balance. PIPETTE 150 $\mu$ L/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	Read Mode: Absorbance Read Type: Endpoint Optics Type: Monochromator Step label: 80 $\mu$ l Read_Disp <1 or 2> Wells: A1–H4 Wavelengths, 2: 405 nm, 750 nm Read Speed: Normal

## 7 Instrument Qualification

### Dispense Module Tests

Parameter	Setting
Read Step	Read Mode: 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 Read Speed: Normal
Analyze Data	Define two Delta OD transformations: 405–750 nm for the 80 uL Read step, columns 1–4 630–750 nm for the 20 and 5 uL Read step, columns 5–12

### Synergy HTX Disp 1 Test No Read.xpt and Synergy HTX Disp 2 Test No Read.xpt

For use with models without Absorbance capability.

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

## 7 Instrument Qualification

### Dispense Module Tests

#### Synergy HTX Disp Test Other Reader.xpt

For use with an Agilent BioTek absorbance-capable reader other than Synergy HTX.

Parameter	Setting
Read Step	Read Mode: 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 Read Speed: Normal
Read Step	Read Mode: 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 Read Speed: Normal
Analyze Data	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

## Required Materials

### NOTE

Manufacturer part numbers are subject to change.

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

### NOTE

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

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200  $\mu$ L hand pipette and disposable tips
- Deionized water
- Supply bottles
- 250 mL beaker
- New 96-well, clear, flat-bottom microplates
- Green Test Dye Solution (part number 7773003) undiluted, or one of the alternate test solutions provided on the next page
- 100 mL graduated cylinder and 10 mL pipettes (if not using the Green Test Dye Solution)
- Gen5 protocol described in [Gen5 Protocol Parameters](#) or Gen6 experiment described in [Gen6 Experiment Parameters](#):

## 7 Instrument Qualification

### Dispense Module Tests

Test	Description
Synergy HTX Disp 1 Test.prt/.xpt	For models with Absorbance capabilities
Synergy HTX Disp 2 Test.prt/.xpt	
Synergy HTX Disp 1 Test No Read.prt /.xpt	For models without Absorbance capabilities
Synergy HTX Disp 2 Test No Read.prt/.xpt	
Synergy HTX Disp Test Other Reader.prt/.xpt	For if you will use Gen5/Gen6 with another Agilent BioTek Absorbance-capable reader

## Alternate Test Solutions

If you do not have the Agilent Green Test Dye Solution (part number 7773003), prepare a dye solution using one of the following methods:

**NOTE**

80  $\mu$ L of test solution with 150  $\mu$ L of deionized water should read between 1.300 and 1.700 OD at 405/750 nm. The solutions should be at room temperature.

### Using Agilent's Blue and Yellow Concentrate Dye Solutions:

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

### Using FD&C Blue and Yellow Dye Powder:

Item	Quantity per Liter
FD&C Blue No. 1	0.200 grams
FD&C Yellow No. 5	0.092 grams
Tween 20	1.0 mL
Sodium Azide N <sub>3</sub> Na	0.100 gram
Deionized water	make to 1 liter

## Test Procedure for Models with Absorbance Capability

- 1 Prime both dispensers with **4000  $\mu$ L** of deionized or distilled water.
- 2 Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to **2000  $\mu$ L**. 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  $\mu$ L** of the solution. When finished, remove the priming plate from the carrier.
- 4 Create a Gen5/Gen6 experiment based on **Synergy HTX Disp 1 Test**.
- 5 Place a new 96-well microplate on the balance and tare the balance.
- 6 Place the plate on the microplate carrier.

## 7 Instrument Qualification

### Dispense Module Tests

#### NOTICE

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



#### IMPORTANT

**When each dispense step is finished, 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/Gen6 will prompt you to empty the tip priming trough.
- 8 Proceed with the experiment when ready. The sequence is as follows:
  - a 80  $\mu$ L/well is dispensed to columns 1–4.
  - b When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
  - c 20  $\mu$ L/well is dispensed to columns 5–8.
  - d When prompted, remove the plate and weigh it. Record the weight and tare the balance. Place the plate on the carrier.
  - e 5  $\mu$ L/well is dispensed to columns 9–12.
  - f When prompted, remove the plate and weigh it. Record the weight.
  - g Manually pipette **150  $\mu$ L** of deionized or distilled water into all 12 columns, on top of the green test dye solution.
  - h 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 **Synergy HTX Disp 2 Test** and a new microplate.
- 12 When the tests are complete:
  - Prime both dispensers with at least **5000  $\mu$ L** of deionized water to flush out the dye solution.
  - Refer to the instructions in [Results Analysis](#) to perform calculations and determine pass/fail.

## 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 $\mu$ L Read	20 and 5 $\mu$ L 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  $\mu$ L** of deionized or distilled water.
- 2 Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to **2000  $\mu$ L**. 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  $\mu$ L** of the solution. When finished, remove the priming plate from the carrier.
- 4 Create an experiment based on **Synergy HTX Disp 1 Test No Read**.

## 7 Instrument Qualification

### Dispense Module Tests

- 5 Place a new 96-well microplate on the balance and tare the balance.
- 6 Place the plate on the microplate carrier.

#### NOTICE

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



#### IMPORTANT

When each dispense step is finished, 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/Gen6 will prompt you to empty the tip priming trough.
- 8 Proceed with the experiment when ready. The sequence is as follows:
  - a Dispense 80  $\mu$ L/well to columns 1–4.
  - b Remove the plate and weigh it. Record the weight and tare the balance.
  - c Place the plate on the carrier and dispense 20  $\mu$ L/well to columns 5–8.
  - d Remove the plate and weigh it. Record the weight and tare the balance.
  - e Place the plate on the carrier and dispense 5  $\mu$ L/well to columns 9–12.
  - f Remove the plate and weigh it. Record the weight.
  - g Manually pipette **150  $\mu$ L** of deionized or distilled water into all 12 columns, on top of the green test dye solution.
  - h Carefully set the plate aside and press OK.
- 9 Close the experiment without saving it.
- 10 Shake the plate for 15 seconds to ensure the dye and water are mixed adequately.
- 11 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 [Results Analysis](#).  
If you *are* using an Agilent BioTek absorbance reader, configure Gen5/Gen6 to communicate with the reader.
- 12 Create an experiment based on [Synergy HTX Disp Test Other Reader](#) and read the plate.
- 13 When the experiment is complete, save the file with an identifying name.
- 14 Remove the plate from the carrier and set it aside.
- 15 Repeat the procedure using [Synergy HTX Disp 2 Test No Read](#) and a new microplate.
- 16 When the tests are complete:
  - Prime both dispensers with at least **5000  $\mu$ L** of deionized water to flush out the dye solution.
  - Refer to the instructions in [Results Analysis](#) to perform calculations and determine pass/fail.

## 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  $\mu$ L, 20  $\mu$ L, 5  $\mu$ L), for each injector (1, 2):

## 7 Instrument Qualification

### Dispense Module Tests

- 1 Calculate the Standard Deviation of the 32 wells
- 2 Calculate the Mean of the 32 wells
- 3 Calculate the %CV:  
$$(\text{Standard Deviation} / \text{Mean}) \times 100$$
- 4 Calculate the Accuracy % Error:  
$$((\text{ActualWeight} - \text{ExpectedWeight}) / \text{ExpectedWeight}) * 100$$

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

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

If any tests fail, prime the fluid lines and rerun the tests. If the tests fail again, the injectors may require cleaning; see [Clean the Internal Components \(As Applicable\)](#). If tests continue to fail, contact Technical Support.

## Troubleshooting

If a dispense module test fails, try the following. If the test continues to fail, contact Technical Support.

- Check that the dispenser calibration values were entered correctly into Gen5/Gen6.
- Ensure that the dispense module is set up correctly:
  - Make sure the tubing is connected to the correct ports. The outlet tube connected to the **SYRINGE 1** port on the dispenser should also be connected to the **SYRINGE 1** port on the back of the instrument.
  - Check all tubing fittings to ensure there are no leaks. If leaks are detected, tighten the fittings.
  - Make sure the syringes are fully screwed into the syringe valves (finger tighten only).
- Check the tubing for crimps or bends, which may be impeding liquid flow. If a crimp is found, the tubing may need to be replaced. Contact Technical Support.

# Appendix A: Specifications

This appendix contains the published specifications for the Synergy HTX.

---

General Specifications .....	150
Environmental Conditions .....	150
Dispense/Read Specifications .....	151
Absorbance Specifications .....	151
Fluorescence Specifications .....	153
Luminescence Specifications .....	154

## General Specifications

---

### Microplates

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

Gen6 software does not support Take3 or Take3 Trio. Gen6 users can access these functions through the Take3 app.

### Hardware

Light Source	Absorbance: Xenon flash light source, 10W maximum average power Fluorescence: Tungsten halogen, 20W power
Dimensions	Approximately 16" D x 16" W x 10" H (40.6 cm x 40.6 cm x 25.4 cm) Note: For dimensions that include installation with a BioStack, refer to the <i>BioStack User Manual</i>
Weight	Approximately 38 lbs. (17 kg)
Power Supply	24-volt external power supply compatible with 100–240 V~; @50–60 Hz
Mains Supply Voltage Fluctuation	Up to +/-10% of the nominal voltage
Power Consumption	100 VA max, 130 VA max with injectors
Incubation	Temperature control range from 4° over ambient to 50°C. Temperature variation $\pm 0.5^{\circ}\text{C}$ @37°C, tested with Innovative Instruments, Inc. temperature test plate. Top and bottom incubation controlled via software-adjustable gradient.

## Environmental Conditions

---

For indoor use only. Not for use in wet conditions.

### Environmental

Temperature	Operational: 18°C to 40°C (64.4°F to 104°F) Storage: -25°C to 50°C (-13°F to 122°F)
Humidity	Operational: 10% to 85% relative humidity (non-condensing) Storage: 10% to 80% relative humidity (non-condensing)
Overvoltage category	II
Pollution degree	2

## Appendix A: Specifications

### Dispense/Read Specifications

#### Dispense/Read Specifications

Plate Type	Dispenses to standard 6-, 12-, 24-, 48-, 96-, and 384-well microplates with 128 x 86 mm geometry
Detection Method	Absorbance, Fluorescence (including TRF), Luminescence
Volume Range	5–1000 $\mu$ L with a 5–20 $\mu$ L tip prime
Accuracy	$\pm 1 \mu$ L or 2.0%, whichever is greater
Precision	Dispensing a 200 $\mu$ L solution of deionized water, 0.1% Tween 20, and dye at room temperature: $\leq 2.0\%$ for volumes of 50–200 $\mu$ L $\leq 4.0\%$ for volumes of 25–49 $\mu$ L $\leq 7.0\%$ for volumes of 10–24 $\mu$ L $\leq 10.0\%$ for volumes of 5–9 $\mu$ L
Injection Speeds	225, 250, 275, and 300 $\mu$ L/sec
Maximum delay between the end-of-dispense and start-of-read processes (96-/384-well plates, default probe heights only):	
<ul style="list-style-type: none"><li>• Absorbance: <math>T \leq 3</math> sec</li><li>• Top Filter Fluorescence: <math>T \leq 1</math> sec</li><li>• Bottom Filter Fluorescence: <math>T \leq 1</math> sec</li><li>• Luminescence: <math>T \leq 0.5</math> sec</li></ul>	

#### Absorbance Specifications

#### Optics

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

## Appendix A: Specifications

### Absorbance Specifications

## Performance

All qualifications were conducted using 96-/384-well, flat-bottom microplates. For the performance specifications described in this section, the Gain on the Optics Test should be below 8.0

### Accuracy

#### *Tested with certified neutral density glass*

96-well plate, normal read speed:	0.000 to 2.000 OD: $\pm 1.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
-----------------------------------	--

2.000 to 3.000 OD: $\pm 3.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
--

384-well plate, normal read speed:	0.000 to 2.000 OD: $\pm 2.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
------------------------------------	--

2.000 to 2.500 OD: $\pm 3.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
--

96- and 384-well plate, sweep read speed	0.000 to 1.000 OD: $\pm 1.0\% \pm 0.010$ OD
--	---

### Linearity

#### *Tested by liquid dilution*

96-well plate, normal read speed:	0.000 to 2.000 OD: $\pm 1.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
-----------------------------------	--

2.000 to 3.000 OD: $\pm 3.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
--

384-well plate, normal read speed:	0.000 to 2.000 OD: $\pm 2.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
------------------------------------	--

2.000 to 2.500 OD: $\pm 3.0\% \pm 0.010$ OD, delay after plate movement = 100 ms
--

96- and 384-well plate, sweep read speed:	0.000 to 1.000 OD: $\pm 1.0\% \pm 0.010$ OD
---	---

### Repeatability (Standard Deviation [STD])

#### *Tested with certified neutral density glass*

#### *Measured by one standard deviation (8 measurements/data points)*

96- and 384-well plate, normal read speed:	0.000 to 2.000 OD: $\pm 1.0\% \pm 0.005$ OD, delay after plate movement = 100 ms
--	--

2.000 to 3.000 OD: $\pm 3.0\% \pm 0.005$ OD, delay after plate movement = 100 ms
--

96- and 384-well plate, sweep read speed:	0.000 to 1.000 OD: $\pm 2.0\% \pm 0.010$ OD
---	---

### Read Timing

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

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

# Fluorescence Specifications

## Optics

Optics	
Optic Probes	Top, 3 mm probe Bottom, 5 mm probe
Detection	PMT, low-noise standard; red-shifted (850 nm) option available

## Sensitivity

The following specifications apply to the Normal mode of reading.

Sensitivity	
5 mm optical probe, bottom reading	DL Sodium Fluorescein in PBS, Excitation 485/20, Emission 528/20 ≤ 30 pM
	DL Propidium Iodide in PBS, Excitation 485/20, Emission 645/40 ≤ 62.5 ng/mL
3 mm optical probe, top reading	DL Sodium Fluorescein in PBS, Excitation 485/20, Emission 528/20 ≤ 53 pM
	DL Methylumbellifereone in CBB, Excitation 360/40, Emission 460/40 ≤ 0.16 ng/mL

## Read Timing

Because of the possible wide variations in setup, the following benchmark conditions are specified: Excitation Filter 485/20 nm, Emission Filter 528/20 nm; 10 measurements per data point; 100 ms delay after plate movement.

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

## Time-Resolved Fluorescence

*For "T" Models*

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

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

Granularity: 10- $\mu$ s step

## Luminescence Specifications

---

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

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

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

# Appendix B: Error Conditions

This appendix describes error conditions and provides troubleshooting tips.

---

Error Codes Overview .....	156
Error Codes .....	156

## Error Codes Overview

---

When a problem occurs during operation with the Synergy HTX, an error code may appear in Gen5/Gen6. Error codes typically contain four characters, such as "4168," and are usually accompanied by descriptive text. With some 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 service personnel. This appendix lists the most common and easily resolved error codes that you may encounter.

**NOTE**

Error codes beginning with "A" (e.g., A100) are fatal errors. These 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 Technical Support.

If an error code appears in Gen5/Gen6, you should run a system test for diagnostic purposes. See [System Test](#). Having the system test report before calling Technical Support can speed up the resolution of the error.

**NOTE**

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.

Use this appendix to diagnose problems and solve them if possible. If you need further assistance, contact [Technical Support](#).

## Error Codes

---

If an error code appears, look for it here. If you find the code, follow the suggestions provided for solving the problem. If you cannot find the code, or if you are unable to solve the problem, please contact [Technical Support](#). The Gen5/Gen6 Help system also provides troubleshooting tips.

Error Codes Description and Resolution	
0100	<p><b>Abort Error</b> <b>Assay process aborted</b></p> <p>Also known as the Halt command that aborts the running process without executing the Failure Mode &amp; Effects Analysis (FMEA) task</p> <p>The carrier is moved outside, and other axes are moved to safe positions. The aborted process returns an error status, indicating that it was aborted</p> <p>User aborted protocol or instrument operation. This is an informational error.</p> <p>Turning the instrument off and on may resolve the problem.</p>
0200	<p><b>24VDC dropped below safe level</b></p> <ul style="list-style-type: none"><li>External power supply has failed.</li><li>Verify connection to AC mains.</li><li>Power supply connection to instrument is loose or broken.</li></ul> <p>Contact <a href="#">Technical Support</a>.</p>

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
03xy	<b>Memory allocation error</b> Turn the instrument off and on. If the error reoccurs, contact <a href="#">Technical Support</a> .
04xy	<b>A/D (analog/digital) data collection failure</b> A/D converter for measurement circuits has failed or is generating inconsistent or noisy data for absorbance, fluorescence, or voltage reference measurements Turning the instrument off and on may resolve the problem. If the error reoccurs, contact <a href="#">Technical Support</a> .
05xy	<b>Measurement channel A/D standby transition never occurred</b> Basecode may need to be reloaded. Turning the instrument off and on may resolve the problem. If the error reoccurs, contact <a href="#">Technical Support</a>
06xy	<b>Voltage reference failures</b> Note: These are checked only during the system self-test Turning the instrument off and on may resolve the problem. If the error reoccurs, contact <a href="#">Technical Support</a> . Error code 0601 indicates that the tungsten halogen lamp requires replacement; see <a href="#">Install the Fluorescence Lamp Assembly</a> .
080x	<b>Configuration checksum errors</b> Basecode may need to be reloaded. Turning the instrument off and on may resolve the problem. If the error reoccurs, contact <a href="#">Technical Support</a> .
150X 151X 152X	<b>Incubator errors</b> Contact <a href="#">Technical Support</a> .

### 21xy errors

Invalid parameter error

These errors will occur when the instrument is sent protocol parameters that are incompatible with it. Because Gen5/Gen6 checks most of these parameters against the instrument model when the protocol is being defined, these errors generally serve as backup.

If one of these errors occurs repeatedly, the user should contact [Technical Support](#) with the following information:

- Instrument serial number
- Gen5/Gen6 version (available under Help > About Gen5/Gen6)
- An overview of the protocol definition
- The plate type used and whether it is standard or custom defined

2101	<b>Microplate geometry: invalid number of rows or columns</b>
2102	Must be > 1 and < 99 Ensure that the plate type is defined correctly, especially if it is a custom plate. If the plate type is based on an imported XML file, ensure that the XML file was defined correctly.
2109	<b>Plate height is out of range.</b> The specified plate height exceeds the maximum for this instrument. Verify that dimensions for the specific plate are correctly defined in the Gen5/Gen6 plate definition table.
22xy	<b>Hardware mismatch error</b> Autocalibration is being requested for an instrument configuration that does not exist. If error is repeatable, contact <a href="#">Technical Support</a> .

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
2302	<b>Plug not found in excitation filter wheel</b>
2303	<b>Plug not found in emission filter wheel</b> The protocol contains a plug in the 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	<b>Empty hole not found in emission filter wheel</b> The protocol requires a "hole" in the emission filter wheel, but no empty locations were detected. Verify that the emission filter wheel contains an empty location and that this is accurately reflected (as a hole) in the filter table.
2326	<b>TRF cartridge not installed</b> The protocol is defined for time-resolved fluorescence (TRF) but the TRF cartridge is not installed in the excitation filter wheel slot. Verify that the TRF cartridge is installed in the reader and that this is accurately reflected in the filter table.
2327	<b>Excitation filter wheel not installed</b> Verify that the excitation filter wheel is installed in the reader.
<b>24xy errors</b>	
X/Y limit error	
Movement requested exceeds defined dimensions of selected plate. Specific error indicates which dimension is incorrect.	
2403	<b>Invalid first row dimension</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2404	<b>Invalid last row dimension</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2405	<b>Invalid first column dimension</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2406	<b>Invalid last column dimension</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2407	<b>Invalid plate width</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2408	<b>Invalid plate length</b> Verify that dimensions for specific plate are correctly defined in Gen5/Gen6 plate definition table.
2500	<b>Tungsten halogen lamp is off when expected to be on</b> The lamp or lamp power supply has failed.
2B0x	<b>Syringe failure</b> x=1-4 Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. (Refer to <a href="#">Install the Dispense Module</a> for instructions.) Restart the reader.

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
2B0A	<b>Priming plate not detected</b> Place the priming plate on the carrier.
2C0x	<b>Dispenser configuration error</b> Verify that the dispenser configuration data is set correctly. Refer to <a href="#">Set Dispenser Calibration Values</a> .
2Dxy	<b>Assay errors</b> These errors are generated when a protocol entry is incorrect (setpoint, volume, etc.). These errors should not occur because Gen5/Gen6 should trap them during protocol creation. If a 2Dxy error is repeatable, contact <a href="#">Technical Support</a> .
2D09	<b>Tip prime volumes specified could overflow the tip prime trough</b>
2D0A	<b>Tip prime trough is full or may overflow</b> Empty the tip priming trough. Ensure the specified volume is not larger than the trough volume.
2D16	<b>Assay timer is past the read setup time</b> Verify that no PC sleep or hibernate modes are enabled on your computer.
2D2A	<b>Dispenser not primed successfully</b> Prime the dispenser again. If the error is repeatable, checked for pinched tubing or an obstruction at the syringe drive.
2D46	<b>Invalid wavelength selected</b> Verify that the selected wavelength is supported by your reader.
3700	<b>Absorbance reference channel failed noise test</b>
3710	<b>Absorbance measurement channel failed noise test</b> Possible causes: <ul style="list-style-type: none"><li>• Too much light in read chamber.</li><li>• The instrument enclosure is not completely installed and secured.</li><li>• The carrier door is not closing fully.</li><li>• The access door is not closed.</li><li>• Humidity is outside environmental specification of instrument.</li></ul>
3800	<b>Absorbance reference channel failed offset test</b>
3810	<b>Absorbance measurement channel failed offset test</b> Possible causes: <ul style="list-style-type: none"><li>• Too much light in read chamber.</li><li>• The instrument enclosure is not completely installed and secured.</li><li>• The carrier door is not closing fully.</li><li>• The access door is not closed.</li><li>• Humidity is outside environmental specification of instrument.</li></ul>

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
39xy	<b>Absorbance reference or measurement channel dark range outside limits.</b> Humidity is outside environmental specification of instrument. Note specification in user manual and move to area of lower humidity. Too much light in read chamber. Ensure that: <ul style="list-style-type: none"><li>• The instrument enclosure is completely installed and secured.</li><li>• The carrier door is closing fully.</li><li>• The access door is closed.</li></ul>
3A1x	<b>Absorbance gain out of range</b> Inspect all filters in the filter wheel and replace any that have failed. Possible intermittent flash failure. Contact <a href="#">Technical Support</a> .
3Exy	<b>Absorbance read saturated. Fail if measurement is <math>\geq 65535</math>. Either absorbance reference or measurement channel has saturated</b> Too much light in read chamber. Ensure that: <ul style="list-style-type: none"><li>• Instrument enclosure is completely installed and secured.</li><li>• The carrier door is fully closed.</li><li>• The access door is closed.</li></ul>
3Fxy	<b>Absorbance signal errors</b> <ul style="list-style-type: none"><li>• Ensure that the door is completely closed.</li><li>• Ensure that instrument enclosure is completely installed and secured.</li><li>• Flash lamp missing flashes or too much flash to flash intensity variation.</li><li>• Check for spill in chamber. Contamination on the lower or the upper optical assembly in the absorbance light path can cause these errors. A system test will typically show elevated gains in the 230 nm wavelength if the absorbance optics is contaminated.</li></ul> <p><b>Note:</b> This error may not occur during a system test but can occur when the first read is attempted.</p>

## Appendix B: Error Conditions

### Error Codes

Error Codes		Description and Resolution																																																															
40XX	<b>PMT well overload</b> Gain too high or chemistry too concentrated. Verify that the physical filter configuration matches the Gen5/Gen6 filter table.	Well location/error code for 1-well plate <table border="1"><tr><td></td><td>1</td></tr><tr><td>A</td><td>4001</td></tr></table>		1	A	4001																																																											
	1																																																																
A	4001																																																																
		Well location/error code for 6-well plate <table border="1"><tr><td></td><td>1</td><td>2</td><td>3</td></tr><tr><td>A</td><td>4001</td><td>4002</td><td>4003</td></tr><tr><td>B</td><td>4004</td><td>4005</td><td>4006</td></tr></table>		1	2	3	A	4001	4002	4003	B	4004	4005	4006																																																			
	1	2	3																																																														
A	4001	4002	4003																																																														
B	4004	4005	4006																																																														
		Well location/error code for 12-well plate <table border="1"><tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td></tr><tr><td>A</td><td>4001</td><td>4002</td><td>4003</td><td>4004</td></tr><tr><td>B</td><td>4005</td><td>4006</td><td>4007</td><td>4008</td></tr><tr><td>C</td><td>4009</td><td>400A</td><td>400B</td><td>400C</td></tr></table>		1	2	3	4	A	4001	4002	4003	4004	B	4005	4006	4007	4008	C	4009	400A	400B	400C																																											
	1	2	3	4																																																													
A	4001	4002	4003	4004																																																													
B	4005	4006	4007	4008																																																													
C	4009	400A	400B	400C																																																													
		Well location/error code for 24-well plate <table border="1"><tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr><tr><td>A</td><td>4001</td><td>4002</td><td>4003</td><td>4004</td><td>4005</td><td>4006</td></tr><tr><td>B</td><td>4007</td><td>4008</td><td>4009</td><td>400A</td><td>400B</td><td>400C</td></tr><tr><td>C</td><td>400D</td><td>400E</td><td>400F</td><td>4010</td><td>4011</td><td>4012</td></tr><tr><td>D</td><td>4013</td><td>4014</td><td>4015</td><td>4016</td><td>4017</td><td>4018</td></tr></table>		1	2	3	4	5	6	A	4001	4002	4003	4004	4005	4006	B	4007	4008	4009	400A	400B	400C	C	400D	400E	400F	4010	4011	4012	D	4013	4014	4015	4016	4017	4018																												
	1	2	3	4	5	6																																																											
A	4001	4002	4003	4004	4005	4006																																																											
B	4007	4008	4009	400A	400B	400C																																																											
C	400D	400E	400F	4010	4011	4012																																																											
D	4013	4014	4015	4016	4017	4018																																																											
		Well location/error code for 48-well plate <table border="1"><tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td></tr><tr><td>A</td><td>4001</td><td>4002</td><td>4003</td><td>4004</td><td>4005</td><td>4006</td><td>4007</td><td>4008</td></tr><tr><td>B</td><td>4009</td><td>400A</td><td>400B</td><td>400C</td><td>400D</td><td>400E</td><td>400F</td><td>4010</td></tr><tr><td>B</td><td>4011</td><td>4012</td><td>4013</td><td>4014</td><td>4015</td><td>4016</td><td>4017</td><td>4018</td></tr><tr><td>C</td><td>4019</td><td>401A</td><td>401B</td><td>401C</td><td>401D</td><td>401E</td><td>401F</td><td>4020</td></tr><tr><td>D</td><td>4021</td><td>4022</td><td>4023</td><td>4024</td><td>4025</td><td>4026</td><td>4027</td><td>4028</td></tr><tr><td>E</td><td>4029</td><td>402A</td><td>402B</td><td>402C</td><td>402D</td><td>402E</td><td>402F</td><td>4030</td></tr></table>		1	2	3	4	5	6	7	8	A	4001	4002	4003	4004	4005	4006	4007	4008	B	4009	400A	400B	400C	400D	400E	400F	4010	B	4011	4012	4013	4014	4015	4016	4017	4018	C	4019	401A	401B	401C	401D	401E	401F	4020	D	4021	4022	4023	4024	4025	4026	4027	4028	E	4029	402A	402B	402C	402D	402E	402F	4030
	1	2	3	4	5	6	7	8																																																									
A	4001	4002	4003	4004	4005	4006	4007	4008																																																									
B	4009	400A	400B	400C	400D	400E	400F	4010																																																									
B	4011	4012	4013	4014	4015	4016	4017	4018																																																									
C	4019	401A	401B	401C	401D	401E	401F	4020																																																									
D	4021	4022	4023	4024	4025	4026	4027	4028																																																									
E	4029	402A	402B	402C	402D	402E	402F	4030																																																									
		<b>Note:</b> Follow the same patterns for 96-well and 384-well plates.																																																															
47x0	<b>PMT Fluorescence noise test failure</b> Ensure that instrument case is completely installed and secured and that the access door is closed.																																																																
48x0	<b>Fluorescence offset test failure</b> Too much light in chamber. Ensure that instrument case is completely installed and secured and that the access door is closed.																																																																

## Appendix B: Error Conditions

### Error Codes

Error Codes		Description and Resolution
4A0y	<b>PMT gain out of range</b>	<p>Too much light in read chamber.</p> <p>Ensure that instrument case is completely installed and secured and that the access door is closed.</p>
4Bxy	<b>PMT operations test error</b>	<p>Ensure that instrument case is completely installed and secured and that the access door is closed. This error may also be caused by a hole in the excitation or emission filter wheel that is not reflected in the Gen5/Gen6 filter table.</p>
4Exy	<b>Detector saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).</b>	<ul style="list-style-type: none"> <li>These errors can indicate one of several scenarios. It is possibly due to incorrect chemistry, e.g., the fluorescence standards dispensed to the plate exceed expectations.</li> <li>Try lowering the gain/sensitivity in your Read step(s).</li> <li>There may be too much light in the read chamber. Ensure that all doors are closed and that the casing is completely installed and secured.</li> <li>For models with the dispense module, the internal chamber may require cleaning (contact Technical Support).</li> <li>Ensure that the Gen5/Gen6 filter table accurately reflects the contents of the excitation and emission filter wheels.</li> </ul>
4Fxy	<b>Fluorescence signal out of range</b>	<ul style="list-style-type: none"> <li>Verify that there is no filter wavelength overlap between the emission/excitation positions.</li> <li>Verify that the microplate door is fully closing, and the instrument cover is properly installed and sealed.</li> <li>Try lowering the Gain in your Read step(s).</li> <li>The reading chamber may be contaminated by a spill that is fluorescing.</li> </ul>
5000	<b>Carrier X failed to home</b>	
5200		<ul style="list-style-type: none"> <li>Ensure there are no objects obstructing the path.</li> <li>X-axis rails are dusty or rusty. Dirt in the roller bearings is causing them to jam.</li> </ul>
5001	<b>Carrier Y failed to home</b>	
5201		<ul style="list-style-type: none"> <li>Verify that the shipping hardware has been removed.</li> <li>Ensure there are no objects obstructing the path.</li> <li>Y-axis rails are dusty or rusty. Dirt in the roller bearings is causing them to jam.</li> </ul>
5002-5003	<b>Excitation filter wheel failed to home</b>	
5202-5203	<b>Emission filter wheel failed to home</b>	<ul style="list-style-type: none"> <li>Verify that the filter wheel is inserted correctly.</li> <li>Verify that the filter wheel is not obstructed.</li> <li>The gear teeth of the filter wheel may be binding with the gear teeth of the motor. Remove the filter wheel, spin the wheel by hand, and reinsert it.</li> </ul>
5006	<b>Probe Z height failed to home</b>	
5206		Verify that the shipping hardware has been removed.

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
5402 5403	<b>Excitation filter wheel failed positional verify</b> <b>Emission filter wheel failed positional verify</b> Verify that filter is fully inserted into filter wheel.
5700 5701	<b>Carrier X-Axis move attempted outside physical limits of travel</b> <b>Carrier Y-Axis move attempted outside physical limits of travel</b> <ul style="list-style-type: none"><li>Verify that dispenser tip, priming trough, microplate lid, or other object has not become dislodged in instrument.</li><li>Verify plate definition dimensions are correct, especially for custom defined plates.</li><li>Verify plate height matches selected plate type.</li><li>Verify that nothing is preventing the dispenser syringes from moving.</li></ul>
5702 5703	<b>Excitation filter wheel obstructed</b> <b>Emission filter wheel obstructed</b> <ul style="list-style-type: none"><li>Verify that the filter wheel is inserted correctly .</li><li>Verify that the filter wheel is not obstructed.</li><li>The gear teeth of the filter wheel may be binding with the gear teeth of the motor. Remove the filter wheel, spin the wheel by hand, and reinsert it.</li></ul>
5706	<b>Vertical Z-Axis move attempted outside physical limits of travel</b> <ul style="list-style-type: none"><li>Verify plate height matches selected plate type.</li><li>Verify that a plate lid is physically installed, that the protocol plate type selection reflects this.</li><li>Verify that the shipping hardware has been removed.</li><li>Manually move the Z-axis up, remove any microplates from the carrier, and attempt a successful system test.</li></ul>
5708 5709	<b>Axis is obstructed for a dispenser syringe</b> Verify that nothing is blocking the syringes.
5800 5801	<b>Carrier X failed sensor positional verify</b> <b>Carrier Y failed sensor positional verify</b> <ul style="list-style-type: none"><li>For 5801: Verify the correct plate height is being used and attempt retry with correct plate height. If retry is unsuccessful, run a system test and try once more using the correct plate height.</li><li>Verify there is nothing obstructing the carrier's path.</li><li>Ensure the shipping hardware has been removed.</li></ul>
5905	<b>Missed flash on absorbance sweep mode when reading more than one wavelength</b> <ul style="list-style-type: none"><li>Increase step size for spectral sweep modes.</li><li>If possible, place wavelengths in ascending or descending order.</li></ul>

## Appendix B: Error Conditions

### Error Codes

Error Codes	Description and Resolution
5Axy	<p><b>Plate jam error during plate move inside</b></p> <ul style="list-style-type: none"><li>• Plate has hit something.</li><li>• Plate cover not accounted for when creating plate dimension file or “Use lid” in the procedure was not selected.</li><li>• Wrong plate type (height) was selected for procedure. Correct plate type and rerun; no system test required.</li><li>• If a custom plate definition is being used in the protocol, verify that the plate dimensions are correct and reflect the actual physical dimensions of the plate.</li><li>• Tip prime trough has become dislodged.</li></ul>
5B00	<p><b>Plate height violation: Z-Axis probe told to move to position that would result in hitting plate.</b></p> <p>This error can occur if the carrier is inside and the newly-defined plate height is different from the most-recently specified plate height. To resolve this error, eject the carrier prior to running the experiment.</p> <p>This error may also occur if the plate is inside the chamber when it should be outside, or if the read was aborted and ‘home all axis’ not performed.</p>

# Appendix C: Safety Information

Veiligheidsmededelingen

Avis de sécurité

Sicherheitshinweise

Avvisi di sicurezza

Avisos de seguridad

This appendix contains safety information for the Synergy HTX, translated into Dutch, French, German, Italian, and Spanish.

---

Safety Notices .....	166
Safety Hazards .....	167

## Safety Notices

---

Veiligheidsmededelingen

Avis de sécurité

Sicherheitshinweise

Avvisi di sicurezza

Avisos de seguridad

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

Besteed in alle productdocumentatie speciale aandacht aan de volgende veiligheidsmededelingen.

Portez une attention particulière aux avis de sécurité suivants dans toute la documentation du produit.

Beachten Sie in allen Produktdokumentationen bitte insbesondere die folgenden Sicherheitshinweise.

Prestare particolare attenzione alle seguenti indicazioni di sicurezza in tutta la documentazione di prodotto.

Ponga especial cuidado a los siguientes avisos de seguridad que se encuentran en toda la documentación del producto.



A DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.

Een GEVAAR duidt op een gevaarlijke situatie die, indien deze niet wordt vermeden, de dood of ernstig letsel tot gevolg zal hebben.

Un DANGER indique une situation dangereuse qui entraîne la mort ou de graves blessures si elle n'est pas évitée.

Eine GEFAHR weist auf eine gefährliche Situation hin, die, wenn sie nicht vermieden wird, zum Tod oder zu schweren Verletzungen führen kann.

PERICOLO indica una situazione di rischio che, se non evitata, provoca morte o gravi lesioni.

Un PELIGRO indica una situación peligrosa que, si no se evita, provocará la muerte o lesiones graves.



A WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury.

Een WAARSCHUWING duidt op een gevaarlijke situatie die, indien deze niet wordt vermeden, de dood of ernstig letsel tot gevolg kan hebben.

Un AVERTISSEMENT indique une situation dangereuse qui peut entraîner la mort ou de graves blessures si elle n'est pas évitée.

Eine WARNUNG weist auf eine gefährliche Situation hin, die, wenn sie nicht vermieden wird, zum Tod oder zu schweren Verletzungen führen könnte.

AVVERTENZA indica una situazione di rischio che, se non evitata, potrebbe provocare morte o gravi lesioni.

## Appendix C: Safety Information

### Safety Hazards

Una ADVERTENCIA indica una situación peligrosa que, si no se evita, podría provocar la muerte o lesiones graves.

#### ▲CAUTION

A CAUTION is used with the safety alert symbol, indicating a hazardous situation which, if not avoided, could result in minor or moderate injury.

LET OP wordt gebruikt in combinatie met het veiligheidswaarschuwingssymbool, dat een gevaarlijke situatie aangeeft die, indien niet vermeden, licht of gering letsel kan veroorzaken.

Une MISE EN GARDE est utilisée avec le symbole d'alerte de sécurité, indiquant une situation dangereuse qui peut entraîner des blessures mineures ou modérées si elle n'est pas évitée.

Der Sicherheitshinweis VORSICHT in Verbindung mit dem Sicherheitswarn-symbol weist auf eine gefährliche Situation hin, die, wenn sie nicht vermieden wird, zu leichten oder mittelschweren Verletzungen führen könnte.

ATTENZIONE, insieme al simbolo di avviso di sicurezza, indica una situazione di rischio che, se non viene evitata, potrebbe provocare lesioni lievi o moderate.

Una PRECAUCIÓN se utiliza con el símbolo de alerta de seguridad para indicar una situación peligrosa que, si no se evita, podría provocar lesiones leves o moderadas.

## Safety Hazards

---

### Electrical Hazards

Elektrische gevaren

Risques électriques

Elektrische Gefahren

Rischi elettrici

Peligros eléctricos

#### ▲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.

**Vermogen.** De voeding of het netsnoer van het instrument moet worden aangesloten op een stopcontact dat spanning en stroom levert binnen de voor het systeem aangegeven vermogen. Het gebruik van een niet-compatibel stopcontact kan elektrische schokken en brandgevaar veroorzaken.

**Alimentation nominale.** Le bloc d'alimentation ou le cordon d'alimentation de l'instrument doit être connecté à une prise de courant fournissant une tension et un courant conformes aux spécifications du système. L'utilisation d'une prise de courant incompatible peut entraîner des risques de choc électrique et d'incendie.

**Nennleistung.** Das Netzteil oder der Netzstecker des Geräts muss an eine Steckdose angeschlossen werden, die eine für das System zulässige Spannung und Stromstärke liefert. Die Verwendung einer nicht kompatiblen Steckdose kann zu Stromschlägen und Bränden führen.

**Alimentazione nominale.** L'alimentazione o il cavo dello strumento devono essere collegati a una presa elettrica avente tensione e corrente entro i valori nominali specificati per il sistema. L'uso di

## Appendix C: Safety Information

### Safety Hazards

una presa elettrica non compatibile può provocare scosse elettriche e rischio di incendio.

**Potencia de salida.** La fuente o el cable de alimentación del instrumento debe estar conectado a una toma de corriente que proporcione tensión y corriente dentro de los valores nominales del sistema. Si se usa una toma de corriente incompatible, se podría producir un riesgo de descarga eléctrica y de incendio.

#### ⚠ 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.

**Elektrische aarding.** Gebruik nooit een stekkeradapter om de primaire voeding aan te sluiten op de externe voeding. Bij gebruik van een adapter wordt de aarding onderbroken, waardoor een ernstig gevaar voor elektrische schokken ontstaat. Sluit het netsnoer altijd aan op een geschikt stopcontact met een werkende aarding.

**Mise à la terre électrique.** N'utilisez jamais un adaptateur de prise pour connecter l'alimentation primaire à l'alimentation externe. L'utilisation d'un adaptateur déconnecte la mise à la terre du service public, ce qui peut créer un grave risque de choc. Branchez toujours le câble d'alimentation directement sur une prise de courant appropriée avec une mise à la terre fonctionnelle.

**Elektrische Erdung.** Verwenden Sie niemals einen Steckeradapter, um die Primärstromversorgung an das externe Netzteil anzuschließen. Durch die Verwendung eines Adapters wird die Erdung des Netzes unterbrochen, was zu einem schweren Stromschlag führen kann. Schließen Sie den Netzstecker immer direkt an eine geeignete Steckdose mit einer funktionierenden Erdung an.

**Collegamento a massa.** Non utilizzare mai un adattatore per collegare l'alimentazione primaria alla finta di energia esterna. L'uso di un adattatore annulla la massa dell'impianto creando un grave rischio di scosse elettriche. Collegare sempre il cavo di alimentazione a una presa appropriata dotata di massa funzionante.

**Conexión a tierra.** Nunca use un adaptador de enchufe para conectar la energía eléctrica primaria a la fuente de alimentación externa. Si se usa un adaptador, se desconecta la conexión a tierra, lo que crea un grave riesgo de descarga eléctrica. Siempre conecte el cable de alimentación directamente a una toma de corriente adecuada con una conexión a tierra funcional.

#### ⚠ WARNING

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

**Voeding.** Gebruik alleen de voeding die bij het instrument wordt geleverd, en gebruik deze binnen het gebruik van de netspanning die hierop is vermeld.

**Alimentation électrique.** Utilisez uniquement le bloc d'alimentation livré avec l'instrument et faites-le fonctionner dans la plage de tensions de ligne indiquée sur celui-ci.

**Netzteil.** Verwenden Sie nur das mit dem Gerät gelieferte Netzteil und betreiben Sie es innerhalb des auf dem Netzteil angegebenen Spannungsbereichs.

**Alimentatore.** Utilizzare solo l'alimentatore fornito insieme allo strumento e utilizzarlo nel range di tensioni di linea su di esso elencati.

**Fuente de alimentación.** Use exclusivamente la fuente de alimentación que se envió con el instrumento y utilícela dentro del rango de tensiones de línea que están enumeradas en este.

#### ⚠ WARNING

**Power Cords.** Do not replace detachable Mains power cords with inadequately rated cords. Always replace with power cords purchased from Agilent.

## Appendix C: Safety Information

### Safety Hazards

**Netsnoeren.** Vervang afneembare netsnoeren niet door snoeren met onvoldoende nominale vermogen. Vervang netsnoeren altijd door netsnoeren van Agilent.

**Câbles d'alimentation.** Ne remplacez pas les câbles d'alimentation secteur par des câbles dont la valeur nominale n'est pas adaptée. Remplacez-les systématiquement par des câbles d'alimentation achetés auprès d'Agilent.

**Netzkabel.** Ersetzen Sie abnehmbare Netzkabel nicht durch Kabel mit unzureichender Nennleistung. Ersetzen Sie sie immer durch Netzkabel, die von Agilent bezogen wurden.

**Cavi di alimentazione.** Non sostituire i cavi di alimentazione staccabili con cavi non idonei. Sostituire sempre con cavi di alimentazione acquistati da Agilent.

**Cables de alimentación.** No sustituya los cables de alimentación de red desmontables por cables inadecuados. Sustitúyalos siempre por cables de alimentación adquiridos en Agilent.

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

**Interne spanning.** Schakel altijd de stroomschakelaar uit en haal de stekker uit het stopcontact voordat u het buitenoppervlak van het instrument reinigt.

**Tension interne.** Avant de nettoyer la surface extérieure de l'instrument, mettez toujours l'appareil hors tension et débranchez l'alimentation électrique.

**Interne Spannung.** Schalten Sie immer den Ein/Aus-Schalter aus und ziehen Sie den Netzstecker, bevor Sie die Außenflächen des Geräts reinigen.

**Tensione interna.** Spegnere sempre l'interruttore di alimentazione e scollegare l'alimentazione di rete prima di eseguire la pulizia delle superfici esterne dello strumento.

**Tensión interna.** Siempre apague el interruptor principal y desconecte la fuente de alimentación antes de limpiar la superficie exterior del instrumento.

**⚠️ 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.

**Vloeistoffen.** Zorg dat u geen vloeistoffen op het instrument morst: vloeistof die in de interne onderdelen terechtkomt kan potentieel schokken of beschadiging van het instrument tot gevolg hebben. Als u vloeistof morst terwijl een programma bezig is, stopt u het programma en schakelt u het instrument uit. Veeg alle gemorste vloeistof onmiddellijk van het instrument. Gebruik het instrument niet als interne onderdelen aan vloeistof zijn blootgesteld.

**Liquides.** Évitez de renverser des liquides sur l'instrument ; l'infiltration de liquide dans les composants internes crée un risque potentiel d'électrocution ou d'endommagement de l'instrument. Si un déversement se produit alors qu'un programme est en cours, arrêtez le programme et éteignez l'instrument. Essuyez immédiatement tout ce qui a été renversé. Ne faites pas fonctionner l'instrument si les composants internes ont été exposés à un liquide.

**Flüssigkeiten.** Vermeiden Sie das Verschütten von Flüssigkeiten auf das Gerät; wenn Flüssigkeit in interne Komponenten eindringt, besteht die Gefahr eines Stromschlags oder einer Beschädigung des Geräts. Wenn während der Ausführung eines Programms Flüssigkeit verschüttet wird, stoppen Sie das Programm und schalten das Gerät aus. Wischen Sie sofort die gesamte verschüttete Flüssigkeit auf. Das Gerät darf nicht betrieben werden, wenn die internen Komponenten mit Flüssigkeit in Berührung gekommen sind.

## Appendix C: Safety Information

### Safety Hazards

**Liquidi.** Evitare di rovesciare liquidi sullo strumento; l'infiltazione di liquidi sui componenti interni crea un potenziale rischio di scosse elettriche o danni allo strumento. Se si rovesciano dei liquidi durante l'esecuzione di un programma, arrestare il programma e spegnere lo strumento. Asciugare immediatamente i liquidi. Non azionare lo strumento se i componenti interni sono stati esposti a liquidi.

**Líquidos.** Evite derramar líquidos sobre el instrumento; la penetración de fluidos en los componentes internos crea un posible riesgo de descarga eléctrica o de daño al instrumento. Si hay un derrame mientras se ejecuta un programa, deténgalo y apague el instrumento. Limpie todos los derrames inmediatamente. No utilice el instrumento si los componentes internos se han expuesto a fluidos.

#### ⚠ WARNING

**Disconnection.** The electrical connection at the back of the instrument is the primary disconnection point. The instrument should be positioned to allow access to the power cord for easy disconnection.

**Loskoppeling.** De elektriciteitsaansluiting aan de achterkant van het instrument is het primaire punt voor loskoppeling. Het instrument moet zo worden geplaatst dat het netsnoer gemakkelijk kan worden losgekoppeld.

**Débranchement.** Le raccordement électrique à l'arrière de l'instrument est le point principal de débranchement. L'instrument doit être installé à un emplacement permettant d'accéder au câble d'alimentation et de le débrancher rapidement.

**Trennung von der Stromversorgung.** Der elektrische Anschluss an der Rückseite des Geräts ist die primäre Trennvorrichtung für das Gerät. Das Gerät sollte so positioniert werden, dass der Zugang zum Netzstecker zum einfachen Trennen gewährleistet ist.

**Disconnessione.** Il collegamento elettrico sul retro dello strumento è il punto di disconnectione principale. Lo strumento deve essere posizionato in modo da consentire l'accesso al cavo di alimentazione affinché possa essere facilmente disconnesso.

**Desconexión.** La conexión eléctrica situada en la parte posterior del instrumento es la desconexión principal del instrumento. El instrumento debe colocarse de forma que se pueda acceder al cable de alimentación para desconectarlo fácilmente.

#### ⚠ CAUTION

**EMC.** The shielding and length of USB and other ports cables are critical to Electromagnetic Compatibility performance. Use only the cables provided from Agilent.

**EMC.** De afscherming en lengte van USB- en andere poortkabels zijn essentieel voor de prestaties met betrekking tot elektromagnetische compatibiliteit. Gebruik alleen de kabels van Agilent.

**CEM.** La protection et la longueur du câble USB et des autres câbles de ports sont essentielles aux performances de compatibilité électromagnétique. Utilisez uniquement les câbles fournis par Agilent.

**EMV.** Die Abschirmung und Länge von USB- und anderen Anschlusskabeln sind für die elektromagnetische Verträglichkeit entscheidend. Verwenden Sie nur die von Agilent gelieferten Kabel.

**Compatibilità elettromagnetica (EMC).** La schermatura e la lunghezza dei cavi USB e di altro tipo sono fondamentali per le prestazioni di compatibilità elettromagnetica. Utilizzare esclusivamente i cavi forniti da Agilent.

**EMC.** La protección y la longitud de los cables USB y de otros puertos son fundamentales para el rendimiento de la compatibilidad electromagnética. Utilice únicamente los cables suministrados

## Appendix C: Safety Information

### Safety Hazards

por Agilent.

## Biohazards

Biologische gevaren

Risques biologiques

Biologische Gefahren

Rischi biologici

Riesgos biológicos

**⚠ 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.

**Mogelijke biologische gevaren.** Bepaalde assays of specimens kunnen een biologisch gevaar vormen. Neem passende veiligheidsmaatregelen zoals beschreven in de bijsluiter van de assay. Draag altijd een veiligheidsbril en passende beschermingsuitrusting, zoals tegen chemicaliën bestendige rubberen handschoenen en schort.

**Risques biologiques potentiels.** Certaines analyses ou certains échantillons peuvent présenter un risque biologique. Des précautions de sécurité adéquates doivent être prises, comme indiqué dans la notice de l'analyse. Portez toujours des lunettes de sécurité et un équipement de protection approprié, comme des gants en caoutchouc résistant aux produits chimiques et un tablier.

**Potenzielle biologische Gefahren.** Einige Tests oder Proben können eine biologische Gefahr darstellen. Es müssen geeignete Vorsichtsmaßnahmen ergriffen werden, wie in der Packungsbeilage des Tests beschrieben. Tragen Sie immer eine Schutzbrille und eine geeignete Schutzausrüstung, wie etwa chemikalienbeständige Gummihandschuhe und eine Schürze.

**Potenziali rischi biologici.** Alcune analisi e campioni possono esporre a rischio biologico. Adottare le precauzioni di sicurezza riportate dell'inserto del pacchetto di analisi. Indossare sempre occhiali protettivi e dispositivi di protezione adeguati, quali grembiali e guanti in gomma resistenti alle sostanze chimiche.

**Posibles riesgos biológicos.** Es posible que algunos ensayos o muestras supongan un riesgo biológico. Se deben adoptar las precauciones de seguridad adecuadas, tal como se indican en el prospecto del ensayo. Siempre lleve gafas de protección y equipo de protección adecuado, como guantes y delantal de goma resistentes a productos químicos.

## Component Hazards

Gevaren van componenten

Risques liés aux composants

Gefahren durch Komponenten

Rischi per i componenti

Riesgos de componentes

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

## Appendix C: Safety Information

### Safety Hazards

instrument.

**Accessoires.** Alleen accessoires die voldoen aan de specificaties van de fabrikant mogen samen met het instrument worden gebruikt.

**Accessoires.** Seuls les accessoires conformes aux spécifications du fabricant doivent être utilisés avec l'instrument.

**Zubehör.** Für das Gerät darf nur Zubehör verwendet werden, das den Spezifikationen des Herstellers entspricht.

**Accessori.** Utilizzare solo gli accessori che soddisfano le specifiche del costruttore.

**Accesos.** Con este instrumento, solo deben utilizarse los accesorios que cumplan con las especificaciones del fabricante.

#### ⚠ WARNING

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

**Service.** Alleen gekwalificeerd technisch personeel mag onderhoudsprocedures aan interne onderdelen uitvoeren.

**Maintenance.** Seul un personnel technique qualifié doit effectuer les procédures de maintenance des composants internes.

**Service.** Servicearbeiten an den internen Komponenten dürfen nur von qualifizierten Technikern vorgenommen werden.

**Assistenza.** Le procedure di assistenza sui componenti interni devono essere eseguite solo da personale qualificato.

**Servicio.** Solo el personal técnico cualificado puede efectuar los procedimientos de servicio en componentes internos.

#### ⚠ WARNING

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

**Heet oppervlak.** De fluorescentielamp is heet wanneer het instrument wordt ingeschakeld. Zet het leesapparaat uit en laat de lamp ten minste 15 minuten afkoelen alvorens te proberen deze te vervangen.

**Surface chaude.** La lampe à fluorescence est chaude lorsque l'instrument est allumé. Éteignez le lecteur et laissez l'ampoule refroidir pendant 15 minutes au moins avant de la remplacer.

**Heiße Oberflächen.** Bei eingeschaltetem Gerät ist die Leuchtstofflampe heiß. Schalten Sie den Reader aus und lassen Sie die Glühbirne mindestens 15 Minuten lang abkühlen, bevor Sie versuchen, sie auszutauschen.

**Superficie molto calda.** Il gruppo lampada fluorescente diventa molto caldo quando lo strumento è acceso. Prima di tentare di sostituirlo, spegnere il lettore e lasciare raffreddare la lampadina per almeno 15 minuti.

**Superficie caliente.** El conjunto de piezas de la lámpara fluorescente está caliente cuando el instrumento está encendido. Apague el lector y deje que la bombilla se enfríe durante al menos 15 minutos antes de proceder a cambiarla.

#### ⚠ CAUTION

**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

## Appendix C: Safety Information

### Safety Hazards

operating.

**Beknellingsgevaar.** Sommige delen van de externe uitgiftemodule kunnen beknellingsgevaar opleveren wanneer het instrument in bedrijf is. Houd handen en vingers uit de buurt van deze gebieden wanneer het instrument in bedrijf is.

**Risque de pincement.** Certaines zones du module de dispense externe peuvent présenter des risques de pincement lors du fonctionnement de l'instrument. Gardez vos mains et vos doigts à l'écart de ces zones lors du fonctionnement de l'instrument.

**Quetschgefahr.** In einigen Bereichen des externen Dispenser-Moduls können beim Betrieb des Geräts Quetschgefahren auftreten. Hände und Finger von diesen Bereichen fernhalten, wenn das Gerät in Betrieb ist.

**Rischio di pizzicamento.** Alcune aree del modulo di erogazione esterno possono presentare rischi di pizzicamento quando lo strumento è in funzione. Tenere le mani e le dita lontane da queste aree quando lo strumento è in funzione.

**Peligro de atrapamiento.** Algunas áreas del módulo dispensador externo pueden presentar riesgos de atrapamiento cuando el instrumento está en funcionamiento. Mantenga las manos y los dedos alejados de estas áreas cuando el instrumento esté en funcionamiento.

#### ▲ CAUTION

**Filters.** The Synergy HTX is shipped with a set of excitation and emission filters installed. The reader's onboard software is preconfigured with the filter values and their locations. If you change the contents of a filter wheel, you must update Gen5/Gen6's filter table and then download the information to the reader. The Synergy HTX does not automatically detect which filters are installed.

**Filters.** De Synergy HTX wordt geleverd met een set excitatie- en emissiefilters geïnstalleerd. De ingebouwde software van het leesapparaat is voorgeconfigureerd met de filterwaarden en hun locaties. Als u de inhoud van een filterwiel wijzigt, moet u de filtertabel van Gen5/Gen6 bijwerken en vervolgens de informatie naar het leesapparaat downloaden. De Synergy HTX detecteert niet automatisch welke filters geïnstalleerd zijn.

**Filtres.** Le Synergie HTX est expédié avec un ensemble de filtres d'excitation et d'émission installés. Le logiciel embarqué du lecteur est préconfiguré avec les valeurs des filtres et leurs emplacements. Si vous modifiez le contenu d'une roue à filtres, vous devez mettre à jour le tableau des filtres du Gen5/Gen6, puis télécharger les informations sur le lecteur. Le Synergy HTX ne détecte pas automatiquement les filtres installés.

**Filter.** Der Synergy HTX wird mit einem Satz eingebauter Anregungs- und Emissionsfilter geliefert. Die Onboard-Software des Readers ist mit den Filterwerten und ihren Positionen vor konfiguriert. Wenn Sie den Inhalt eines Filterrads ändern, müssen Sie die Filtertabelle von Gen5/Gen6 aktualisieren und dann die Informationen auf den Reader herunterladen. Der Synergy HTX erkennt nicht automatisch, welche Filter installiert sind.

**Filtri.** Synergy HTX viene spedito con una serie di filtri di eccitazione e di emissione installati. Il software a bordo del lettore è preconfigurato con i valori dei filtri e le loro posizioni. Se si modifica il contenuto di una ruota filtri, è necessario aggiornare la tabella dei filtri di Gen5/Gen6 e quindi scaricare le informazioni nel lettore. Synergy HTX non rileva automaticamente quali filtri sono installati.

**Filtros.** El envío de Synergy HTX incluye una serie de filtros de excitación y emisión instalados. El software a bordo del lector está preconfigurado con los valores de filtro y sus ubicaciones. Si cambia el contenido de una rueda de filtro, debe actualizar la tabla de filtros de Gen5/Gen6 y, a continuación, descargar la información al lector. Synergy HTX no detecta automáticamente los

## Appendix C: Safety Information

### Safety Hazards

filtros que hay instalados.

## Intended Product Use

Beoogd gebruik van het product

Utilisation prévue du produit

Bestimmungsgemäße Verwendung des Produkts

Uso previsto

Uso previsto del producto

### ⚠️ 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.

**Software-kwaliteitscontrole.** Bij het wijzigen van softwareparameters en het bepalen van uitleesmethoden moet de operator de bijsluiter van de assay-verpakking volgen. Het wordt als een goede laboratoriumpraktijk beschouwd om laboratoriummonsters te nemen volgens de instructies en specifieke aanbevelingen in de bijsluiter van de assay-verpakking voor de uit te voeren test. Het niet uitvoeren van kwaliteitscontroles kan foutieve testgegevens tot gevolg hebben.

**Contrôle qualité du logiciel.** L'opérateur doit suivre la notice d'analyse du fabricant de l'analyse lorsqu'il modifie les paramètres du logiciel et établit les méthodes de lecture. Il est considéré comme une bonne pratique de laboratoire de réaliser les échantillons de laboratoire selon les instructions et les recommandations spécifiques incluses dans la notice d'analyse du test à réaliser. Le fait de ne pas effectuer de contrôles de qualité peut entraîner des données d'essai erronées.

**Qualitätskontrolle der Software.** Der Bediener muss die Packungsbeilage des Testherstellers beachten, wenn Softwareparameter geändert und Messmethoden festgelegt werden sollen. Es gilt als gute Laborpraxis, Laborproben gemäß den Anweisungen und spezifischen Empfehlungen in der Packungsbeilage des jeweiligen Tests zu untersuchen. Wenn keine Qualitätskontrollen durchgeführt werden, kann dies zu fehlerhaften Testdaten führen.

**Controllo qualità del software.** Quando si modificano i parametri del software e si definiscono i metodi di lettura, seguire le istruzioni riportate nell'inserto del pacchetto di analisi. È considerata una buona pratica l'esecuzione di campioni di laboratorio conformi alle istruzioni e alle specifiche raccomandazioni incluse nel pacchetto di analisi per il test da eseguire. La mancata esecuzione di controlli di qualità può causare dati di test errati.

**Control de calidad del software.** El usuario debe seguir el prospecto del ensayo del fabricante al modificar parámetros del software y determinar métodos de lectura. Se considera que analizar las muestras de laboratorio según las instrucciones y recomendaciones específicas incluidas en el prospecto del ensayo para realizar el análisis es una práctica adecuada de laboratorio. Si no se realizan las comprobaciones de control de calidad, se podrían obtener datos erróneos del análisis.

### ⚠️ 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

## Appendix C: Safety Information

### Safety Hazards

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.

**Gegevensreductie.** Er zijn geen limieten van toepassing voor de ruwe meetgegevens. Gegevens die via computerbesturing worden geëxporteerd, moeten door de operator worden geanalyseerd. De prestatiekenmerken van de software voor gegevensreductie zijn niet vastgesteld door middel van diagnostische laboratoriumassays. Gebruikers moeten dit instrument en pc-gebaseerde software beoordelen in combinatie met hun specifieke assay(s). Deze beoordeling moet een bevestiging omvatten dat aan de prestatiekenmerken voor de specifieke assay(s) wordt voldaan.

**Réduction des données.** Aucune limite n'est appliquée aux données de mesure brutes. Les données exportées par le contrôle informatique doivent être analysées par l'opérateur. Les caractéristiques de performance du logiciel de réduction des données n'ont pas été établies avec une analyse de diagnostic de laboratoire. Les utilisateurs doivent évaluer cet instrument et le logiciel basé sur PC en fonction de leurs analyses spécifiques. Cette évaluation doit inclure la confirmation que les caractéristiques de performance pour la ou les analyses spécifiques sont respectées.

**Datenreduktion.** Es gibt keine Einschränkungen für die rohen Messdaten. Die unter der Steuerung des Computers exportierten Daten müssen vom Bediener analysiert werden. Die Leistungsmerkmale der Software zur Datenreduktion wurden bisher für keinen labordiagnostischen Test ermittelt. Der Benutzer muss dieses Gerät und die PC-basierte Software in Verbindung mit den zu verwendenden Tests evaluieren. Im Rahmen dieser Evaluierung muss bestätigt werden, dass die Leistungsmerkmale der jeweiligen Tests eingehalten werden.

**Riduzione dei dati.** Non sussistono limiti ai dati di misurazione non elaborati. I dati esportati tramite controllo del computer devono essere analizzati dall'operatore. Le caratteristiche di prestazione del software di riduzione dei dati non sono state definite con nessuna analisi diagnostica di laboratorio. L'utente deve valutare lo strumento e il software su PC unitamente alle proprie analisi specifiche. Questa valutazione deve includere la conferma che siano soddisfatte le caratteristiche di prestazione per l'analisi specifica.

**Reducción de datos.** No se aplican límites a los datos primarios de medición. El usuario debe analizar los datos exportados mediante el control informático. Las características de rendimiento del software de reducción de datos no se han determinado con ningún ensayo de diagnóstico de laboratorio. Los usuarios deben evaluar este instrumento y el software para PC junto con sus ensayos específicos. Esta evaluación debe incluir la confirmación de que se cumplen las características de rendimiento de los ensayos específicos.

#### ⚠ 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.

**Niet-gespecificeerd gebruik.** Als apparatuur niet wordt bediend in overeenstemming met de richtlijn en veiligheidsmaatregelen die in de gebruikersdocumentatie van het product zijn vermeld, kan dit tot gevaarlijke situaties leiden.

**Utilisation non spécifiée.** Le fait de ne pas utiliser l'équipement conformément aux directives et aux mesures de protection spécifiées dans la documentation de l'utilisateur du produit peut entraîner une situation dangereuse.

**Nicht spezifizierte Verwendung.** Wird das Gerät nicht gemäß den in der Benutzerdokumentation des Produkts angegebenen Richtlinien und Sicherheitsmaßnahmen betrieben, kann dies zu einem gefährlichen Zustand führen.

**Uso non previsto.** Il mancato rispetto delle linee guida e indicazioni di sicurezza specificate nella

## Appendix C: Safety Information

### Safety Hazards

documentazione per l'utente del prodotto può esporre a condizioni di pericolo.

**Uso no especificado.** Si no usa el equipo de conformidad con las directrices y los métodos de protección especificados en la documentación del usuario sobre el producto, se podría ocasionar una condición peligrosa.

# In This Book

## Document Revision History

Part Number	Revision	Date	Modifications
1341000N	E	October 2024	<ul style="list-style-type: none"><li>• Updated symbol definitions.</li><li>• Added the IFU symbol.</li><li>• Removed the Conformance to Standards and Directives topic.</li><li>• Updated the EMC topic.</li><li>• Removed the Ingress Protection Code.</li><li>• Converted hazards to the ANSI system.</li><li>• Added additional electrical and safety information.</li><li>• Removed the errata notice.</li><li>• Updated a note in the Internal Components section with a link to the online chemical compatibility chart.</li><li>• Added requirement of 6" between rear panel and wall.</li><li>• Added rear panel diagram in the External Components section.</li><li>• Updated dispenser installation instructions with new images.</li><li>• Updated System Test recommendation to use the 6 wavelengths you use most frequently.</li><li>• Added troubleshooting for the dispense module tests.</li><li>• Removed the shake step from the parameters for Synergy HTX Disp Test Other Reader.prt/.xpt and added a step in the procedure to shake the plate.</li><li>• Formatting improvements throughout.</li></ul>

Original Language – EN

[www.agilent.com](http://www.agilent.com)

Manufactured by Agilent Technologies, Inc.  
5301 Stevens Creek Blvd.  
Santa Clara, CA 95051  
© Agilent Technologies, Inc. 2024

