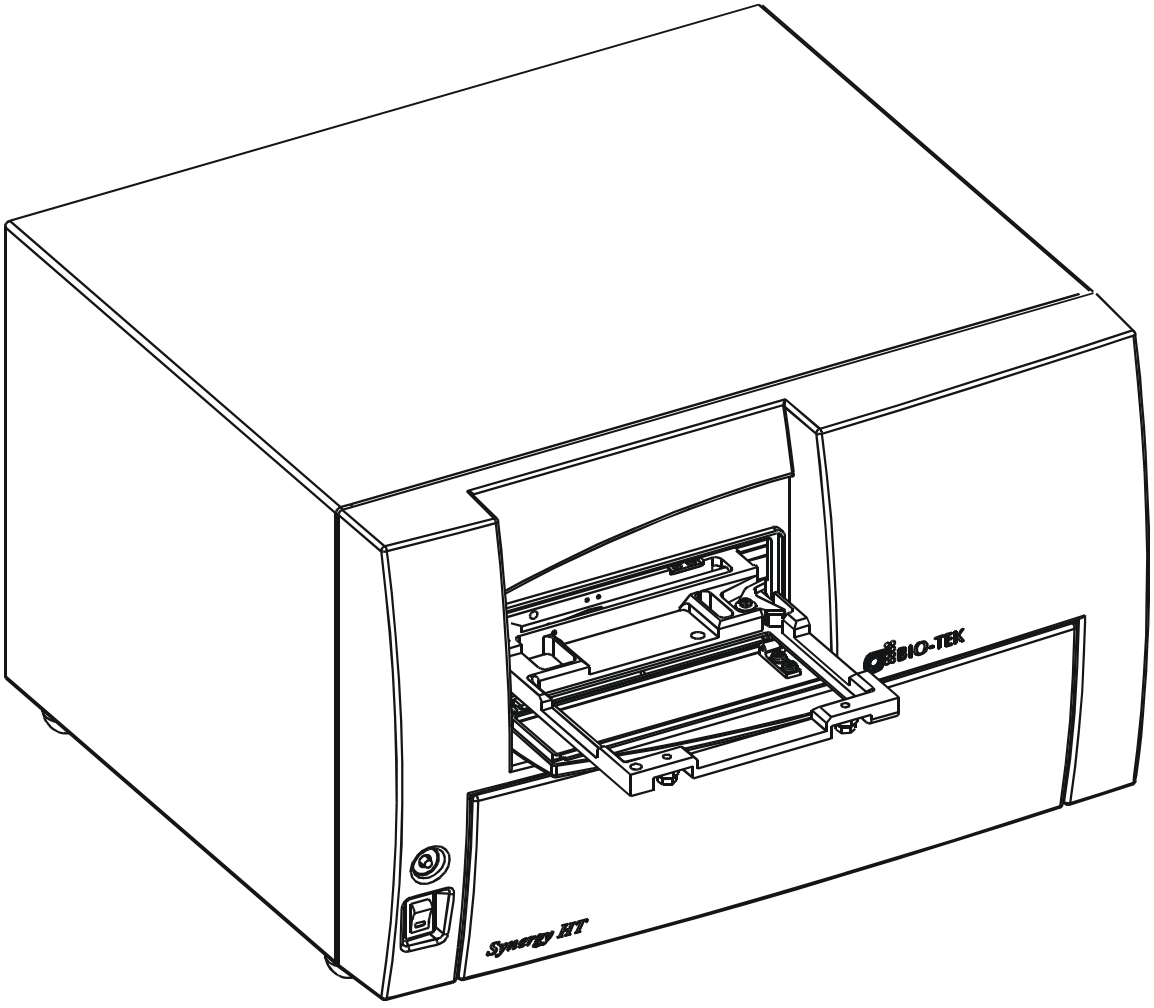
Synergy HT

Multi-Detection Microplate Reader

Operator's Manual



Synergy HT™

Multi-Detection Microplate Reader
Operator's Manual

For R & D Use

and

***in vitro* Diagnostic Use**

AUGUST 2002

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REVISION B

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Revision History

Revision	Date	Changes
A	4/02	First Issue
B	8/02	Added Time-Resolved Mode

Document Conventions

This manual uses the following typographic conventions.

Example	Description
	This icon calls attention to important safety notes.
Warning!	A **Warning** indicates the potential for bodily harm and tells you how to avoid the problem.
Caution:	A **Caution** indicates potential damage to the instrument and tells you how to avoid the problem.
Note	Bold text is primarily used for emphasis.
	This icon calls attention to important information.

Warnings

The *Synergy HT* should be operated on a flat surface away from direct sunlight or strong incandescent light. Excessive humidity should be avoided.

Hazards

Warning! Power Rating. The *Synergy HT* 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! Internal Voltage. Always turn off the power switch and unplug the power cord before cleaning the instrument's outer surface.

Warning! Liquids. Avoid spilling liquids on the reader; fluid seepage into internal components creates a potential shock hazard. Do not operate the instrument if internal components are exposed to fluid.

Precautions

The following precautions are provided to help you avoid damaging the system:

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

Caution: Environmental Conditions. Do not expose the system to temperature extremes. Ambient temperatures should remain between 15-35°C. System performance may be adversely affected if temperatures fluctuate above or below this range.

Caution: Sodium Hypochlorite. Do not expose any part of the instrument to Sodium Hypochlorite solution (bleach) for more than 30 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Caution: Power Supply. Only use the correct line voltage when operating the Automated Microplate Reader.

Caution: Shipping Screw. The shipping bracket must be removed prior to operating the device.

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

Warning: Changes or modifications to this unit not expressly approved by the manufacturer could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. Like all similar equipment, this equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause interference, in which case the user will be required to correct the interference at his own expense.

Canadian Department of Communications Class A

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

Le present appareil numerique n'met pas du bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.

User Safety

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

- **Underwriters Laboratories UL 3101-1 1st edition, 1993**

  "Electrical Equipment for Laboratory Use; Part 1: General Requirements"

- **Canadian Standards Association CAN/CSA C22.2 No. 1010.1-1992**

  "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use; Part 1: General Requirements"

CE **Based on the testing described below, this instrument bears the CE mark.**

EMC EC DIRECTIVE 89/336/EEC Electromagnetic Compatibility

- **Emissions – CLASS A**

  The system has been type tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1:1998 for Radiated Emissions and Line Conducted Emissions. Verification of compliance was conducted to the limits and methods of the following:

  CISPR 16-1:1993 and CISPR 16-2:1996

- **Immunity**

  The system has been type tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1:1998 for Immunity. Verification of compliance was conducted to the limits and methods of the following:

  EN 61000-4-2 (1991) Electrostatic Discharge

  EN 61000-4-3 (1995) Radiated EM Fields

  EN 61000-4-4 (1995) Electrical Fast Transient/Burst

  EN 61000-4-5 (1995) Surge Immunity

  EN 61000-4-6 (1996) Conducted Disturbances

  EN 61000-4-11 (1994) Voltage Dips, Short Interruptions and Variations

EC Directive 73/23/EEC Low Voltage (Safety)

The system has been type tested by an independent testing laboratory and was found to meet the requirements of EC Directive 73/23/EEC for Low Voltage. Verification of compliance was conducted to the limits and methods of the following:

- **EN 61010-1 (2001) 2nd Edition**

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

Safety Symbols

The following warning and informational symbols may be found in various locations on the *Synergy HT*. Only qualified personnel who recognize shock hazards and are familiar with the safety precautions should use this instrument. Read the manual carefully before operating this instrument.

Alternating current
Courant alternatif
Dreiphasen-Wechselstrom
Corriente Alterna
Corrente alternata

Earth ground terminal
Borne de terre
Erde (Bettriebserde)
Borne de Tierra
Terra (di funzionamento)

Protective conductor terminal
Borne de terre de protection
Schutzleiteranscluss
Borne de Tierra de Protección
Terra di protezione

On (Supply)
Marche (alimentation)
Ein (Verbindung mit dem Netz)
Conectado
Chiuso

Off (Supply)
Arrêt (alimentation)
Aus (Trennung vom Netz)
Desconectado
Aperto (sconnessione dalla rete di alimentazione)

Caution (refer to accompanying documents)

Attention (voir documents d'accompanement)

Achtung siehe Begleitpapiere

Atención (vease los documentos incluidos)

Attenzione, consultare la doc annessa

Caution, risk of electric shock

Attention, risque de choc electrique

Gefährliche elektrische Spannung

Atención, riesgo de sacudida eléctrica

Alta tensione (in questo documento Alta tensione non significa “tensione pericolosa” come definito in IEC 417)

Intended Use Statement

The *Synergy HT* is a single-channel absorbance, fluorescence, and luminescence microplate reader that utilizes a dual-optics design to perform measurements of samples in a microplate format. It is designed to perform *in-vitro* diagnostic and R&D analyses of a variety of samples. The Performance Characteristics of the data reduction software have not been established with any laboratory diagnostic assay. The user must evaluate this instrument and PC-based software in conjunction with the specific assay. This evaluation must include the confirmation that performance characteristics for the specific assay are met.

This system is designed for use with PC-based software only. Bio-Tek software packages, such as KC4™ for Windows®, will provide the user with instrument control.

Specimen Preparation

Samples should be obtained, treated, and stored following instructions and recommendations determined by the user's laboratory.

Quality Control

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

About This Manual

The intent of this Operator's Manual is to quickly instruct the new user how to set up and operate Bio-Tek's *Synergy HT*. To help you read and understand this manual, certain document conventions have been used.

Major topic headings start a new page (such as **About This Manual**, above) to give you a visual and style clue that a new major subject is being introduced. One or more subheadings may appear below each major heading.

Registration Card

Once the *Synergy HT* has been set up and is running successfully, please take a moment to fill out and mail the postage-paid Warranty Registration card. By sending in the registration card, you will be assured of receiving prompt information on product enhancements.

Warranty

This Warranty is limited and applies only to new products, except for computer-based software, which is covered under a separate Warranty Policy, manufactured by Bio-Tek Instruments, Inc. ("Bio-Tek"). Bio-Tek makes no warranty whatsoever regarding the condition of used products.

Bio-Tek warrants the instrument (hereinafter collectively referred to as "Products" or "Product") for a period of one (1) year from the original purchase date against defective materials or workmanship. This Warranty is limited to the original purchaser (the "Purchaser") and cannot be assigned or transferred. All claims under this Limited Warranty must be made in writing to Bio-Tek, Attention: Service Department. Purchaser must ship the Product to Bio-Tek, postage pre-paid. Bio-Tek shall either repair or replace with new or like new, at its option and without cost to the Purchaser, any Product which in Bio-Tek's sole judgment is defective by reason of defects in the materials or workmanship.

This Warranty is VOID if the Product has been damaged by accident or misuse, or has been damaged by abuse or negligence in the operation or maintenance of the Product, including without limitation unsafe operation, operation by untrained personnel, and failure to perform routine maintenance. This Warranty is VOID if the Product has been repaired or altered by persons not authorized by Bio-Tek, or if the Product has had the serial number altered, effaced, or removed. This Warranty is VOID if any of the Products has not been connected, installed or adjusted strictly in accordance with written directions furnished by Bio-Tek. Batteries, fuses, light bulbs, and other "consumable" items used in any of the Products are not covered by this Warranty. Software utilized in conjunction with any of the Products is not covered by the terms of this Warranty but may be covered under a separate Bio-Tek software warranty.

We will continue to stock parts for a maximum period of five (5) years after the manufacture of any equipment has been discontinued. Parts shall include all materials, charts, instructions, diagrams, and accessories that were furnished with the standard models.

THIS WARRANTY CONTAINS THE ENTIRE OBLIGATION OF BIO-TEK INSTRUMENTS, INC., AND NO OTHER WARRANTIES, EXPRESSED, IMPLIED, OR STATUTORY ARE GIVEN. PURCHASER AGREES TO ASSUME ALL LIABILITY FOR ANY DAMAGES AND/OR BODILY INJURY OR DEATH THAT MAY RESULT FROM THE USE OR MISUSE OF ANY EQUIPMENT OR INSTRUMENT BY THE PURCHASER, HIS EMPLOYEES, AGENTS, OR CUSTOMERS, OTHER THAN THE EXPRESS WARRANTY CONTAINED HEREIN. WE SHALL NOT BE RESPONSIBLE FOR ANY DIRECT OR CONSEQUENTIAL DAMAGES OF ANY KIND. THIS WARRANTY SHALL NOT BE CHANGED OR MODIFIED IN ANY WAY WITHOUT THE EXPRESS WRITTEN PERMISSION OF AN OFFICER OF BIO-TEK INSTRUMENTS, INC.

THIS WARRANTY IS VOID UNLESS THE PURCHASE REGISTRATION CARD HAS BEEN COMPLETED AND MAILED TO US WITHIN TEN (10) DAYS OF PURCHASE.

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Chapter 1
Introduction

This chapter introduces the Synergy HT and describes its hardware and software features and technical specifications. Instructions on how to contact Bio-Tek for Technical Assistance are included on page 1-9.

Introducing the *Synergy HT*

The *Synergy HT* is a single-channel absorbance and fluorescence reader for research and development and *in vitro* diagnostic use. The reader is completely computer controlled via KC4™ PC software for all operations including data reduction and analysis. The *Synergy HT* is robot accessible.

Unlike many multi-detection microplate readers, the *Synergy HT* utilizes a unique dual-optics design that allows the investigator to have two different reading modes without compromise of either capability. When making fluorescence determinations, the *Synergy HT* uses a tungsten quartz halogen lamp with interference filters for wavelength specificity in conjunction with a photomultiplier (PMT) tube detector. The tungsten quartz halogen lamp produces a large amount of light at a constant intensity, providing increased sensitivity and repeatability. Specially designed user-accessible fluorescent interference filters prevent light leakage from excitation wavelength to the emission wavelength, particularly important with fluorescent compounds with small Stoke's shifts. In addition, the ability to use filters with different bandpasses allows the investigator to tailor their fluorescence measurements to their experiment more effectively. The *Synergy HT* has both top and bottom probes for fluorescence measurements. The top probe can be automatically adjusted for the correct reading height.

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

The Synergy Time-Resolved (TR) option allows time-resolved measurements by using the xenon flash light source in conjunction with the PMT measurement detector.

The *Synergy HT* has a 4-Zone™ temperature control from 4° over ambient to 50°C that ensures superior temperature uniformity necessary for kinetic assays. Internal plate shaking is also supported.

Synergy HT models support the reading of 6-, 12-, 24-, 48-, 96-, and 384-well microplates with standard 128 x 86 mm geometry. Absorbance mode reads plates up to 20.3 mm in height; fluorescence mode reads plates up to 31.75 mm. PCR tubes to 31.75 mm are also readable with the use of existing adapter plates.

Hardware Features

- Dual optics design, with separate fluorescence and absorbance channels
- 3-mm top and 5-mm bottom fluorescence probes (standard)
- An absorbance λ range of 200 to 999 nm
- Absorbance OD range from 0-4 OD
- A fluorescence excitation λ range of 300 to 650 nm and an emission λ range of 350 to 800 nm
- Low, Medium, High and Variable plate shake frequencies with adjustable durations
- Reads 6-, 12-, 24-, 48-, 96- and 384-well microplates
- Operates from 100-240 VAC (± 10%) @ 50-60 Hz
- One serial COM port (25-pin male connector)
- 4-Zone™ incubation to 50ºC
- Optional Time-Resolved fluorescence capability

Software Features

This instrument is controlled using computer control commands sent via the RS-232 interface.

KC4™ is the primary operating software for the *Synergy HT*.

Package Contents

- Microplate Reader
- Power supply
- Power cord
- Serial Cable (PN 75053)
- Operator's Manual (PN 7091000) and Warranty Registration Card

Optional Accessories

- Service Manual for all *Synergy HT* models (PN 7091007)
- Universal Test Plate (PN 7260522) for absorbance measurement testing
- Product Validation (IQ-OQ-PQ) package (PN 7090522)
- PCR tube adaptor (PN's 6002072 and 6002076)

Spares

- Replacement tungsten quartz halogen bulb with cable for fluorescence (PN 7080500)

Specifications

- **Microplates**

  All models accommodate standard 6-, 12-, 24-, 48-, 96- and 384-well microplates with 128 x 86 mm geometry for:

  - **Absorbance mode plates** up to .8" high (20.30 mm)
  - **Fluorescence mode plates** up to 1.25" high (31.75 mm)
  - **PCR tube trays** up to 1.25" high (requires an adaptor)

- **Absorbance Speed of Reading**

  The actual plate read time and accuracy are dependent on the method of reading and wavelength(s) selected:

  - **Normal** mode contains provisions for extended optical density range read operation. Extended optical density read operation is automatic when a sample well in a column produces a signal of less than 400 A/D counts. (This would be at 2.000 Abs with a Blank of 40,000 counts.) At Optical Densities greater than 2.000 Abs, the reader takes 64 reads per well for the best accuracy.
  - **Rapid** mode is similar to normal mode but will not use the extended read.
  - **Sweep** mode is the fastest of the three modes. One read is taken on each well. The carrier does not stop in this mode.

  The following read times are based on a single or dual wavelength measurement. Actual reading speeds may vary, depending upon the reading wavelength selected. Each wavelength has a unique location within the monochromator, and the different locations require varying amounts of time to position.

- **Absorbance 96-Well Read Timing**

	630 nm	630/450 nm
Normal Read Mode	Single	Dual
Endpoint	57 sec	106 sec
Rapid Read Mode	Single	Dual
Endpoint	47 sec	86 sec
Sweep Read Mode	Single	Dual
Endpoint	23 sec	37 sec

Kinetics:

All three read modes are available in Kinetics mode. Single Wavelength reads are limited to the following minimum times.

- 49 seconds from A1 to A1 in Normal mode, single wavelength, depending upon optical density of solution.
- 39 seconds from A1 to A1 in Rapid mode, single wavelength.
- 14 seconds from A1 to A1 in Sweep mode, single wavelength.

- **Absorbance 384-Well Read Timing**

	630 nm	630/450 nm
Normal Read Mode	Single	Dual
Endpoint	159 sec	309 sec
Rapid Read Mode	Single	Dual
Endpoint	121 sec	232 sec
Sweep Read Mode	Single	Dual
Endpoint	38 sec	65 sec

Kinetics:

All three read modes are available in Kinetics mode. Single Wavelength reads are limited to the following minimum times.

- 150 seconds from A1 to A1 in Normal mode, single wavelength, depending upon density of solution.
- 111 seconds from A1 to A1 in Rapid mode, single wavelength.
- 26 seconds from A1 to A1 in Sweep mode, single wavelength.

- **Fluorescence Read Timing**

Because of wide variation in setup, the following benchmark conditions are specified:

Samples per well	10
Delay before sampling	350 ms
Delay between samples	1 ms
96 -well read	89 sec
384-well read	275 sec

- **Absorbance Performance**

Measurement Range: 0.000 to 4.000 Abs

All qualifications were conducted with 96- and 384-well flat bottom plates.

Accuracy:

0.000 to 2.000 Abs ± 1% ± 0.010 Abs Normal and Rapid modes, 96-well plates

0.000 to 2.000 Abs ± 2% ± 0.010 Abs Normal and Rapid modes, 384-well plates

2.000 to 2.500 Abs ± 3% ± 0.010 Abs Normal and Rapid modes, 96- and 384-well plates

2.500 to 3.000 Abs ± 3% ± 0.010 Abs Normal mode, 96-well plates

0.000 to 1.000 Abs ± 1% ± 0.010 Abs Sweep mode, 96- and 384-well plates

Linearity:

0.000 to 2.000 Abs ± 1% Normal and Rapid modes, 96-well plates

0.000 to 2.000 Abs ± 2% Normal and Rapid modes, 384-well plates

2.000 to 2.500 Abs ± 3% Normal and Rapid modes, 96- and 384-well plates

2.500 to 3.000 Abs ± 3% ± 0.010 Abs Normal mode, 96-well plates

0.000 to 1.000 Abs ± 1% Sweep mode, 96- and 384-well plates

Repeatability:

0.000 to 2.000 Abs ± 1% ± 0.005 Abs Normal and Rapid modes, 96- and 384-well plates

2.000 to 2.500 Abs ± 3% ± 0.005 Abs Normal and Rapid modes, 96- and 384-well plates

2.500 to 3.000 Abs ± 3% ± 0.005 Abs Normal mode, 96-well plates

0.000 to 1.000 Abs ± 2% ± 0.010 Abs Sweep mode, 96- and 384-well plates

For the above performance, the Gain on Optics test should be below 10.0.

Optical Specifications

λ range:	200 to 999 nm
λ accuracy:	± 2 nm
λ repeatability:	± 0.2 nm
λ bandpass:	2.4 nm
Detector:	Photo diodes (2)

- **Fluorescence Performance**

Sensitivity (all data for normal mode of reading):

Bottom-reading 5-mm probe:

Sodium Fluorescein

- 10 pg/ml solution of SF in PBS
- 150 µl per well signal-to-noise ratio greater than 2
- Excitation 485/20, emission 530/25
- Hellma 96-well quartz plate

Propidium Iodide

- 62.5 ng/ml solution of PI in PBS
- 50 µl per well signal-to-noise ratio greater than 2
- Excitation 485/20, emission 645/40
- Costar 96-well black sides, clear bottom plate

Top-reading 3-mm probe:

Methylumbelliferone

- 0.16 ng/ml solution of Methylumbelliferone in CBB
- 300 µl per well signal-to-noise ratio greater than 2
- Excitation 360/40, emission 460/40
- Costar black strips

- **Optional Time-Resolved Performance**

- Delay 0 or 20 ms to 16000 ms
- Integration interval 20 to 16000 ms
- Times adjustable in 10 ms increments

- **Incubation**

- From 4º over ambient to 50ºC
- Variation ± 0.50ºC

- **Shake**

- Low, Medium, High and Variable
- 17 Hz, 18 Hz, 19 Hz; Variable = composite of three rates

- **Hardware and Environmental Specifications**

Display:	None
Light Source:	**Absorbance:** Xe flash light source 10 W max average power Life: 1 billion flashes **Fluorescence:** Tungsten quartz halogen 20 W Life: 1000 hours
Dimensions:	16” D x 15" W x 10" H (40.6 cm x 38 cm x 25.4 cm)
Weight:	38 lb. maximum (17 kg)
Environment:	Operational temperature 15º-35°C
Humidity:	10-85%, non-condensing
Power Consumption:	100 VA
Incubation option: Temperature Control: Temperature Variation:	 4° over ambient to 50°C***** ± 0.50°C across the plate @ 37°C (250 µl per well with the plate sealed) ***** Rise over ambient will be within a degree if the filter wheel access door is left open (@ 23°C ambient).

Technical Support

Bio-Tek's *Synergy HT* is backed by a superior support staff. If the *Synergy HT* ever fails to work perfectly, please contact Bio-Tek's Technical Assistance Center.

Whichever method of contact you choose, please be prepared to provide the following information:

- Product name and serial number
- The specific steps that produce your problem
- Any error codes displayed in the controlling software (i.e., KC4). (See *Appendix C* for information on error codes.)
- A daytime phone number
- Your name and company information
- A fax number and/or an email address, if available
- If you need to return the reader to Bio-Tek for service, contact Bio-Tek for a Return Materials Authorization (RMA) number, and be sure to repack the reader properly (see *Chapter 3, Installation*).

Phone Support

You can telephone the Technical Assistance Center between 8:30 AM and 5:30 PM Eastern Standard Time (EST), Monday through Friday, excluding holidays.

Bio-Tek Instruments Main Number:	802-655-4040
Technical Assistance Center:	800-242-4685

Written Communication

You may write a letter with your comments and send it to:

Bio-Tek Instruments, Inc.
Technical Assistance Center
Highland Park, Box 998
Winooski, Vermont 05404-0998
USA

Facsimile Support

You may send a fax with your questions or requests for help 24 hours a day to the following numbers:

Technical Assistance Center:	802.655.3399

Electronic Communication

Electronic communication is available via the following:

E-Mail:	labtac@biotek.com
Internet Site:	www.biotek.com

Chapter 2
Instrument Description

This chapter includes principles of operation and descriptions of important external and internal components of the absorbance and fluorescence reader.

Principles of Operation

The *Synergy HT* is a PC-controlled absorbance and fluorescence plate reader that is also capable of conducting luminescence assays. It has been designed with a dual optical measurement system to maximize its capabilities as an absorbance or fluorescence reader. The Synergy HT utilizes two light sources: (1) continuous tungsten halogen for fluorescence measurements, and (2) xenon flash for absorbance and for optional time-resolved fluorescence measurements. The *Synergy HT* can also handle a variety of microplate formats to meet the needs of various applications.

The *Synergy HT* has a 4-Zone™ incubation system and an XY transport system that is designed for robot access. The transport system positions the plate accurately, based upon input plate geometries from the controlling software. When all measurements have been taken, the data is output via the serial port to an external computer.

External Components

The external components of the *Synergy HT* are illustrated in *Figure 2-1*.

- The power switch is located on the front of the instrument. The switch is labeled "I/O", indicating on and off, respectively. An LED indicates that the power is on.
- The **microplate carrier** supports the microplates and adapter plates. A spring clip holds the plate securely in place. The microplate carrier access door helps to ensure a light-impermeable measurement chamber. The carrier may be ejected by using the button on the front of the instrument.
- To use the optional Time-Resolved Fluorescence feature, remove the excitation filter wheel and replace it with the time-resolved cartridge.
- When a plate read is initiated, the plate carrier is drawn into the measurement chamber and then moves in the X and Y axes to align each microwell with the top or bottom fluorescence probe, or bottom absorbance probe as specified in the assay. When the read is complete, the plate carrier is returned to its full-out position.
- The **back panel** contains the power inlet and serial port.
- The hinged door on the front of the instrument is held in place with two magnets and provides access to the lamp assembly and excitation and emission filter wheels. To open the door, press on its lower right and lower left corners, and the door will open 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.
- The fluorescence reader features top- and bottom-probe capability; top probe height is adjustable using motor control.

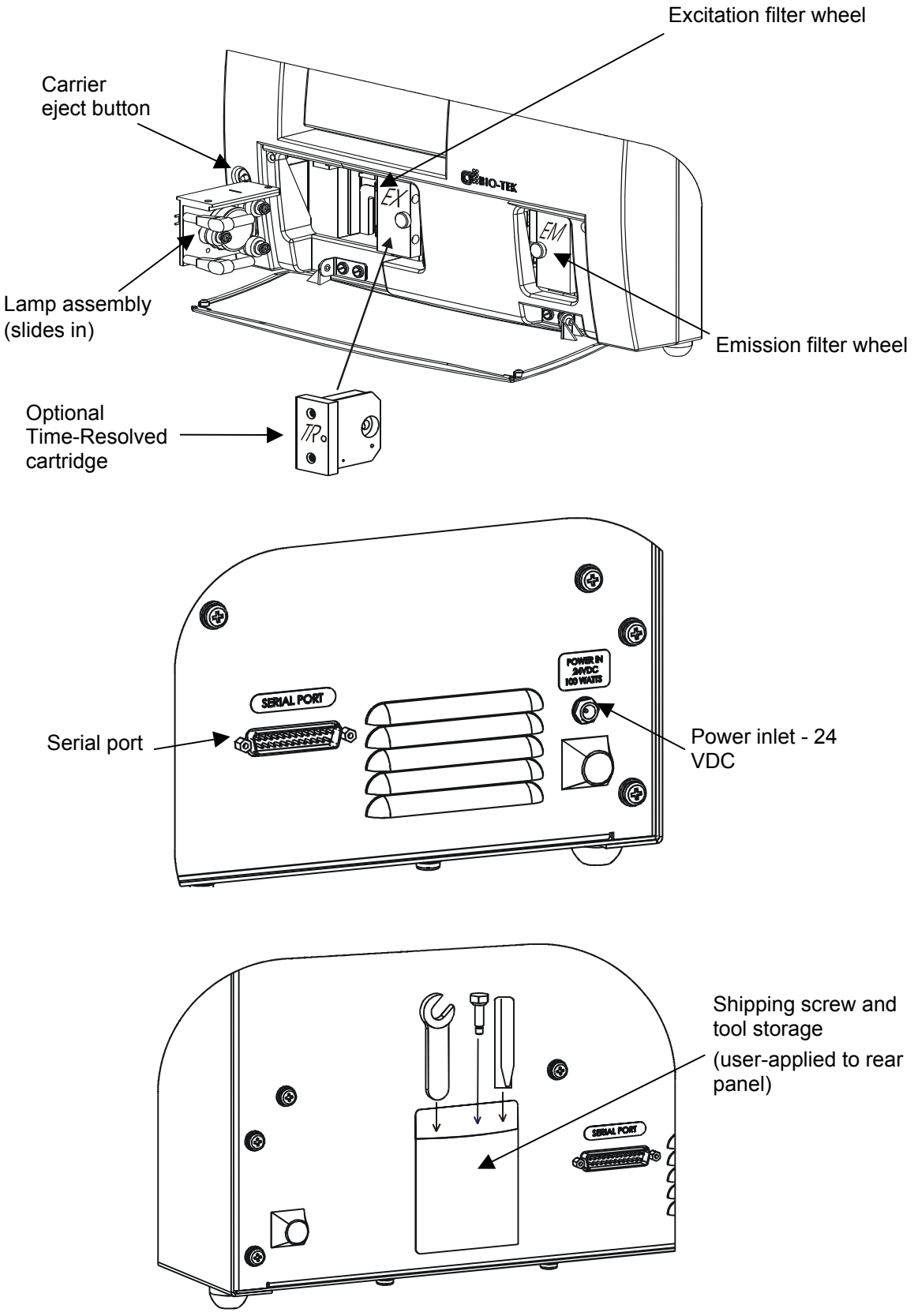


Figure 2-1: *External components*

Excitation and Emission Filter Wheels

All *Synergy HT* models are equipped with one excitation filter wheel and one emission filter wheel. (See *Figures 2-2a* and *2-2b* below.) The excitation filter selects the narrow band of light to which the sample will be exposed. The emission filter selects the band of light with the maximum fluorescence signal, to be measured by the photomultiplier (PMT).

Each filter wheel is labeled EX or EM, and can contain up to four filters and/or black "plugs." Each filter and plug is held securely in place with a C-clip filter retainer.

Note: Each filter has an arrow printed on its side to indicate the proper direction of light through the filter.

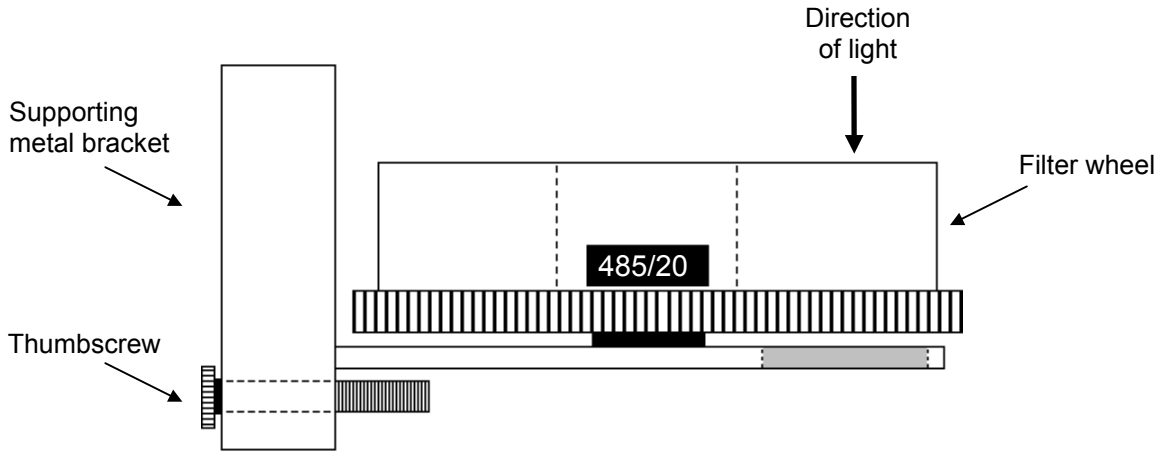


Figure 2-2a: *Side view of excitation filter wheel, showing proper filter orientation*

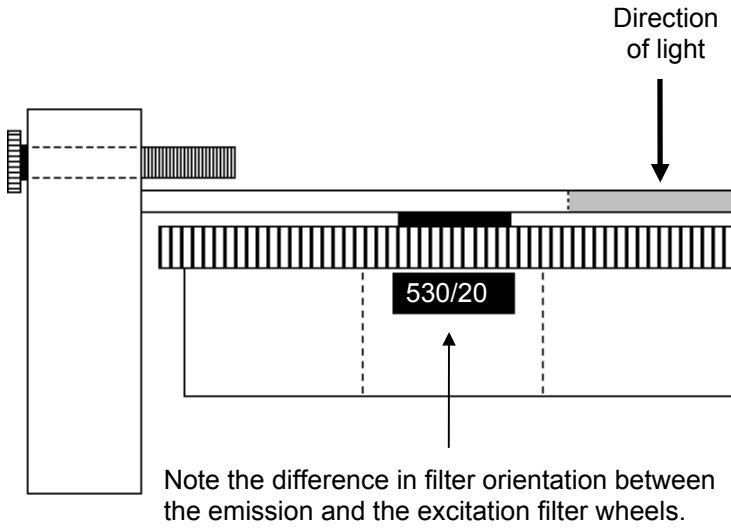


Figure 2-2b: *Side view of emission filter wheel, showing proper filter orientation*

Removing the Filter Wheels

The filter wheels can be removed if their contents need to be changed. It is important to note that

- The excitation and emission filter wheels are not interchangeable, and are labeled as follows:

  EX = Excitation

  EM = Emission

- Filter direction within a filter wheel is important, and the direction differs depending on the filter wheel. There is an overlay on the front panel door indicating this.
- Each filter is marked with an arrow indicating the proper direction of light.
- If a filter's wavelengths are changed, the KC4™ software filter tables must be updated accordingly.

Refer to *Figures 2-2a* and *2-2b* on the previous page for proper filter orientation. See also Configuring the Excitation and Emission Filter Tables in the KC4 manual.

To remove a filter wheel and change its contents:

1. **Important!** Turn off the instrument.
2. Using both hands, push down on the bottom of the hinged door on the front of the instrument. Observe the two thumbscrews within the compartment. The left thumbscrew holds the excitation filter wheel in place; the right secures the emission filter wheel. The Emission filter wheel will "spring" out when removed. This is because a shutter behind the wheel closes quickly to protect the PMT.
3. Remove the thumbscrew, then slide the filter wheel's supporting metal bracket straight out of the compartment.
4. *To remove a filter or plug*:
   - Turn the filter wheel to align the desired filter with the hole in the supporting bracket.
   - Place the bracket on a flat surface, with the filter wheel facing down.
   - Prepare a multi-layered "cushion" of lens paper. Using your finger covered with the lens paper, gently push against the filter until it and its C-clip retainer pop out.

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

5. *To replace a filter or plug*:

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

6. *To replace a filter wheel:*

   - Ensure that all filters and/or plugs are inserted properly (see step 5 above).
   - Slide the filter wheel back into its chamber.
   - Replace the thumbscrew.
   - Close the front door.
   - Power on the instrument.

Optional Time-Resolved Cartridge

The optional time-resolved fluorescence (TRF) cartridge is shown in *Figure 2-3a* below. The time-resolved (TR) cartridge allows the Synergy HT to be used for making time-resolved measurements. The cartridge allows light from the xenon flash bulb to be input to the fluorescence optical system within the Synergy instrument. The Synergy instrument detects the TR cartridge once it is installed, which allows use of the TR feature.

The time-resolved cartridge, labeled "TR," is installed in place of the excitation filter as described on the next page.

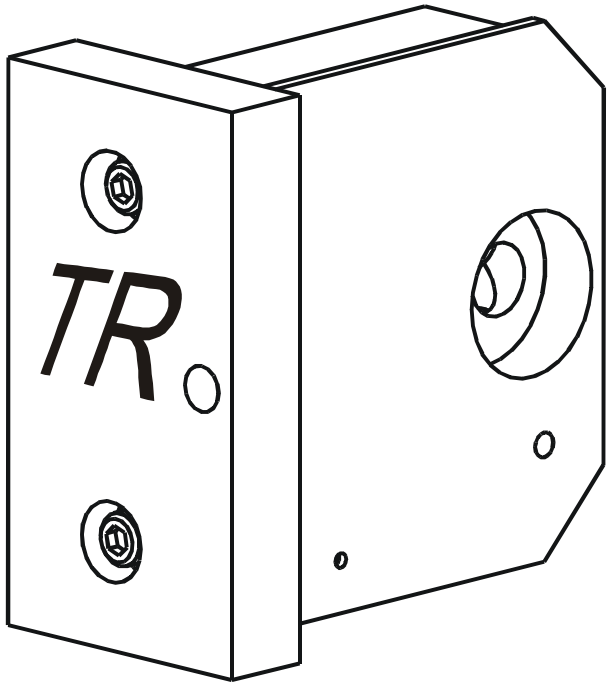


Figure 2-3a: *Optional time-resolved cartridge*

Installing the Time-Resolved Cartridge

To use the time-resolved fluorescence feature, you must remove the excitation filter (labeled "EX") and replace it with the time-resolved cartridge (labeled "TR"). Refer to *Figure 2-3b* below for proper cartridge orientation.

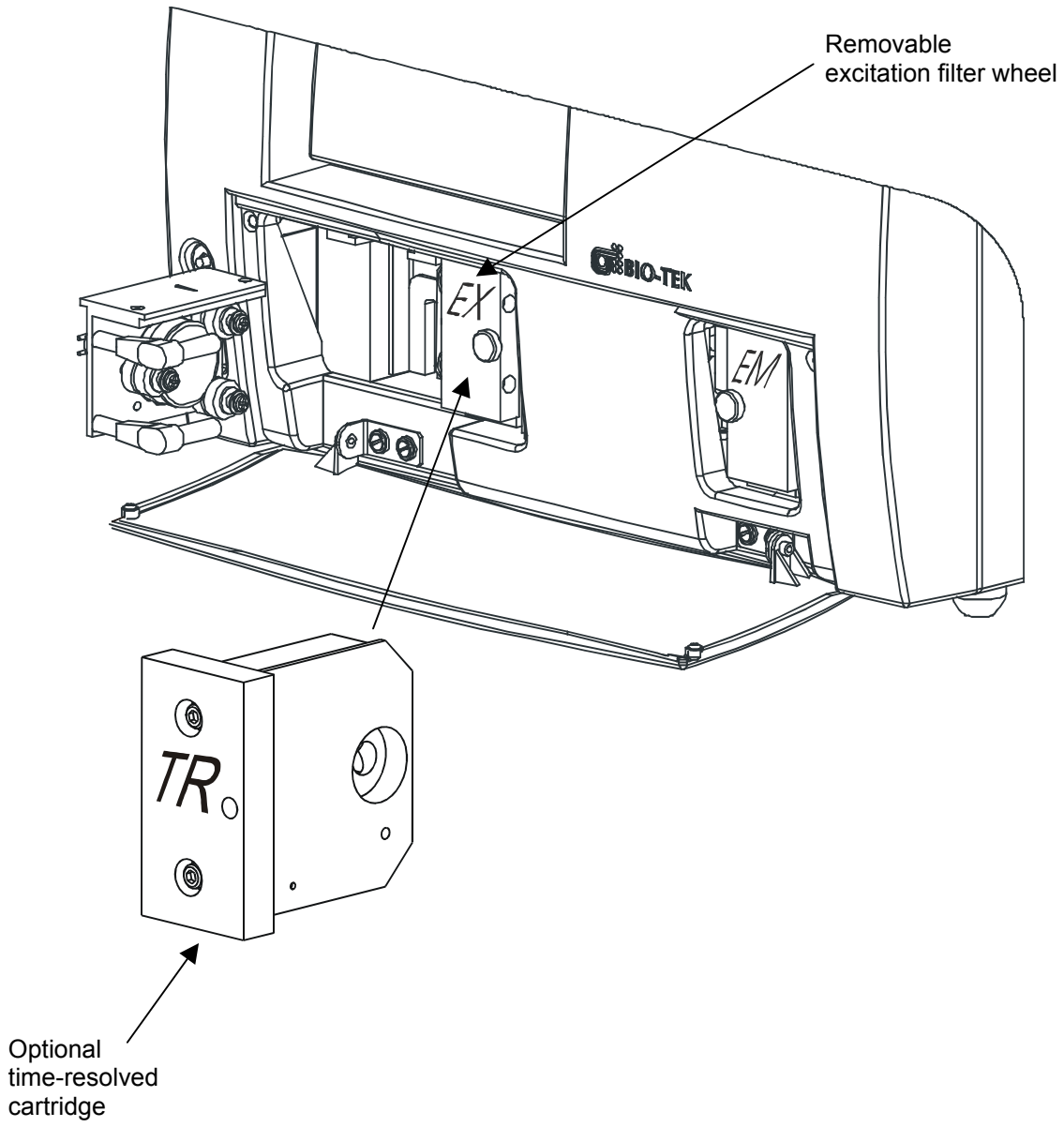


Figure 2-3b: *Orientation of time-resolved cartridge*

To remove the excitation filter wheel and replace it with the time-resolved cartridge:

1. **Important!** Turn off the instrument.
2. Using both hands, push down on the bottom of the hinged door on the front of the instrument. Observe the two thumbscrews within the compartment. The left thumbscrew holds the excitation filter wheel in place.
3. Remove the left thumbscrew, then slide the filter wheel's supporting metal bracket straight out of the compartment.
4. Slide the time-resolved cartridge into the compartment.
5. Replace the thumbscrew.
6. Close the front door.
7. Power on the instrument.

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

Important! To reinstall the excitation filter wheel, follow steps 5 and 6 on page 2-6.

Fluorescence Lamp Assembly

The lamp assembly consists of a 20-watt tungsten quartz halogen bulb, with two 6" cables soldered onto the back. It is accessible through the hinged door on the front of the instrument. The lamp is shipped in a bag with the reader and must be inserted into the unit as illustrated in *Figure 2-4* below. The lamp is attached to a removable metal bracket that also holds the condenser lens and heat absorber. The metal bracket is held in place by a detent mechanism. The cables are plugged into a power source located to the left of the excitation filter wheel.

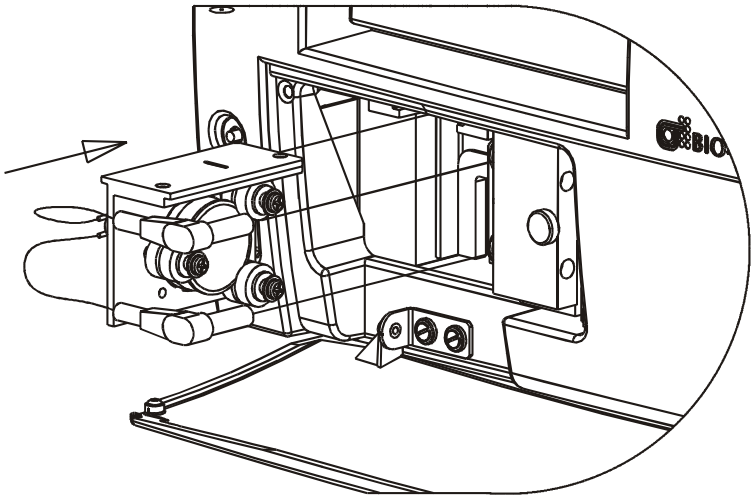


Figure 2-4: *Installing the lamp assembly*

The bulb is expected to operate without replacement for a minimum of 1000 hours. The intensity of the bulb will slowly drop over time until the instrument's run-time self-check detects a low signal and displays an error message. The lamp assembly should be replaced at this time. A replacement lamp assembly is available from Bio-Tek Instruments, part number **7080500**.

Replacing the Lamp Assembly

	**Caution!** Before replacing the lamp assembly, turn off and unplug the instrument, then allow the lamp to cool down for a minimum of 15 minutes before proceeding.

1. Open the hinged door on the front of the instrument.
2. Unplug the two cables connecting the lamp to its power source.
3. Gently grasp the metal bracket housing the lamp and glass lenses, then pull it straight out of the lamp compartment. Do not touch the lenses!

¡¡¡	**Important!** When removing the lamp from or reattaching it to the metal bracket, do not touch the glass lenses! Fingerprints on the condenser lens or heat absorber may negatively affect performance.

4. Remove the two thumbscrews that attach the lamp to the metal bracket.
5. Remove the wire bracket from the lamp assembly.
6. Place the wire bracket on the replacement lamp assembly.
7. Seat the replacement lamp in its circular cutout, against the metal bracket.
8. Replace the thumbscrews. When they are partially tightened, hold the metal bracket in its upright position and gently twist the lamp so that the cables hang downward. Fully tighten the thumbscrews.
9. Slide the metal bracket into its slot in the lamp compartment.
10. Plug the lamp cables into the lamp's power source. Either cable can be plugged into either socket.
11. Close the hinged door.
12. Plug the instrument in and turn it on. If the system test passes, lamp replacement is complete. If the test fails, note the error code and turn to *Appendix C, Error Codes.*

Lamps

Absorbance and Time-Resolved Fluorescence

The xenon flash lamp life is rated at an average of 1 billion flashes. This bulb should outlive the useful life of the reader. If there is a problem with the lamp, however, the intensity may drop and the run-time self check will detect a low signal level and generate an error message. If this happens, the instrument will require service; contact Bio-Tek for assistance.

Standard Fluorescence

The tungsten halogen lamp life is rated at an average of 1000 hours and 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 displays an error message. The lamp assembly should be replaced at this time. A replacement lamp (PN 7080500) is available from Bio-Tek.

Cooling Fan

The internal cooling fan is used to keep the internal electrical circuits operating at a proper temperature.

Power Supply

The *Synergy HT* requires a 24-VDC, 4.0-A universal power supply (PN 76053). The input is 100-240 VAC (± 10%) at 50-60 Hz, with an output of 24 VDC at 3.75 A. Connect the power cord into the power supply and the power supply output plug in the 24-VDC connector on the rear of the unit. Tighten the plug barrel to retain the plug to the instrument. See *Figure 2-1* on page 2-3.

Power ON/OFF Switch and Carrier Eject Button

The power ON/OFF switch has an internal LED lamp that illuminates when the power is on. The carrier eject button (when depressed) ejects the carrier out of the unit or pulls the carrier back in the instrument to the carrier home position. The button is disabled during plate reads, autocal, and self-test functions. It only functions when the unit is in standby mode waiting for a computer control command. See *Figure 2-5* below.

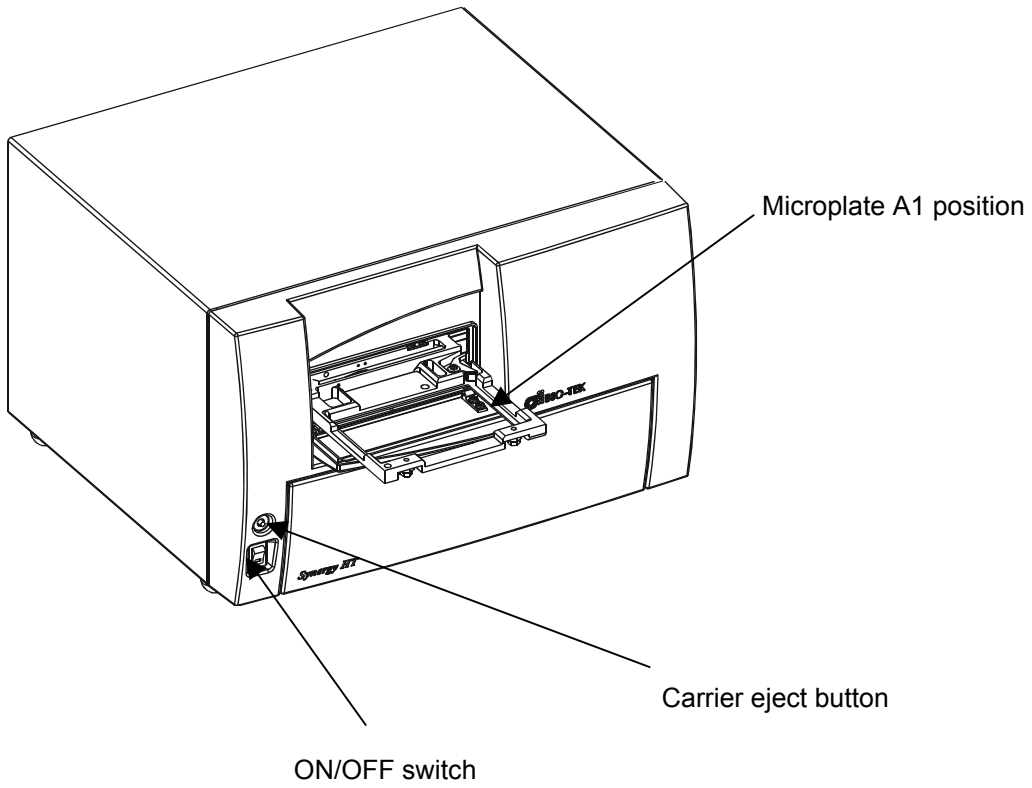


Figure 2-5: *Power ON/OFF switch and carrier eject button*

Chapter 3
Installation

This chapter includes instructions for unpacking and setting up the Synergy HT as well as instructions for connecting a PC.

Operating Environment

For optimal operation, install the *Synergy HT* on a level surface in an area where ambient temperatures between 15º and 35ºC can be maintained. The reader is sensitive to extreme environmental conditions. Conditions to avoid are:

- **Excessive humidity:** Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks.
- **Excessive ambient light:** Bright sunlight or strong incandescent light can reduce the linear performance range and affect the instrument's readings.
- **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.

Unpacking and Repackaging the Instrument

1. Inspect the packaging and instrument for shipping damage.
2. If the reader is damaged, notify the carrier and your manufacturer's representative.

¡¡¡	**Important!** Keep the shipping cartons and the packing material for the carrier's inspection. If the reader is shipped to the factory for repair or replacement, it must be carefully repackaged using the original packing materials. Shipping with improper packaging materials may **void your warranty.** If the original packing materials have been damaged, replacements are available from Bio-Tek.

3. Contact Bio-Tek's Technical Assistance Center for an RMA (Return Materials Authorization) before returning equipment for service. Mark the RMA number on the outside of the shipping container. See page 1-9 for contact information.
4. Carefully open the top of the shipping container and remove the accessories box containing the power cord, power supply, Operator's Manual, and serial cable.
5. Remove the top end caps from the reader. See *Figure 3-1*.

6. Lift the reader out of the box, and place it on a level surface. Remove the reader from the plastic bag.

7. Place all shipping material back into the shipping box for reuse if the instrument needs to be shipped again.

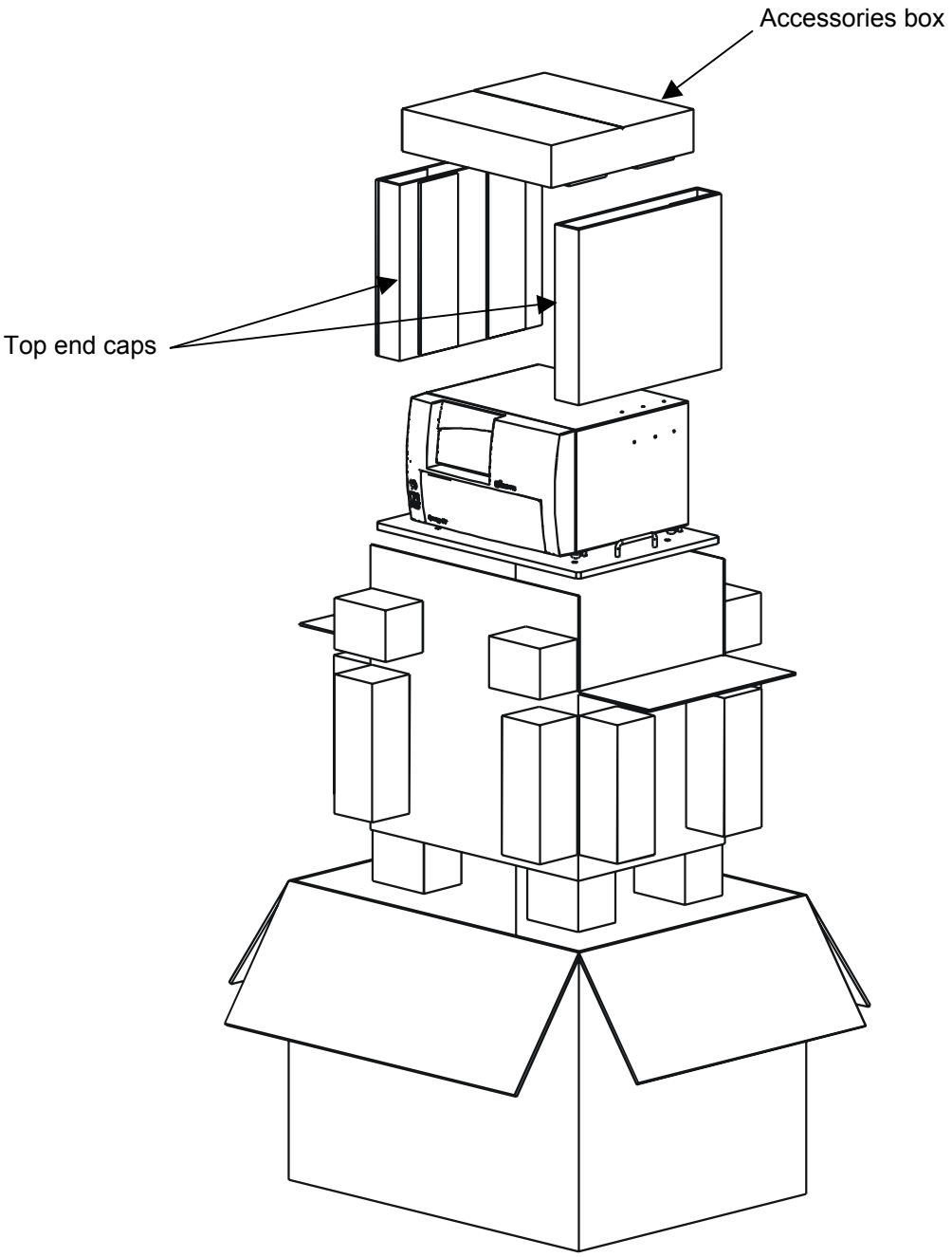


Figure 3-1: *Removing the top end caps from the reader*

Removal of the Shipping Panel

Using a Phillips head screwdriver, remove all four screws attaching the shipping panel to the front of the reader. Place the screws in the plastic tool storage bag and use the supplied Velcro strip to attach the bag to the back of the reader for storage. Place the panel in the inner box.

¡¡¡	**Important!** Re-attach the shipping panel prior to repackaging the unit for shipment.

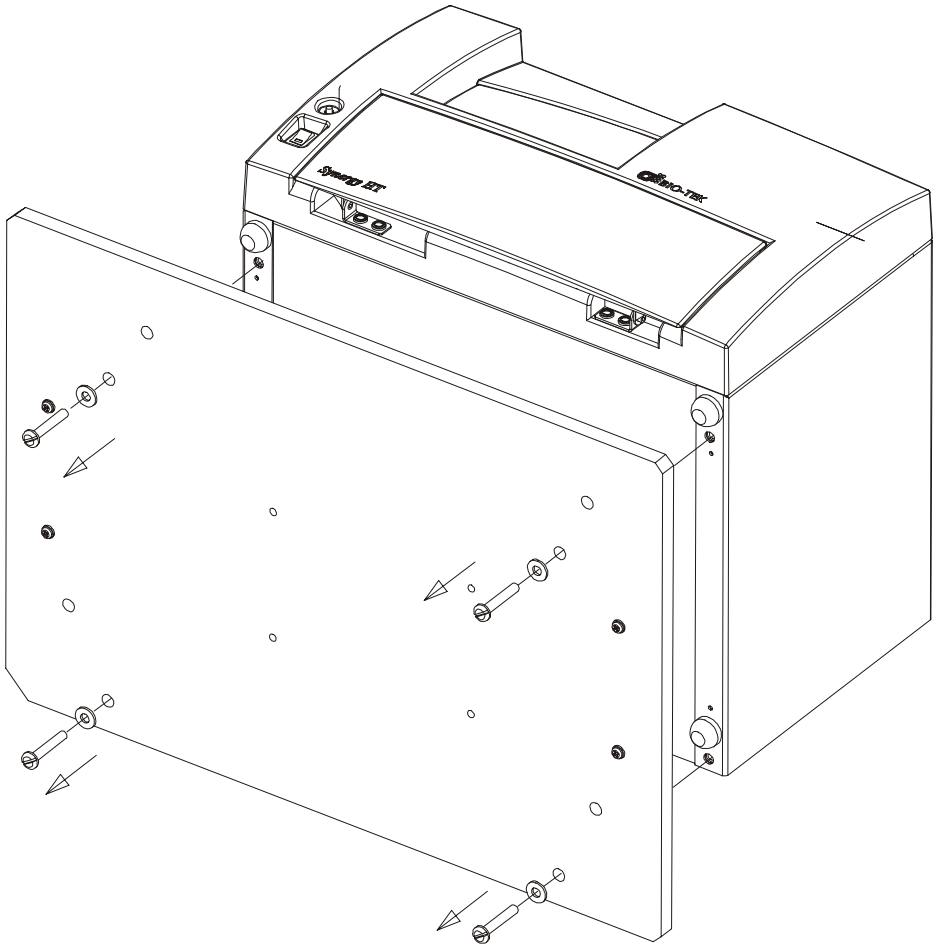


Figure 3-2: *Removing the shipping panel*

Microplate Carrier Shipping Screw Removal Instruction

¡¡¡	The *Synergy HT* model is shipped with a **carrier shipping screw** that must be removed before the reader is used. See *Figure 3-3* below.

Open the top front door. Remove the carrier shipping screw and place the screw in the plastic tool storage bag that is fastened to the back of the reader.

¡¡¡	**Important!** Replace the screw prior to shipment.

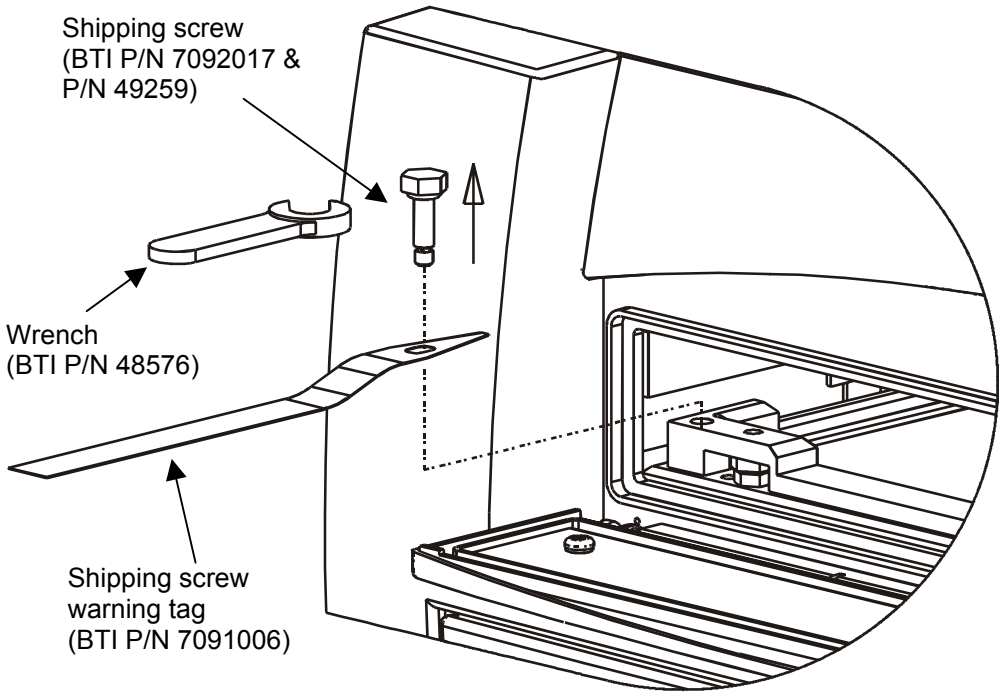


***Figure 3-3**: Removing the carrier shipping screw*

After Unpacking, Verify Performance

Before using the *Synergy HT* for the first time, verify that it is operating properly by following the steps listed below, *Attaching the Serial Cable*.

Before Repackaging the Instrument

- Decontaminate the reader prior to shipping (see *Appendix A, Decontamination*).
- Remove the lamp assembly (*Figure 2-7*) and pack it in the bubble wrap.
- Replace the carrier shipping screw. See *Figure 3-3* for an illustration.
- Once the reader is clean, attach the shipping panel (*Figure 3-2*). Be sure to align the bevel with the beveled edge. Pack the reader in its original shipping box, using original packing materials. This shipping system was designed to be used no more than five times. If the container is damaged and/or has been used more than five times, contact Bio-Tek for a new set of shipping materials (BTI P/N 7090003).

Serial Port for Communications

The *Synergy HT* has a 25-pin serial (RS-232) port located on the rear panel of the instrument (see *Figure 2-4*). The serial port allows the reader to communicate with a computer, using standard communications software and/or RS-232 protocols.

Appendix B contains information on required protocols for computer control of the reader.

Attaching the Serial Cable

- Always power down the computer and the reader before attaching or removing any cables.
- Connect the appropriate serial cable to both machines. The serial port on the reader is a DTE configuration with a 25-pin (male) D-sub connector. The connector's pinout is illustrated in *Table 3-1*.
- Connect the power supply to the reader per the instructions on page 2-15.
- Power up the reader and the computer. Install KC4™ on the computer's hard drive. (See page B-1 of *Appendix B, Computer Control*.)
- Each time the reader is turned on, it performs a system self-test. If the self-test completes successfully, the reader is ready for use. If the test fails, note any error codes and contact Bio-Tek. (Error codes are displayed with KC4.)

  Refer to *Chapter 4, Performance Verification/Qualification Tests* for a recommended maintenance schedule, which includes Installation, Performance, and Operational Qualification Tasks.

Serial Port Pinout Description

Table 3-1 describes the reader's serial/RS-232 pin connection.

Table 3-1
Serial Pinout Description

Serial Pin Description			
Pin	**Signal**	**Pin**	**Signal**
1	NC	14	NC
2	TX	15	NC
3	RX	16	NC
4	RTS	17	NC
5	CTS	18	NC
6	DSR	19	NC
7	GND	20	DTR
8	DCD	21	NC
9	NC	22	RI
10	NC	23	NC
11	NC	24	NC
12	NC	25	NC
13	NC		

Setting Up Communication Parameters

Before serial communication can be initiated between the *Synergy HT* and another device (such as a host PC running KC4™), the communication parameters must match on both devices. The reader's default communication parameters are:

- Baud Rate: 9600
- Data Bits: 8
- Stop Bits: 2
- Parity: None

Chapter 4
Performance Verification/ Qualification Tests

This chapter discusses the tasks and procedures necessary for verifying and qualifying instrument performance on an ongoing basis. A convenient Recommended Test Schedule arranges tasks into Installation, Performance, and Operational Qualification categories.

Recommendations for Achieving Optimum Performance

- Microplates should be perfectly clean and free of 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 when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.
- Although the *Synergy HT* supports all flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with optically clear, flat-bottomed wells.
- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µl per well in a 96-well plate and 25 µl in a 384-well plate.
- Dispensing solution into 384-well plates often traps air bubbles in the wells. Dual wavelength reads will cancel most of these errors; however, for best results, they should be removed by degassing the plate in a vacuum chamber prior to reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Agitate the microplate before reading to help bring this problem within acceptable limits. Use Tween® 20, if possible (or some other wetting agent) to normalize the meniscus for absorbance measurements. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change. The meniscus shape will stabilize over time.

Recommended Test Schedule

The schedule shown in *Table 4-1* defines the factory-recommended intervals for performance testing for a microplate reader used two to five days a week.

Note: The risk factors associated with your tests may require that the Performance and Operational Qualification procedures be performed more or less frequently than shown below.

Table 4-1
Recommended Test Schedule

	Installation Qualification	**Performance Qualification / *Monthly***	**Operational Qualification / *6 Months***	**Operational Qualification / *Annually***
Absorbance				
System Self-Test, p. 4-4	✓	✓		✓
Universal Plate Test, p. 4-7	✓*	✓		✓*
Liquid Test 1, p. 4-14	✓*		✓*	✓*
Liquid Test 2, p. 4-16	✓*		✓*	✓*
Liquid Test 3, p. 4-19 Optional for 340 nm	✓			✓
Fluorescence				
System and Checksum Tests, p. 4-22	✓	✓	✓	✓
Corners Test, p. 4-23	✓	✓		
Sensitivity Test, p. 4-23	✓	✓		✓
Inspect/Clean Wavelength Filters			✓	✓

* Run Liquid Test 2 only if you do not have a Universal Test Plate. If you run Liquid Test 2, you do not have to also run Liquid Test 1.

Installation, Operational and Performance Qualification Procedures

Tests outlined in this section may be utilized to confirm initial and ongoing performance of the *Synergy HT*.

Your *Synergy HT* reader was fully tested at Bio-Tek prior to shipment and should operate properly upon initial setup. If it is suspected that problems may have occurred during shipment, if you reshipped the device, or if regulatory requirements dictate that Performance Qualification Testing is necessary, the following tests should be performed.

Absorbance

- **System Self-Test:** Verifies proper gains, bulb operation, low electronic noise, fluorescence probe positions, and incubator functionality. This test also prints the reader Serial Number and onboard basecode part and version numbers.
- **Universal Plate Test:** Confirms the optical accuracy, linearity, alignment, and wavelength accuracy of the instrument.
- **Liquid Testing:** Quantifies the repeatability variation of the instrument using liquids, which verifies operation in a way that glass test filters cannot.

Fluorescence

- **System Test:** Checks voltage levels, motor control, and the integrity of the photomultiplier.
- **Checksum Test:** Compares the onboard basecode with internally recorded checksum values to ensure that no corruption has occurred.
- **Corners Test:** Uses fluorescence compounds to verify that the plate carrier is properly aligned in relation to the optical probe(s).
- **Sensitivity Test:** Uses fluorescence compounds to verify that the difference between the means of wells with known lower limits of concentration of the substance under investigation is statistically distinguishable from the mean of wells with pure diluent.

Absorbance Testing

Routine Procedure

To ensure proper operation of the *Synergy HT* on an ongoing basis, the System Self-Test and the Universal Plate Test should be conducted monthly.

- Select Reader System Test to verify that the absorbance light levels and electronic noise at all set wavelengths fall within factory acceptance criteria. The report will also contain the part and version numbers of the software installed on the unit.
- Select Universal Test Plate to run the calibration plate test to confirm the alignment, repeatability, and accuracy/linearity of the reader.
- Perform a wavelength scan to confirm wavelength accuracy of the reader.

System Self-Test

The System Self-Test confirms that the absorbance module's light levels and electronic noise at all set wavelengths fall within factory acceptance criteria. To accomplish this, measuring the air and dark readings, and evaluate them to ensure they fall within specified ranges.

The reader automatically runs an internal System Test each time it is powered on. The power-on System Test will not be initiated and the instrument will beep if the shipping screw has not been removed from the carrier. If the unit fails the Self Test, refer to the error message displayed in KC4™. To run the System Test from KC4, use the Optics Test feature.

To obtain a report of the System Test values for either periodic testing documentation or troubleshooting (*Figure 4-1*), follow the instructions in KC4. The instrument's System Test will be conducted and the results reported in a pass/fail format. This report will include results of both the absorbance and fluorescence tests.

See *Appendix C* for a list of possible error codes.

Photodiodes

The Optics portion of the System Self-Test confirms that the absorbance reading channel has adequate signal range without saturating the electronics.

Flash Lamp

The Optics test also indicates if the flash lamp is within operational limits.

Incubation

With the 4-Zone™ incubation installed, the System Self-Test verifies the four thermistors and compares these readings to internal voltage references to confirm proper operation.

Operator ID:_____

Notes:_____

_____

SYSTEM SELF TEST

7090202 Version 1.00 128793 1101 0000

Bias current offset	0 counts	PASS
Offset voltage	1552 counts	PASS
750V measurement	234 counts	PASS
750V noise	49 counts	
500V measurement	15 counts	
500V noise	2 counts	
750V / 500V ratio	15.6	PASS

Lambda: 340 Gain: 2.39 Resets: 4

Channel:	Ref	1
Air:	12577	39522
Dark:	9877	9900
Delta:	2700	29622

Lambda: 630 Gain: 1.95 Resets: 1

Channel:	Ref	1
Air:	12498	39909
Dark:	9881	9911
Delta:	2617	29998

Lambda: 977 Gain: 2.69 Resets: 2

Channel:	Ref	1
Air:	12782	39368
Dark:	9881	9912
Delta:	2901	29456

Lambda: 900 Gain: 1.58 Resets: 1

Channel:	Ref	1
Air:	12689	39503
Dark:	9878	9903
Delta:	2811	29600

Lambda: 405 Gain: 1.91 Resets: 4

Channel:	Ref	1
Air:	12487	39838
Dark:	9874	9893
Delta:	2613	29945

Lambda: 450 Gain: 1.64 Resets: 4

Channel:	Ref	1
Air:	12496	39838
Dark:	9873	9889
Delta:	2623	29949

Channel:	Ref	1
Noise Max:	9905	9993
Noise Min:	9905	9992
Delta:	0	1

Voltage Reference:	Lamp	24V	Mtr	Min	Low	High	Max
	1702	1941	2051	1428	1739	2164	2475

Figure 4-1a: *Sample output for the System Test (Sheet 1 of 2)*

```
                              INCUBATOR SELF TEST

Temperature Setpoint:  0.0     Current Average: 22.6      A/D Test: PASS

Zone 1: 22.6   Min: 22.6   Max: 22.6     Range: PASS    Thermistor: PASS
Zone 2: 22.7   Min: 22.7   Max: 22.7     Range: PASS    Thermistor: PASS
Zone 3: 22.6   Min: 22.6   Max: 22.6     Range: PASS    Thermistor: PASS
Zone 4: 22.6   Min: 22.6   Max: 22.6     Range: PASS    Thermistor: PASS


AUTOCAL ANALYSIS

PROBE:          TOP

Upper Left Corner:  x=  9724  y=  268
Lower Left Corner:  x=  9716  y= 5796
Lower Right Corner: x=  1032  y= 5792
Upper Right Corner: x=  1036  y=  268
Delta 1:  9724 - 9716=    +8
Delta 2:  1036 - 1032=    +4
Delta 3:   268 -  268=    +0
Delta 4:  5792 - 5796=    -4

PROBE:          BOTTOM

Upper Left Corner:  x=  9720  y= 1848
Lower Left Corner:  x=  9712  y= 7376
Lower Right Corner: x=  1032  y= 7380
Upper Right Corner: x=  1032  y= 1848
Delta 1:  9720 - 9712=    +8
Delta 2:  1032 - 1032=    +0
Delta 3:  1848 - 1848=    +0
Delta 4:  7380 - 7376=    +4

PROBE:          ABSORB

Upper Left Corner:  x= 11268  y= 1832
Lower Left Corner:  x= 11268  y= 7356
Lower Right Corner: x=  2576  y= 7356
Upper Right Corner: x=  2584  y= 1836
Delta 1: 11268 -11268=    +0
Delta 2:  2584 - 2576=    +8
Delta 3:  1836 - 1832=    +4
Delta 4:  7356 - 7356=    +0

Probe Height:          33.83

Middle Sensor:      y= 11968
Tested:                11968
Delta:                    +0

Back Sensor:        x= 11556  y= 7916
Tested:                11560     7920
Delta:                    +4       +4


SYSTEM TEST PASS
```

Figure 4-1b: *Sample output for the System Test (Sheet 2 of 2)*

Calibration Verification

It is considered good laboratory practice to periodically verify the calibration of the *Synergy HT*. Verification should be performed per the schedule shown in *Table 3-1*, using the tests in this section:

- Universal Calibration Plate Test (see below)
- Liquid Tests (see page 4-11)

Universal Plate Test

The Universal Plate Test (also referred to as the Calibration Plate Test) confirms the alignment, repeatability, accuracy/linearity, and wavelength accuracy of the *Synergy HT*. An alternative method that may be used to determine accuracy, repeatability, and linearity is Liquid Test 2, described on page 4-16.

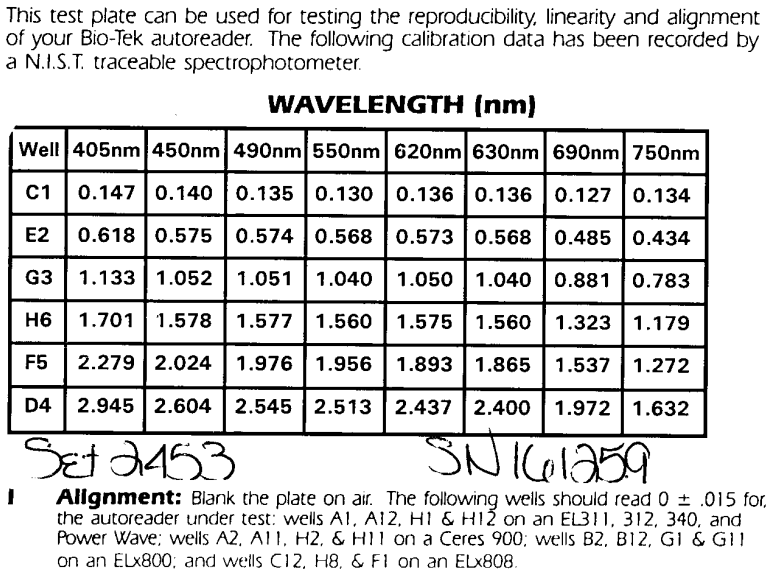
The Universal Test Plate (Part Number 7260522) allows the comparison of the reader's optical density measurements and mechanical alignment to NIST-traceable values. Accuracy/linearity, repeatability, alignment, and wavelength accuracy are tested. Specific standard calibration values must be entered for each wavelength to be tested.

The Universal Plate Test confirms the following:

- **Accuracy:** Accuracy of the Optical Density readings at specific wavelengths is confirmed by comparison of the readings with those given with the Universal Test Plate insert.
- **Linearity:** Linearity of the Optical Density readings is confirmed by default if the optical density readings are accurate.
- **Alignment:** Alignment of the plate carrier and standard microplates is confirmed by the four-corner positional accuracy check.
- **Wavelength accuracy:** To check the accuracy of wavelength settings, use Bio-Tek's Universal Test Plate, Part Number 7260522. The Universal Test Plate provides a multi-band test filter (Didymium glass V10), in location C6.

Requirements

To run the Universal Plate Test, you need Bio-Tek's Universal 7-Filter Test Plate (Part Number 7260522), with its accompanying Data Sheet, shown in *Figure 4-2*.



This test plate can be used for testing the reproducibility, linearity and alignment of your Bio-Tek autoreader. The following calibration data has been recorded by a N.I.S.T. traceable spectrophotometer.

WAVELENGTH (nm)

Well	405nm	450nm	490nm	550nm	620nm	630nm	690nm	750nm
C1	0.147	0.140	0.135	0.130	0.136	0.136	0.127	0.134
E2	0.618	0.575	0.574	0.568	0.573	0.568	0.485	0.434
G3	1.133	1.052	1.051	1.040	1.050	1.040	0.881	0.783
H6	1.701	1.578	1.577	1.560	1.575	1.560	1.323	1.179
F5	2.279	2.024	1.976	1.956	1.893	1.865	1.537	1.272
D4	2.945	2.604	2.545	2.513	2.437	2.400	1.972	1.632

Set 2453 SN 161259

Alignment: Blank the plate on air. The following wells should read 0 ± .015 for, the autoreader under test: wells A1, A12, H1 & H12 on an EL311, 312, 340, and Power Wave; wells A2, A11, H2, & H11 on a Ceres 900; wells B2, B12, G1 & G11 on an ELx800; and wells C12, H8, & F1 on an ELx808.

Figure 4-2: *Sample Universal Test Plate data sheet*

Entering the Universal Test Plate Data

Note: Before you define the standard calibration values, set the reader's wavelength settings in KC4™ to correspond with up to six of the wavelengths shown on the Universal Test Plate Data Sheet. Refer to the Data Sheet provided with the Universal Test Plate (see sample above) when entering the standard values.

Running the Universal Plate Test

Before running the Universal Plate Test, ensure that the standard calibration values for the test plate have been defined.

Run the Universal Plate Test per the KC4 program:

- When the test is complete, results will print. See the sample Calibration Plate Analysis (*Figure 4-3*).

Date: 04/03/02 07:54:08
Reader: SynergyHT-I - SN#128798
Universal Plate: 115347 - Universal Test plate
User: Bob
Comment:

Alignment Results:

WELLS	A1	A12	H1	H12
DATA	0.000	0.000	0.000	0.000
TOLERANCE	0.015	0.015	0.015	0.015
RESULT	PASS	PASS	PASS	PASS

Wavelength = 405 nm

Accuracy Results:

WELLS	C1	D4	E2	F5	G3	H6
STANDARD	0.149	0.619	1.194	1.758	2.218	2.825
MIN. VALUE	0.126	0.587	1.150	1.703	2.109	2.692
MAX. VALUE	0.172	0.651	1.238	1.813	2.327	2.958
DATA	0.141	0.614	1.190	1.757	2.235	2.860
RESULT	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results:

WELLS	C1	D4	E2	F5	G3	H6
READ1	0.141	0.614	1.190	1.757	2.235	2.860
MIN. VALUE	0.135	0.603	1.173	1.734	2.163	2.769
MAX. VALUE	0.147	0.625	1.207	1.780	2.307	2.951
READ2	0.141	0.613	1.190	1.757	2.235	2.863
RESULT	PASS	PASS	PASS	PASS	PASS	PASS

Figure 4-3: *Sample printout showing the calibration plate analysis*

The Calibration Plate Analysis Report contains results for the following:

- **Alignment:** This portion of the test measures the alignment of the microplate carrier with the optical path. A reading > 0.015 represents an out-of-alignment condition. Wells A01, A12, H01, and H12 are the only valid alignment holes for the reader on the Part Number 7260522 calibration test plate.
- **Accuracy/Linearity:** Accuracy is a measure of the absorbance (optical density) of Calibration Test Plate wells C01, D04, E02, F05, G03, and H06 with known standard values contained in the Specification Sheet that accompanies each Calibration Test Plate. If the accuracy specifications are met, then the reader also proves to be linear.
- **Repeatability:** Repeatability is a measure of the instrument's ability to read the same well with minimum variation between two reads with the well in the same location.

Wavelength Accuracy

The C6 filter should be scanned between 580 and 590 nm in 1-nm increments using KC4 software. The wavelength of the maximum absorbance should be compared with the wavelength written on the sheet supplied with the test plate (see *Figure 4-4*). The accuracy of the wavelength should be ± 3 nm (± 2 nm instrument, ± 1 nm filter allowance).

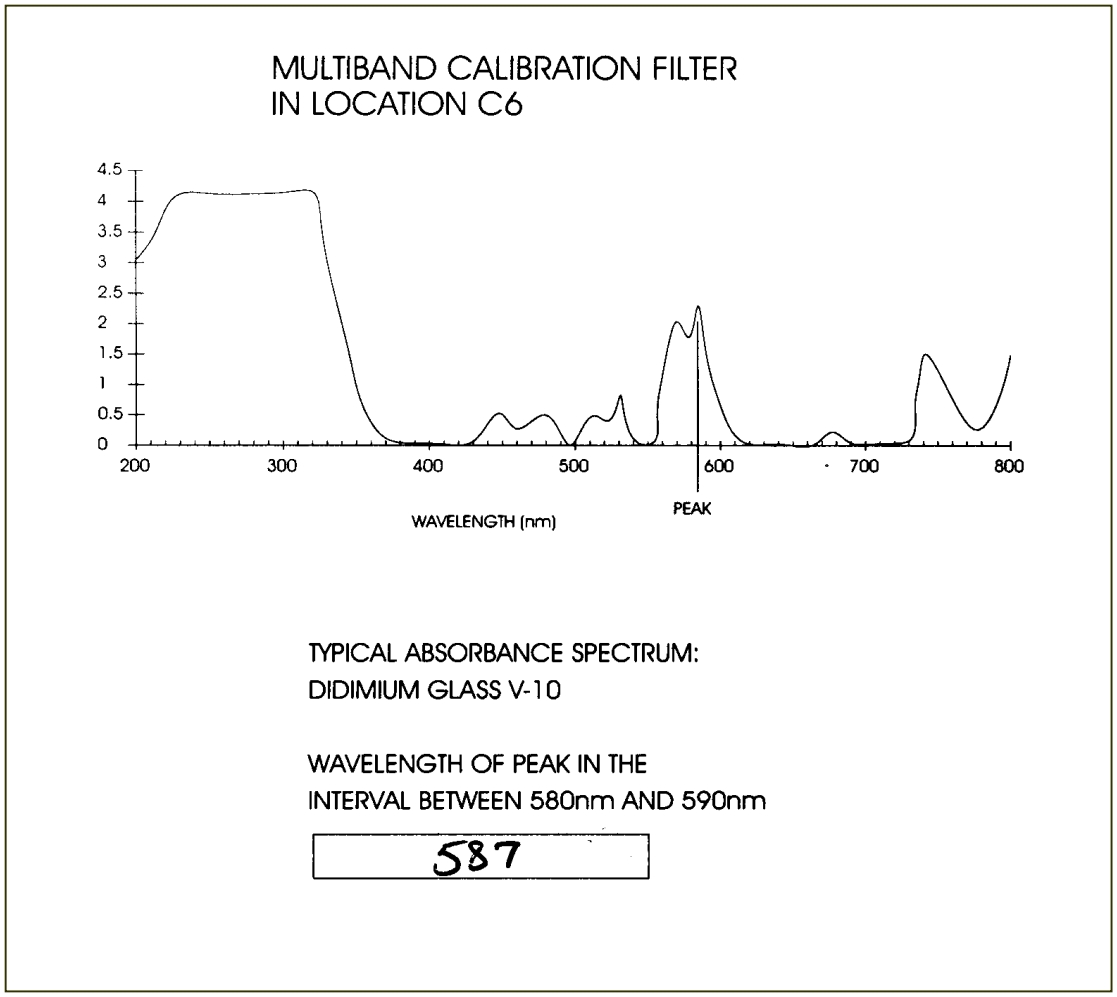


Figure 4-4: *Sample data sheet showing wavelength of peak in the interval between 580 and 590 nm*

Liquid Testing

Liquid testing tests the reader in ways that the Universal Test Plate cannot. The test plate will indicate the absolute amount of light absorbed, which will accurately test the linearity of the electronics. The liquid test will help detect optical defects such as dirt on the lenses or contamination that can contribute to errant readings.

- If you have the Universal Test Plate, you will only need to run the simple Liquid Test 1 for routine testing.
- If you do not have a Universal Test Plate, test the linearity, repeatability, and alignment of the reader by preparing a series of solutions of varying absorbances as described in Liquid Test 2.
- If you prefer, you may use the dye solution described in *Table 4-3*. The purpose of the formulation is to create a solution that absorbs light in a well-defined manner at ~ 2.0 OD full strength when dispensed at 200 µl in a flat-bottom microplate well.
- Alternatively, any solution that gives a stable color will suffice. (This includes substrates incubated with an enzyme preparation and then stopped with an acidic or basic solution.) Some enzyme/substrate combinations that may be used as alternates to the described dye are shown in *Table 4-2*.
- If you must test the reader's performance at 340 nm, run optional Liquid Test 3 (see *Table 4-5*).

Table 4-2
Typical Enzyme-Substrate Combinations and Stopping Solutions

Enzyme	Substrate	Stopping Solution
Alkaline Phosphate	o-nitrophenyl phosphate	3N sodium hydroxide
beta-Galactosidase	o-nitrophenyl -beta-D galactopyranoside	1M sodium carbonate
Peroxidase	2,2'-Azino di-ethylbenzothiazoline-sulfonic acid (ABTS)	citrate-phosphate buffer, pH 2.8
Peroxidase	o-phenylenediamine	0.03N sulfuric acid

Stock Solution Formulation

The stock solution for Liquid Tests No. 1 and No. 2 may be formulated from the chemicals listed below, or by diluting a dye solution available from Bio-Tek. See Procedure A or B outlined below for details.

Procedure A

Required materials:

- Deionized water
- FD&C Yellow No. 5 dye powder (typically 90% pure)
- Tween® 20 (Polyoxyethylenesorbitan Monolaurate, a wetting agent)
- Analytical balance
- 1-liter volumetric flask

Table 4-3
Stock Solution Formulation for Liquid Test Nos. 1 and 2

FD&C Yellow No. 5 powder	0.092 g
Tween® 20	0.5 ml
DI Water to bring volume to:	1000 ml

Preparation of stock solution:

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.
4. Make up to 1 liter with DI water; cap and shake well.
    - This should create a solution with an absorbance of about 2.0 when using 200 µl in a flat-bottom microwell. The OD value result will be proportional to the volume in the well and the amount of FD&C No. 5 dye used. You can use a larger or smaller well volume, or add more dye or water to adjust the solution. Note that too small a well volume may result in increased pipetting-related errors.

Procedure B

Required materials:

- Bio-Tek QC Check Solution No. 1 (P/N 7120779, 25 ml; or 7120782, 125 ml)
- Deionized water
- 5-ml Class A Volumetric Pipette
- 100-ml Volumetric Flask

Preparation of stock solution:

1. Pipette a 5-ml aliquot of Bio-Tek QC Check Solution No. 1 into a 100-ml volumetric flask.
2. Make up to 100 ml with DI water; cap and shake well.
   - This should create a solution with an absorbance of about 2.0 when using 200 µl in a flat-bottom microwell. The OD value result will be proportional to the volume in the well and the amount of QC Check Solution No. 1 used. You can use a larger or smaller well volume, or add more Check Solution or water to adjust the stock solution. Note that too small a well volume may result in increased pipetting-related errors.

Liquid Test 1

This procedure will test for repeatability and consistency, making evident any problems with the optics of the system.

1. Using a freshly prepared stock solution (see Procedure A or Procedure B on page 4-20), 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 will have an optical density of approximately 2.0 Abs. This value is not critical, but should be at the higher end of this absorbance range. Should it exceed the range, simply reduce the volume in the microwell. The diluted solution should have ODs of half of the concentrated solution.
2. Pipette 200 µl of the concentrated solution into the first column of wells of a new 96-well, flat-bottom microplate (Costar® #3590 is recommended). A new microplate is required for this test, as any scratches may cause variations in the turnaround reading.
3. Pipette 200 µl of the diluted solution into the second column of wells.
4. Read the microplate five times at 405 nm using normal mode, single wavelength, no blanking.
5. The plate data can be exported to an Excel spreadsheet using KC4. The mathematical computations described below may then be performed and the template kept for future data reduction.

Repeatability:

6. Calculate the mean and standard deviation for the five readings taken above at each concentration. Only one row of data needs to be analyzed for each concentration.
7. For each mean below 2.0 Abs, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 1.0% ± 0.005 Abs. If above 2.0 Abs, apply the ± 3% ± 0.005 specification.
8. The standard deviation for each set of readings should be less than the allowed deviation.

For example:

Absorbance 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% (1.951 * 0.010) = 0.0195, which, when added to the 0.005 (0.0195 + 0.005) = 0.0245 Abs, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specification:

For comparison in this test, the following repeatability specifications are applied, using Normal mode and a 96-well microplate.

± 1% ± 0.005 Abs from 0 to 2.000 Abs

± 3% ± 0.005 Abs from 2.000 Abs to 3.000 Abs

Liquid Test 2

The recommended method of testing the instrument performance is to use the Universal Test Plate to confirm alignment, repeatability, and accuracy, which will also confirm linearity.

If a Test Plate is not available, Liquid Test 2 can be utilized for these tests.

Preparation of Dilutions:

1. Set up a rack containing 10 tubes, numbered consecutively.
2. Prepare a concentrated stock test solution insert using either Procedure A or Procedure B on page 4-20.
3. Create a percentage dilution series, beginning with 100% of the original concentrated stock solution 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 last tube. Use Class A volumetric pipettes for better accuracy.
4. Dilute using amounts of the remaining 0.05% solution of deionized water and Tween 20, as shown in *Table 4-4***.**

Table 4-4
Test Tube Dilutions

Tube Number	1	2	3	4	5	6	7	8	9	10
Volume of Original 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 µl	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

Plate Preparation:

5. Pipette 200 µl of the concentrated solution from tube 1 into each well of the first column, A1 to H1, of a new flat-bottom microplate (Costar® #3590 is recommended). Next, pipette 200 µl from each of the remaining tubes into the wells of the corresponding column of the microplate (tube 2 into wells A2 to H2, etc.).

Note: The choice of dilutions and the absorbance of the original solution can be varied. Use *Table 4-4* as a model for calculating the expected absorbances of a series of dilutions, given a different absorbance of the original solution.

Linearity – Test A

1. Read the microplate prepared above using a normal mode dual wavelength at 450 nm with 630 nm as the blank. Repeat the read four times for a total of five reads.
2. The plate data can be exported to an Excel spreadsheet using KC4™. The mathematical computations described below may then be performed and the template kept for future data reduction.
3. Calculate the mean absorbance for each well, and average the means for each concentration.
4. Perform a regression analysis on the data to determine if there is adequate linearity.

   For example:

   In Microsoft® Excel, under Tools, select Data Analysis and then Regression. (Prior to opening the regression analysis tool, enter the expected results of the dilutions in a row of cells to use in the analysis.) Use the Regression "Input" box to enter the expected values as the "Input Y Range" and the mean absorbance for each concentration as the "Input X Range".

   Expected results:

   Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R Square value of at least 0.99 is considered adequate.

Repeatability – Test B

1. Calculate the mean and standard deviation for the five readings taken above at each concentration. Only one row of data needs to be analyzed.
2. For each mean below 2.0 Abs, calculate the allowed deviation using the repeatability specification for a 96-well format of ±1.0% ± 0.005 Abs. If above 2.0 Abs, apply the ±3% ± 0.005 specification.
3. The standard deviation for each set of readings should be less than the allowed deviation.

   For example:

   Absorbance 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% (1.951 * 0.010) = 0.0195, which, when added to the 0.005 (0.0195 + 0.005) = 0.0245 Abs, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specification:

±1% ± 0.005 Abs from 0 to 2.000 Abs

±3% ± 0.005 Abs from 2.000 Abs to 3.000 Abs

Alignment – Test C

1. Using the plate prepared for Test A above, conduct a turnaround test by reading the plate with the A1 well in the H12 position five times. This test results in values for the four corner wells that can be used to determine alignment.
2. Calculate the means of the wells A1 and H1 in the normal plate position (data is from Test A) and in the turnaround position (from Step 1 above). 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 the instrument.

   For example:

   If the mean of well A1 in the normal position is 1.902, where the specified accuracy is
   ± 1% ± 0.010 Abs, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 Abs. (1.902 * 1% = 0.019 + 0.010 = 0.029, which is added and subtracted from 1.902 for the range.)
3. If the four corner wells are within the accuracy range, the reader is in alignment.

Liquid Test 3

To verify operation of the *Synergy HT* at 340 nm, perform the Liquid Test 3 Procedure described on page 4-20.

Required Materials:

- Deionized Water
- Pipettes
- Costar® #3590 Flat-Bottom Microplate
- Beakers and Graduated Cylinder
- Analytical balance
- Tween® 20 (Polyoxyethylenesorbitan Monolaurate)
- Phosphate-Buffered Saline with Tween 20 (PBS Buffer Solution). Use Sigma® P 3563 packets, which will be adequate for one liter of PBS solution each (Procedure B), or prepare a 10x concentrate per *Table 4-5* (Procedure A).
- β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma® bulk catalog number N 8129, or pre-weighed 10-mg vials, Sigma number 340-110.

Stock Buffer Solution Formulation:

Table 4-5
Phosphate-Buffered Saline 10X Concentrate Solution

KH_2PO_4 anhydrous	0.2 grams
NaCl	8.0 grams
Na_2HPO4 anhydrous	1.15 grams
KCl	0.2 grams
Tween® 20	0.5 ml
Add Deionized water to bring to	100 ml

Procedure A:

Mix 45 ml of deionized water with 5 ml of the concentrated PBS solution (from *Table 4-5*) in a beaker. Add 10 mg of the β-NADH powder and mix thoroughly. This is the high-level test solution.

Procedure B:

Add 50 ml of a PBS solution (prepared from the Sigma powder) to a beaker. Add 10 mg of the β-NADH powder and mix thoroughly. This is the alternate high-level test solution.

Liquid Test 3 Procedure:

1. Check the absorbance of a sample of either high-level test solution created in Procedure A or B at 340 nm on the microplate reader. This solution will have an optical density (absorbance) of approximately 0.7 to 1.0. This value is not critical, but it should be within this absorbance range. Adjust up by adding β-NADH powder, if low, until the high-level test solution is at least at the lower end of this range. Do not adjust if slightly high.
2. Carefully prepare a mid-level test solution by diluting 15 ml of the high-level test solution with 5 ml of the Sigma PBS solution. (If using the 10X-concentrate PBS solution, you should mix one part of the concentrate with nine parts of deionized water to obtain a low-level buffer similar to the Sigma PBS. Then, use 5 ml of this solution as the diluent.) This will be the mid-level solution.
3. Carefully prepare a low-level test solution by diluting 10 ml of the high-level test solution with 10 ml of the Sigma PBS solution. (If using the 10X-concentrate PBS solution, you should mix one part of the concentrate with nine parts of deionized water to obtain a low-level buffer similar to the Sigma PBS. Then use 10 ml of this solution as the diluent.) This will be the low-level solution.
4. Pipette 150 µl of the concentrated solution into each well of the first two columns, A1 to H1 and A2 to H2, of a flat-bottom microplate (Costar® #3590 is recommended). Next, pipette 150 µl from the mid-level solution into the wells of columns 3 and 4 of the microplate. Finally, pipette 150 µl of the low-level solution into the wells of column 5 and 6 of the microplate.
5. Read the microplate using Normal mode, single wavelength at 340 nm, no blanking (or blank on air). Repeat the read four times for a total of five reads.

Repeatability – Test A

1. The plate data can be exported to an Excel spreadsheet using KC4. The mathematical computations described below may then be performed and the template kept for future data reduction.
2. Calculate the mean and standard deviation for the five readings of each well.
3. For each mean, calculate the allowed deviation using the repeatability specification for a 96-well format of ±1.0% ± 0.005 Abs.
4. The standard deviation for each set of readings should be less than the allowed deviation.

   For example:

   Absorbance readings of 0.802, 0.802, 0.799, 0.798, and 0.801 will result in a mean of 0.8004, and a standard deviation of 0.0018. The mean (0.8004) multiplied by 1% (0.8004 * 0.010) = 0.008, which, when added to the 0.005 (0.008 + 0.005) = 0.013, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Linearity – Test B

1. Obtain an average mean for each concentration by averaging the mean values for each well that were obtained above.
2. Perform a regression analysis on the data to determine if there is adequate linearity.

   For example:

   In Microsoft® Excel, under Tools, select Data Analysis and then Regression. (Prior to opening the regression analysis tool, enter the expected results of the dilutions in a row of cells to use in the analysis.) Use the Regression "Input" box to enter the expected values as the "Input Y Range" and the mean absorbance for each concentration as the "Input X Range".

   Expected results:

   Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R Square value of at least 0.99 is considered adequate.

Fluorescence Testing

Routine Procedure

The System and Checksum tests are performed automatically whenever the instrument is turned on. They can also be performed manually through KC4 using the Optics Test feature.

The Corners and the Sensitivity tests are performed on every *Synergy HT* before it leaves the factory. These tests are critical for verifying instrument performance.

System Test

The System Test begins with a check of the stepper motors and the analog power supplies, to ensure that they have a proper input voltage level. The data flash checksum, motor axis, and analog offset are then verified. PMT dark current, noise, and gain are checked to ensure they fall within specific pass/fail criteria. If equipped, incubation zones are measured and reported.

Checksum Test

The Checksum Test compares the onboard software with internally recorded checksum values to ensure that no corruption has occurred. If this test is run manually, part number and version information is displayed for the software currently loaded on the instrument. This information is useful when contacting Bio-Tek for technical assistance.

If there is a failure, the instrument "beeps." Press the carrier-eject button and run an Optics Test from KC4. See *Appendix C, Error Codes* for more information.

To obtain a report of the System Test values for either periodic testing documentation or troubleshooting (Figure 4-1), follow the instructions in KC4. The instrument's System Test will be conducted and the results reported in a pass/fail format. This report will include results of both absorbance and fluorescence tests.

See Appendix C for a list of possible error codes.

Corners Test

The Corners Test uses fluorescent compounds to verify that the plate carrier is properly aligned in relation to the optical top and bottom probes. Consider running the test twice, once with each probe.

¡¡¡	**Important:** ➢ The *Synergy HT* is specially designed to accommodate plates and PCR tubes with heights up to 31.75 mm. ➢ To effectively read 96-well microplates using the **top** probe, a plate carrier adapter is required (Bio-Tek part number 7080522) to raise the microplate and bring it closer to the probe. Microplates with heights up to 20.3 mm (absorbance mode) and 31.75 mm (fluorescence mode) can be read using this adapter.

Sensitivity Test

The Sensitivity Test uses fluorescent compounds to verify that the difference between the means of wells with known lower limits of concentration of the substance under investigation is statistically distinguishable from the mean of wells with pure diluent.

The following pages describe the procedures used by the factory for performing the Corners and Sensitivity Tests. See page 1-7 for specifications.

Required Materials

- Sodium Fluorescein Powder (1 mg vial, Bio-Tek part no. 98155)
- Phosphate-Buffered Saline (PBS) (1 packet, Sigma catalog no. 1000-3, or equivalent)
- Hellma Quartz 96-well titration plate (catalogue number 730.009.QS, or equivalent)
- Deionized water
- 100% methanol
- Disposable glass 16 x 100 mm test tubes or equivalent
- Various pipettes
- pH meter or pH indicator strips with pH range 4-10
- 0.45 micron filter
- Aluminum foil
- Black polyethylene bag(s) to temporarily store plate(s)
- Excitation filter 485/20 mm
- Emission filter 530/25 mm

Solution Preparation

PBS Solution

Note: Discard the unused PBS solution after seven days.

1. Using a 0.45-micron filter, filter 1 liter of deionized water.
2. Dissolve the contents of 1 packet PBS into the filtered deionized water.
3. Place on a stir table until the powder is completely dissolved.
4. Check the pH with either a pH meter or with pH indicator strips. The pH should be between 7.2 and 7.6 at 25°C.

Sodium Fluorescein Stock Solution for the (SF) Serial Dilution Test

Note: Wrap the vial containing the stock solution in aluminum foil to prevent exposure to light. Discard the unused stock solution after seven days.

1. Add 2.0 ml of the PBS solution to the 1 mg Sodium Fluorescein (SF) vial. This yields a 500 µg/ml stock solution.
2. Ensure that dye has completely dissolved and is well mixed.

Titration Dyes for the (SF) Serial Dilutions Test:

1. Add 0.2 ml of the 500 µg/ml stock solution to 2.3 ml of the PBS solution. This yields a 40 µg/ml solution.
2. Mix the solution well.
3. Dilute the 40 µg/ml solution as follows (note that dilutions D and E are made from the same solution):

   A. 1 ml of 40 µg/ml into 9 ml of PBS = 4000 ng/ml

   B. 1 ml of 4000 ng/ml into 9 ml of PBS = 400 ng/ml

   C. 1 ml of 400 ng/ml into 9 ml of PBS = 40 ng/ml

   D. 0.25 ml of 40 ng/ml into 7.75 ml of PBS = 1.25 ng/ml
   (for Corners Test)

   E. 1 ml of 40 ng/ml into 11.5 ml of PBS = 3.2 ng/ml

   F. 1 ml of 3.2 ng/ml into 9 ml of PBS = 0.32 ng/ml
   (for Sensitivity Test)

Corners Test: Procedure

1. Ensure that the *Synergy HT* has the following filters installed:

   Excitation Filter: 485/20 nm

   Emission Filter: 530/25 nm

2. Create a *Synergy HT* assay with one filterset containing the following parameters:

   Excitation Filter: 485/20 nm

   Emission Filter: 530/25 nm

   Probe Orientation: Bottom

   Sensitivity: 80

3. Prepare the Hellma 96-well quartz plate as indicated below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	SF_D	SF_D	SF_D	PBS					PBS	SF_D	SF_D	SF_D
B	PBS	PBS	PBS	PBS					PBS	PBS	PBS	PBS
C												
D												
E												
F												
G	PBS	PBS	PBS	PBS					PBS	PBS	PBS	PBS
H	SF_D	SF_D	SF_D	PBS					PBS	SF_D	SF_D	SF_D

- Pipette 150 µl of the indicated solution per well
- SF_D = Solution "D" (1.25 ng/ml)
- PBS = PBS Solution
- All other wells should remain empty

Important:

- Store the plate in a black polyethylene bag until use.
- If the base of the plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Prior to placing the plate in the instrument, blow the bottom of the plate with an aerosol duster.

4. Read the plate.
5. Analyze results according to the instructions on page 4-28.

Sensitivity Test: Procedure

1. Ensure that the *Synergy HT* has the following filters installed:

   Excitation Filter: 485/20 nm

   Emission Filter: 528/20 nm

2. Create a *Synergy HT* assay with one filterset containing the following parameters:

   Excitation Filter: 485/20 nm

   Emission Filter: 528/20 nm

   Probe Orientation: Bottom

   Sensitivity: 100

3. Put 2 ml of PBS into a fresh, disposable 16 x 100 mm test tube.

4. Serially dilute the 0.32 ng/ml SF solution (solution "F" from page 4-24) as follows:

   Note: Mix each dilution in a fresh, disposable 16 x 100 mm test tube.

   A. 2 ml of 320 pg/ml into 2 ml of PBS = 160 pg/ml

   B. 2 ml of 160 pg/ml into 2 ml of PBS = 80 pg/ml

   C. 2 ml of 80 pg/ml into 2 ml of PBS = 40 pg/ml

   D. 2 ml of 40 pg/ml into 2 ml of PBS = 20 pg/ml

   E. 2 ml of 20 pg/ml into 2 ml of PBS = 10 pg/ml

   F. 2 ml of 10 pg/ml into 2 ml of PBS = 5 pg/ml

   G. 2 ml of 5 pg/ml into 2 ml of PBS = 2.5 pg/ml

   H. 2 ml of 2.5 pg/ml into 2 ml of PBS = 1.25 pg/ml

   I. 2 ml of 1.25 pg/ml into 2 ml of PBS = 0.625 pg/ml

   J. 2 ml of 0.625 pg/ml into 2 ml of PBS = 0.31 pg/ml

5. Using the prepared solutions (A through J), prepare the 96-well Hellma quartz titration plate as indicated below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
B	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
C	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
D	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
E	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
F	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
G	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS
H	160	80.0	40.0	20.0	10.0	5.00	2.50	1.25	0.62	0.31	PBS	PBS

- Pipette 150 µl of the indicated solution per well
- xxx = Sodium Fluorescein dilution (xxx ng/ml), A through J
- PBS = PBS Solution

Important:

- Store the plate in a black polyethylene bag until use.
- If the base of the plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Prior to placing the plate in the instrument, blow the bottom of the plate with an aerosol duster.

6. Read the plate.
7. Analyze results according to the instructions on page 4-28.

Analysis of Test Results

Corners Test

1. Calculate the mean of wells A1, A2, A3, A10, A11, A12, H1, H2, H3, H10, H11, H12.
2. Calculate the standard deviation of the same wells.
3. Calculate the % CV as **((standard deviation / mean) * 100)**.
4. If the % CV is **less than 3.0**, the test passes.

   A sample Corners Test data sheet is shown on the next page.

Sensitivity Test

The pass/fail criteria are based on the specifications provided on page 4-29.

1. Calculate the mean of the PBS wells (A11 through H12).
2. Calculate the standard deviation of the PBS wells.
3. For each set of 8 dilution wells (columns 1 through 10):

   a. Calculate the mean.

   b. Subtract the mean PBS.

   c. Calculate the standard deviation.

   d. Calculate the total standard deviation:

      SQRT(((standard deviation)^2)+((standard deviation of the PBS wells)^2))

   e. Calculate the signal to noise ratio:

      Divide the results from step b by the **total standard deviation**.

4. For concentrations of 10 pg/ml or greater, the signal to noise ratio must be **greater than 2** to pass (meet the specification).

   A sample Sodium Fluorescein Serial Dilution (i.e., Sensitivity) Test data sheet is shown on the next page.

Synergy HT Corners Test
Pass

Specification: **% Coefficient of Variation < 3**
Test Results: **Pass**

Average: 42363.33
STD Deviation: 13.03
% CV: 0.03

Synergy HT Sodium Fluorescein Serial Dilution Test
Pass

Specification: **Signal/Noise Ratio 2.00 or Greater, down to 10 pg/ml**
Test Results: **Pass**

Conc. (pg/ml)	Mean	Mean-PBS	Standard Deviation	Total STD	Signal/ Noise Ratio	Pass/ Fail	Conc. (pg/ml)	
160	13556.63	9226.63	56.68	91.32	101.03	PASS	160	
80	8919.25	4589.25	91.50	116.19	39.50	PASS	80	
40	6616.88	2286.88	54.67	90.09	25.38	PASS	40	
20	5458.38	1128.38	63.43	95.66	11.80	PASS	20	
10	4904.63	574.63	59.33	92.99	6.18	PASS	10	Specification
5	4653.13	323.13	92.75	117.17	2.76	PASS	5	
2.5	4493.75	163.75	112.53	133.38	1.23	N/A	2.5	
1.25	4449.88	119.88	147.76	164.20	0.73	N/A	1.25	
0.625	4372.88	42.88	82.80	109.47	0.39	N/A	0.625	
0.31	4364.75	34.75	103.95	126.23	0.28	N/A	0.31	
PBS	4330.00	0.00	71.60				PBS	

Figure 4-5: *Sample factory data sheets for the Corners and Sensitivity Tests*

Appendix A
Decontamination

This appendix contains the procedure for decontaminating the Synergy HT.

Decontamination Procedure

If the *Synergy HT* is to be shipped after being exposed to potentially hazardous material, it should be **decontaminated.** The following procedure outlines how to decontaminate the instrument before packaging and shipment.

Purpose

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

General Considerations

- Any laboratory instrument that has been used for clinical analysis is considered a biohazard and should be decontaminated prior to handling. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Prophylactic gloves must be worn when handling instruments that have not been decontaminated. Gloved hands should be considered contaminated at all times and must be kept away from eyes, mouth and nose at all times.
- Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosols.
- Eating and drinking while decontaminating instruments is not advisable**.**

Procedure

Warning! The bleach solution is caustic; wear gloves and eye protection when handling the solution. Do not soak the instrument keypad – this will cause damage. Wipe the keypad with a damp cloth.

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.5% Sodium Hypochlorite (NaClO, or bleach).
   - Be sure to check the % NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, use a 20:1 mixture. Household bleach is typically 5% NaClO; if this is the case use a 10:1 mixture.
3. Wipe down the carrier and all exposed surfaces of the unit with the bleach solution.
4. Discard the used gloves and towels.

Appendix B
Computer Control

The Synergy HT may only be controlled by a computer connected to the reader via the computer's serial port. This section describes the features of computer control, and explains the information necessary to program the computer to control the reader.

Overview

This appendix details the protocols necessary for communicating with the *Synergy HT* reader.

Controlling the Reader with KC4™ *for Windows*

The *Synergy HT* can be operated using a computer running KC4 software. Follow the steps below:

1. Power off the computer and the reader. Connect the appropriate serial cable between the two machines. A Null is required to connect the reader to most computers. If a 25-pin (female) to 9-pin connector is used, the Null is built into the cable. Bio-Tek's serial cable (PN 75053) may be used with no additional Null.
2. Power up both machines.
3. Install KC4 on the computer's hard drive.
4. Once installed, start KC4.
5. Select **System**, then **Readers**.
6. Highlight the desired reader model:
    - Synergy HT
    - Synergy HT-I
    - Synergy HTTR
    - Synergy HTTR-I
7. Click **Port** and choose a **COM Port**.

8. Click **Setup**. To define the communications parameters, choose the following setup parameters:

   Transmission speed: 9600

   Data Bits: 8

   Parity: No

   Stop Bits: 2

9. Click **OK**, **OK**, **Current Reader**. KC4 will attempt to communicate with the reader. If no error messages appear, click **close** to return to KC4's main view.

Refer to the KC4 User's Guide for instructions for setting up an assay, reading a plate, etc.

Problems

- If KC4 fails to run and displays a serial communications error, check the cable plug-in location to make sure it matches the setup choices and is not a Null cable. If this is suspected, add another Null and try again.
- If the system test fails and the instrument beeps upon startup, press the carrier-eject button and run an Optics Test from KC4. To run an Optics Test from KC4, click **System|Diagnostics**, and then run **Optics Test**.

Controlling the Reader Using Serial Protocol

At baud rates of 9600, 19200, and 38400, the *Synergy HT* is capable of sending and receiving data through its serial port (RS-232C). The baud rate used for transmission is held in nonvolatile memory and can be changed by the user. Other serial port parameters, No Parity, 8 Data Bits, and 2 Stop Bits are fixed and cannot be changed.

The *Synergy HT's* RS-232C serial port is configured as a DTE (see the section *Serial Port for Communications* in *Chapter 3, page 3-5*); that is, the unit is wired to resemble a modem. Data is received on Pin 3 (the RX Pin) and transmitted on Pin 2 (the TX pin).

Table B-1

ASCII Control Characters Used in Computer Control Protocol

ASCII Code	Function	Hex Code	Decimal Code	Control Code	Reader <-- >
ACK	Acknowledge	06	06	^F	--->
NAK	Negative acknowledge	15	21	^U	--->
RS	Record separator	1E	30	^^	--->
ETX	End of text	03	03	^C	<-->
DLE	Data link escape	10	16	^P	--->
CR	Carriage return	0D	13	^M	<---
LF	Line feed	0A	10	^J	<---
CTRL-Z	Control Z	1A	26	^Z	<---

Computer Control Commands

A command from the computer to the reader consists of a single ASCII character, and in many cases, subsequent argument data. Upon receipt of a valid command character, the reader returns an <ACK> character, except in certain special cases. Some commands also return response data to the host computer. Upon completion of command processing, the *Synergy HT* transmits a status string to the computer.

While awaiting a command, the *Synergy HT* responds to nulls or other unexpected characters by clearing its input buffer and transmitting a <NAK>. Therefore, if valid commands are preceded by invalid characters, they may be missed.

Refer to ***Table B-1*** for the **ASCII Control Characters** used in the computer control protocol.

All ASCII character strings representing numbers or names are transmitted most significant digit or letter first. Unless indicated, ASCII strings do not include a null terminator. Data values not indicated as ASCII are treated as binary integers, and are transmitted least significant byte first.

Absorbance Commands

The following section describes computer control commands specific to absorbance.

SET READ MODE (‘k’)

This command selects the read mode controlling the speed, accuracy, and precision of all subsequent absorbance plate reads.

host:		valid limits:	response:
‘k’			<ACK>
data bytes:	mode		
		‘0’: slow (enhanced) mode	
		‘1’: fast mode	
		‘2’: sweep mode	
			status string (5)

Area scanning and wellset reads are performed in standard fast mode if sweep mode is selected.

CONFIGURE ABSORBANCE WAVELENGTHS (‘M’)

This command downloads the wavelength setup table for use in future absorbance read cycles, and then calibrates the reader for those wavelengths.

host:	valid limits:	response:
‘M’		<ACK/NAK>
data bytes:		
1 - 3	1st wavelength (“200” - “999”)	
4	comma separator (‘,’)	
5 - 24	remaining wavelengths, commas	
<ETX>		
		status string (5)

Each wavelength is represented by “xxx,” (3 ASCII digits, followed by a comma). Six wavelengths must be sent (24 bytes), followed by ETX after the last comma. If a wavelength is not to be specified, it must be replaced by “000”. The absorbance wavelength setup table is stored in non-volatile memory, and only needs updating when a change in current absorbance wavelength configuration is desired.

After wavelength configuration using this command, the reader automatically runs a sequence to calibrate gains and generate self-check information for the new wavelength configuration.

It is not absolutely necessary to configure and calibrate wavelengths for use, but performance may be compromised slightly if this is not done. The wavelengths stored are automatically calibrated during the instrument power-up self-test. The Self-Test command ('*') can also be used to accomplish this.

SET STATUS FORMAT ('n')

This command selects the format for the status string (see **Status String Format**).

host:	**valid limits:**	**response:**
'n'		<ACK>
data bytes:	format	
	'0': 312 status mode	
	'1': ELx status mode	
		status string (5)

When the status format is set to **ELx status mode**, any error detected during the execution of any command described in this document will be transmitted to the host PC in this format; in addition, absorbance plate read and scan ('c', 'd', 'S', and '&') commands will return an additional status string at the very end of the read sequence. See **Status String Format** for error descriptions, and for restrictions and notes on the use of the **ELx status format**.

READ ABSORBANCE PLATE ('S')

This command causes a microplate to be read according to the currently loaded absorbance assay definition table, downloaded using the Set Absorbance Assay Definition ('V') command. This is the same command as the Read Fluorescence/Luminescence Plate command; an 'S' command issued when an absorbance definition is in place requires a different response protocol to meet the needs of that particular assay type.

host:	**valid limits:**	**response:**
'S'	no arguments	<ACK/NAK>

reader response protocol:

status string (5)		
for each kinetic interval (one if endpoint read):		
for each wavelength expected:	**(312 format: one, ELx format: up to six)**	
wavelength start code (1)		<CR>
timestamp (11)	**** (if specified)**	"hhh:mm:ss.s"
for each row within specified row range:		
for each column (well) within specified column range:		
comma separator (1)		','
sign (1)		'+' or '-'
data (4)		"1234"
row terminator (2)		<CR>, <LF>
temperature (3)	*****(if specified)**	"000" – "999"
assay name (8)	**(312 format only)**	"ABCDEF", <CR>, <LF>
unused extra characters (49)	**(312 format only)**	
wavelength terminator (1)		<^Z>
checksum (1)	**(ELx format only)**	0 – 255
final terminator (1)	**(ELx format only)**	<^Z>
final status string (5)	**(ELx status mode only)**	

** Timestamp included only if requested. See the Include Timestamp command for description. The timestamp indicates the time elapsed between the very first read and the current read for any given well on the microplate in a kinetic read series.

*** Temperature response included only if requested. Format is three ASCII bytes scaled up by ten; i.e., "275"corresponds to 27.5ºC. See the Include Temperature Response command.

Absorbance data is returned in the form of a 5-character ASCII string, with a sign character followed by four digits. A decimal point is not included, but should be inserted by the user after the first digit; i.e., "+1234" should be translated as +1.234 OD. Overrange readings are indicated by the 5-character string "*****" instead of a signed numerical value.

If dual-wavelength subtraction has been selected in the reader definition table, the subtraction is performed by the reader and the result is sent as a single plate representing the difference in ODs per well. Otherwise a complete plate of ODs is sent for each wavelength selected.

If an error is detected during a plate read, or the user aborts the process via serial 'X', 'x', or stop key press, the entire (remaining) data response is replaced by a single <DLE> character (0x10). In the **ELx status mode**, the final status string then indicates the error detected. In the case of reader error, data previously received during the current read sequence should be discarded.

312 Format:

This format is selected by default at reader power-up, and every time a new assay definition is loaded using the Set Absorbance Assay Definition ('V') command. The reader always returns data as an 8 x 12 matrix, regardless of the selected plate geometry, along with the assay name and extra characters. There are no checksums or final terminator.

If geometries other than 8 x 12 have been selected using the Set Plate Geometry ('{') command, the data is upper left justified in the 8 x 12 matrix, and wells outside plate boundaries are marked as invalid. That is, for a 4 x 6 plate, the first six ODs received are valid data, followed by six more marked as invalid ("*****") to fill out the first row of 12. The next three rows also start with six wells' worth of valid data. In the last four rows returned, all wells are marked as invalid.

If a 384-well plate geometry has been selected, data is returned as a series of four 8 x 12 quadrants. Each quadrant is presented as a single 8 x 12 plate in the 312-plate format. Quadrants are returned in the following sequence: A1 - H12, A13 - H24, I1 - P12, I13 - P24.

ELx Format:

If any of the following occurs, the **ELx format** is selected and remains enabled until a new assay definition is loaded:

1. Multi-wavelength mode is selected in the assay definition.
2. A range of rows is set, even if for all rows (see Set Row Range 'r' command).
3. A range of columns is set, even if for all columns (see Set Column Range '%' command).

The reader returns data in a matrix corresponding to the rows and columns selected, for up to six wavelengths. If dual-wavelength subtraction is selected, only one plate will be returned (not available in the multi-wavelength mode).

Checksum calculation starts with the first byte AFTER the wavelength start code, up through and including the <^Z> data terminator code at the end of each wavelength's plate data. The checksum is transmitted as an integer data byte (not ASCII).

The final terminator indicates that the data stream is complete for the corresponding read command. No more data will be transmitted until another command is sent.

Data Handshaking:

If data handshaking has been turned on via the Set Data Handshaking Mode ('N') command, the host is expected to respond after the data for each wavelength has been received. If the host does not receive all the data bytes expected for the wavelength, or the checksum received is incorrect, the host can indicate this to the reader by sending a <NAK>. The reader will respond by re-sending all data for the wavelength, including any optional timestamps or temperatures, from the wavelength start code (<CR>) through the last or only checksum byte. This can be repeated as many times as necessary until the reader receives an <ACK> for the data. If the reader has not received an <ACK> by the time it is ready to send data for the next wavelength or kinetic interval, or ready to send the final terminator at the very end of an endpoint read or kinetic series, the reader will flag an error and terminate the read sequence.

During periods while the reader is expecting an <ACK> response to data sent, the host may also send either abort ('X' or 'x') command, which will cause the reader to terminate the current sequence as defined for those two commands, with no additional results data transmitted.

Data handshaking is only used with the **ELx format**.

312 Status Format:

If the middle character in the status string is not ASCII '0', an error has been detected and no results data is transmitted.

ELx Status Format:

If any character in the initial status string is not ASCII '0', an error has been detected and no results data is transmitted. Otherwise, if an error is detected during the read process, the final status string reflects that error.

See **Status String Format** for error descriptions.

SET ABSORBANCE ASSAY DEFINITION ('V')

This command downloads to the reader an absorbance assay definition table to be used in subsequent plate reads, area scans, or wellset reads ('S', 'c', or 'd' commands) until a new definition replaces it. Only one assay – either absorbance or fluorescence/luminescence – can be in place, so this command will cause any assay definition currently loaded to be replaced. When an absorbance assay definition is in place, response data is always returned in the **312 data mode**.

host:		**valid limits:**	**response:**
'V'			<ACK/NAK>
data bytes:			
1		don't care	
2 - 7		assay name (ASCII characters)	
8		1: offset corners for alignment test, 0: no offset	
9		position in read series (endpoint only)	
		'0': single read	
		'1': first read in series	
		'2': next read in series	
		'3': final read in series	
10 - 29		don't care	
30		subtraction (0x00: none, 0x40: dual wavelength)	
31		encoded byte:	
	bits 0 - 3 (0x0F)	0	
	bit 4 (0x10)	1: shake, 0: no shake	
	bit 5 (0x20)	1: shake before every read, 0: before first read only	
	bit 6 (0x40)	1: continuous shake, 0: timed shake	
	bit 7 (0x80)	1: use total kinetic time, 0: use kinetic read count	
32		1D scanning points (1 – 31, must be odd)	
33 - 49		don't care	
50 – 52		measurement wavelength ("000", "200" - "999")	
53 - 55		reference wavelength ("000", "200" - "999")	
56		read type (0: endpoint, 2: kinetic, 3: 1D scan)	
57 - 59		don't care	
60 - 61		kinetic interval (seconds, 0 - 9999)	
62 - 63		kinetic read count (2 - 9999)	
64 - 66		don't care	
67 - 69		first wavelength ("000", "200" - "999")	
70 - 84		remaining wavelengths ("000", "200" - "999")	
85 - 162		don't care	
163 - 164		shake time (seconds, 0 - 999)	

host:	valid limits:	response:
165	shake speed (0: slow, 1: med, 2: fast, 3: variable)	
166 - 167	total kinetic read time (minutes, 1 - 9999)	
168	delay before first read (0: no, 1: yes)	
169 - 170	delay (seconds, 0 - 999)	
		status string (5)

After the assay definition is received, kinetic or endpoint plate reads may be initiated using the Read Absorbance Plate ('S') command. Area scans are initiated using the Run Area Scan ('c') command, which includes additional information concerning the region for each scanning pass across the plate. Wellset reads are initiated using the Read Well Set ('d') command, which selects individual wells instead of an entire plate.

The multi-wavelength mode returns a plate of data for each wavelength specified. To select the multi-wavelength mode, the "measurement wavelength" must be set to "000", and wavelengths specified starting with "first wavelength". Up to six wavelengths may be specified in this mode. However, if dual-wavelength subtraction is desired, the measurement and reference wavelengths must be specified. A single-wavelength read may use either mode.

If a wavelength is not to be used, it must be replaced by "000".

If the multi-wavelength mode is selected, the **ELx format** for data response will be used until a new assay definition table is specified.

A kinetic interval of zero will cause the plate to be read repeatedly with no pauses between reads.

Continuous shaking is available in the kinetic read mode by selecting a shake for every read and setting the shake time to zero. Shaking will then be performed using any extra time available in the selected kinetic interval while a plate read is not in progress.

If the incubator is enabled and on (setpoint > 0), incubator error trapping is available for running assays where accurate thermal control is critical. Once the trap is set at the beginning of a plate read (or wellset read or area scan), any event that may disrupt accurate incubation is recorded. Such events may include an incubator zone wandering out of range (± 2.0°C from setpoint) or a temperature sensor failure. Trapping is automatically turned off upon completion of the entire read process. Any errors detected are encoded into the final status string, as described in the **Status String Format** section.

The assay definition table must be downloaded before other plate-specific commands are sent, such as Set Plate Geometry('{'), Set Row Range ('r'), and Set Column Range ('%').

To minimize confusion, all "don't cares" should be set to 0.

GET ABSORBANCE WAVELENGTH TABLE ('W')

This command uploads the wavelength setup table from the reader.

host:	**valid limits:**	**response:**
'W'	no arguments	<ACK/NAK>
reader response protocol:		
for each wavelength configured (six):		
wavelength		("000 - "999")
comma terminator		','
status string (5)		

SET PLATE GEOMETRY ('{')

This command selects a microplate geometry to be used with the currently loaded assay definition.

host:	**valid limits:**	**response:**
'{'		<ACK/NAK>
data bytes:		
1	plate type (geometry, 0 – 11)	
	0: 24 wells (4x6)	
	1: 48 wells (8x12)	
	2: 96 wells (8x12)	
	3: 384 wells (16x24)	
	4: 72 well Terasaki (6x12)	
	5: 60 well Terasaki (6x10)	
	6: 96 well Terasaki (8x12)	
	7: 96 well Hellma (8x12)	
	8: 6 well (2x3)	
	9: 12 well (3x4)	
	10: 96 well Metric (8x12)	
	11: previously defined custom geometry	
		status string (5)

If no Set Plate Geometry ('{') command is sent after the assay definition table, the default 96 well plate is assumed. If only a range of wells is desired, the Set Row Range ('r') and/or Set Column Range ('%') commands must be sent AFTER the Set Plate Geometry ('{') command.

This command should be sent to the reader BEFORE a spectral scan or area scan is requested.

NOTE - This command is not intended for use with fluorescence/luminescence assays, since the Set Fluorescence/Luminescence Assay Definition (‘v’) command contains its own geometry parameter. However, using this command after sending a ‘v’ command will cause the defined geometry to be overwritten with the new geometry.

SET ROW RANGE (‘r’)

This command selects a range of adjacent rows (lettered on plate or strip carrier) to be read.

host:	**valid limits:**	**response:**
‘%’		<ACK/NAK>
data bytes:		
1	first row (1 - 16)	
2	last row (1 - 16)	
		status string (5)

By default the instrument is initialized to perform full plate reads, and automatically reverts to this default when a new assay definition table is loaded. The well range is also reset when the Set Plate Geometry (‘{’) command is sent.

Once this command is invoked, the reader will use the **ELx format** for data response until a new assay definition table is specified.

This command has no effect on the read parameters for a wellset read, area scan, or spectral scan.

NOTE - This command is not intended for use with fluorescence/luminescence assays, since the Set Fluorescence/Luminescence Assay Definition (‘v’) command contains its own row range parameters. However, using this command after sending a ‘v’ command will cause the defined row range to be overwritten with the new row range.

SET COLUMN RANGE (‘%’)

This command selects a range of adjacent columns (numbered on plate or strip carrier) to be read.

host:	**valid limits:**	**response:**
‘%’		<ACK/NAK>
data bytes:		
1	first column (1 - 24)	
2	last column (1 - 24)	
		status string (5)

By default the instrument is initialized to perform full plate reads, and automatically reverts to this default when a new assay definition table is loaded. The well range is also reset when the Set Plate Geometry (‘{’) command is sent.

Once this command is invoked, the reader will use the **ELx format** for data response until a new assay definition table is specified.

This command has no effect on the read parameters for a wellset read, area scan, or spectral scan.

NOTE - This command is not intended for use with fluorescence/luminescence assays, since the Set Fluorescence/Luminescence Assay Definition (‘v’) command contains its own column range parameters. However, using this command after sending a ‘v’ command will cause the defined column range to be overwritten with the new column range.

GET MINIMUM ABSORBANCE KINETIC INTERVAL (‘$’)

This command provides a means for the user to determine the minimum kinetic read interval, as dictated by the current assay definition table, plate geometry, well range, and even baud rate selected. This is the same command as the Get Minimum Fluorescence/Luminescence Kinetic Interval command; here, the response protocol is different to maintain compatibility with older absorbance readers.

host:	**valid limits:**	**response:**
‘$’	no arguments	<ACK/NAK>
		kinetic interval (2)
		status string (5)

The reader returns the minimum kinetic interval as a 2-byte integer value (not ASCII, so low byte first).

Minimum kinetic intervals are not available for wellset reads.

SCAN PLATE ('&')

This command causes the reader to perform a spectral scan on the indicated microwell. Absorbance data is always returned in the **312 data mode**.

host:	**valid limits:**	**response:**
'&'		<ACK/NAK>
data bytes:		
1 - 2	row ("01" - "16")	
3 - 4	column ("01" - "24")	
5 - 7	start wave ("200" - "999")	
8 - 10	stop wave ("201" - "999")	
11 - 13	wave step ("001" - "799")	
14	series option ('0' - '3')	
	'0': single scan	
	'1': first scan in series	
	'2': next scan	
	'3': last scan	
15	calibrate option ('0' - '1')	
	'0': calibrate only if necessary	
	'1': calibrate before scanning	
16 - 17	shake time in seconds ("00" - "99")	
18	shake speed	
	'0': slow	
	'1': medium	
	'2': fast	
	'3': cycle through speeds listed above	
<ETX>		<ACK> if data valid

reader response protocol:

status string (5)		
for each wavelength undergoing calibration (none to all wavelengths selected):		
wavelength (3)		"200" - "999"
wave terminator (1)		<CR>
calibration terminator (1)		<^Z>
additional terminator (1)		<^Z>
data start code (1)		<CR>
for each wavelength specified for scan:		
wavelength (3)		"200" - "999"
comma separator (1)	','	
sign (1)		'+' or '-'
data (4)		"1234"
well terminator (2)		<CR>, <LF>
data terminator (1)		<^Z>
checksum (1)		0 - 255
final status string (5)	**(ELx status mode only)**	

If the middle character in the status string is not ASCII '0', an error has been detected and no further data is transmitted. See **Status String Format** for error descriptions.

If an error is detected during the plate read, data transmission is halted, indicated by a single <DLE> character (0x10). In the **ELx status mode**, the final status string then indicates the error detected.

The reader sequentially scans the well from the start wavelength through the stop wavelength in increments dictated by the wavelength step. At least two wavelengths must be scanned. The start must be less than the stop, and the step must be less than the difference.

If more than one well is to be scanned, the series option can be used to make the process more efficient.

If the calibrate option is selected ('1'), all wavelengths indicated for the current spectral scan will be calibrated. If calibration is not selected, any wavelengths selected but not scanned since the instrument was powered up will be calibrated anyway. In addition, any selected wavelengths with previous errors detected will also be calibrated. If no calibration is performed at all, the <^Z> terminator is returned alone with no wavelengths. Calibration is not absolutely necessary with each plate, but is recommended in scans where absolute accuracy is a requirement.

Absorbance data is returned in the form of a 5-character ASCII string, with a sign character followed by four digits. A decimal point is not included, but should be inserted by the user after the first digit; i.e, "+1234" should be translated as +1.234 OD. Over-range readings are indicated by the 5-character string "*****" instead of a signed numerical value.

Checksum calculation starts with the first byte AFTER the data start code, up through and including the <^Z> data terminator code. The checksum is transmitted as an integer data byte (not ASCII).

If any invalid arguments are sent by the host computer, an error response is returned by the reader.

The Set Plate Geometry command must be sent before any Scan Plate commands if a plate geometry other than the most recent plate selected is to be used (96-well plate by default on power-up).

Fluorescence/Luminescence Commands

The following section describes computer control commands specific to fluorescence and luminescence.

GET SENSITIVITY ('f')

This command returns the sensitivity setting for the indicated filterset. It is useful in the event that on-board automatic sensitivity calculation has been performed via serial control.

host:	**valid limits:**	**response:**
'f'		<ACK/NAK>
data bytes:		
1	filterset ("1" – "2")	
reader response protocol:		
sensitivity (3)		"000" – "255"
status string (5)		

SET LAMP ('L')

This command sets the incandescent lamp used in fluorescence to either on or off, and starts the lamp warmup timer at three minutes (180 seconds).

host:	**valid limits:**	**response:**
'L'		<ACK/NAK>
data bytes:		
1	new lamp state:	
	'0': off	
	'1': on	
		status string (5)

The lamp should remain off for time-resolved assays, since illumination is provided by the xenon flash lamp also used for absorbance functions. A TR-equipped Synergy will automatically turn off the lamp if it detects that the special TR block has been installed. If an attempt is made to turn on the lamp while the block is in place, an error will be returned.

Under some circumstances, a TR-equipped reader may also turn off the lamp if it detects that the excitation wheel has been removed. If this command is used to turn the lamp on and the TR block is not detected, the reader will attempt to home the excitation wheel. If successful, the lamp will then be turned on and the warm-up timer started.

GET LAMP STATUS ('l')

This command returns the current state of the incandescent lamp used in fluorescence. It also returns the time left in the lamp warmup period.

host:	**valid limits:**	**response:**
'l'	no arguments	<ACK/NAK>

reader response protocol:

current lamp state (1)	'0': off
	'1': on and ready
	'2': on and warming up
warmup seconds remaining (4)	"0000" – "9999"
status string (5)	

CONFIGURE EXCITATION/EMISSION FILTERS ('m')

This command downloads a filter configuration table to the reader, and then homes both filter wheels if installed.

host:	**valid limits:**	**response:**
'm'		<ACK/NAK>
data bytes:		
for each excitation filter installed (four):		
wavelength (4)	"0000", "0001", "0200" – "9999"	
bandpass (3)	"000" – "099"	
for each emission filter installed (four):		
wavelength (4)	"0000", "0001", "0200" – "9999"	
bandpass (3)	"000" – "099"	
<ETX>		
		status string (5)

Each wavelength is represented by "xxxx," (4 ASCII digits), followed by the bandpass for that wavelength (3 ASCII digits). Eight total wavelength/bandpass combinations must be sent (56 bytes), followed by ETX after the last emission bandpass. The fluorescence wavelength setup table is stored in non-volatile memory, and only needs updating when a change in current wavelength configuration is desired.

A filter plug must be indicated by “0000”. No filter installed (empty hole) must be indicated by “0001”. The bandpass in these two cases should be “000”.

Whenever a filter wheel is replaced in the instrument, fluorescence wavelength configuration should always be was performed using this command. This will cause the fluorescence filter wheels to be homed, along with other possible calibration functions. The Self-Test (‘*’) command can also be used to accomplish this.

On TR-equipped readers, it is not necessary to send a new excitation wavelength configuration for use in time-resolved assays. Wavelengths can be selected directly for use in the Set Fluorescence/Luminescence Assay Definition (‘v’) command. The monochromator has a band pass of 10 nm when used in the TR mode.

GET PROBE CONFIGURATION (‘P’)

This command returns the current probe configuration for the instrument.

host:	**valid limits:**	**response:**
‘P’	no arguments	<ACK/NAK>

reader response protocol:	
bottom probe size (3), in units of 0.10 mm	“000” – “999”
top probe size (3) , in units of 0.10 mm	“000” – “999”
current probe position (1)	(‘0’: bottom, ‘1’: top)
status string (5)	

READ FLUORESCENCE/LUMINESCENCE PLATE (‘S’)

This command causes a microplate to be read according to the currently loaded fluorescence or luminescence assay definition table, downloaded using the Set Fluorescence/Luminescence Assay Definition (‘v’) command. This is the same command as the Read Absorbance Plate command; an ‘S’ command issued when a fluorescence/luminescence definition is in place requires a different response protocol to meet the needs of that particular assay type.

NOTE – The fluorescence lamp is automatically turned on upon receipt of this command when fluorescence is the selected assay type. Ideally though, the lamp should remain on for three full minutes before reading to ensure ideal accuracy and repeatability. Therefore, before sending this command (or other fluorescence read commands for area scanning and wellset reads), it is recommended that the lamp be turned on three minutes in advance using the Set Lamp State (‘L’)

command. The Get Lamp Status ('l') command can be used to determine the time remaining in the warmup period. If running an incubated assay, the lamp should not be turned on until the incubator is up to temperature. The lamp remains on until an absorbance, luminescence, or time-resolved fluorescence assay is selected, or a new incubator setpoint is selected that is more than two degrees above the current incubator temperature.

host:	**valid limits:**	**response:**
'S'	no arguments	<ACK/NAK>

reader response protocol:

initial status string (5)
for each kinetic interval (one if endpoint read):
    for each filterset or auto-sensitivity block:
        filterset start code (1) <CR>
        timestamp (11) **** (if specified)** "hhh:mm:ss.s"
        for each row within specified range of rows:
            for each column (well) within specified range of columns:
                comma separator (1) ','
                data (5) "00000" – "99998"
            row terminator (2) <CR>, <LF>
        temperature (3) *****(if specified)** "000" – "999"
        filterset terminator (1) <^Z>
        checksum (3) "000" – "255"
final data terminator (1) <^Z>
final status string (5)

** Timestamp included only if requested. See the Include Timestamp ('Q') command for description. The timestamp indicates the time elapsed between the very first read and the current read for any given well on the microplate in a kinetic read series.

*** Temperature response included only if requested. See the Include Temperature Response ('i') command for description, and the Get Temperature ('h') command for data format.

If an error is detected before the plate read starts, indicated by an error code in the initial status string, no further information is transmitted.

If an error is detected during a plate read, no fluorescence data is transmitted for that read. Instead of the data start code, the final data terminator is returned, followed by the final status string containing the error detected. In the case that an instrument failure is detected after the data has been

transmitted, indicated by an error code in the final status string, the data should be considered invalid.

Overrange wells are indicated by the 5-character string "*****" instead of a numerical value.

Total quantity of data returned per plate is dependent on plate geometry and row/column range defined.

If auto-sensitivity via serial control is selected and any of the three available auto-sensitivity blocks has a first well row defined as 0 ("00"), nothing will be returned for that block.

Checksum calculation starts with the first byte AFTER the data start code, up through and including the <^Z> plate data terminator code at the end of each plate's data. The checksum is then converted into three ASCII digits and transmitted, most significant digit first.

Data Handshaking:

If data handshaking has been turned on via the Set Data Handshaking mode ('N') command, the host is expected to respond after the data for each wavelength has been received. If the host does not receive all the data bytes expected for the wavelength, or the checksum received is incorrect, the host can indicate this to the reader by sending a <NAK>. The reader will respond by re-sending all data for the wavelength, including any optional timestamps or temperatures, from the wavelength start code (<CR>) through the last or only checksum byte. This can be repeated as many times as necessary until the reader receives an <ACK> for the data. If the reader has not received an <ACK> by the time it is ready to send data for the next wavelength or kinetic interval, or ready to send the final terminator at the very end of an endpoint read or kinetic series, the reader will flag an error and terminate the read sequence.

During periods while the reader is expecting an <ACK> response to data sent, the host may also send either abort ('X' or 'x') command, which will cause the reader to terminate the current sequence as defined for those two commands, with no additional results data transmitted.

SET TIME-RESOLVED PARAMETERS ('T')

This command sets the parameters used specifically for reading in the time-resolved fluorescence mode.

host:	**valid limits:**	**response:**
'T'		<ACK/NAK>
data bytes:		
1 - 5	time-resolved delay, filterset #1 (microseconds)	
	"00000", "00020" – "16000"	
6 - 10	data collection time, filterset #1 (microseconds)	
	"00020" – "16000"	
11 - 15	time-resolved delay, filterset #2 (microseconds)	
	"00000", "00020" – "16000"	
16 - 20	data collection time, filterset #2 (microseconds)	
	"00020" – "16000"	
<ETX>		
		status string (5)

The time-resolved delay is measured from the cessation of illumination to the start of data collection.

If "fluorescence" or "luminescence" is selected as the assay type in the current assay definition, parameters set by this command will be saved, but not used until a time-resolved assay is specified.

If a read process is started with "time-resolved fluorescence" specified as the current assay type, and the special TR block is not installed, an error will be flagged. The same will occur if a standard fluorescence read is started and the excitation filter wheel is not in place.

CONFIGURE PROBE HEIGHT ('t')

This command sets the probe height to be used for a top-probe fluorescence or luminescence plate read. It can also be used to adjust the current probe height positioning. Probe height indicates distance in 0.01mm from the bottom of the top probe assembly down to the bottom of a microplate on the carrier.

host:	**valid limits:**	**response:**
't'		<ACK/NAK>
data bytes:		
1	plate height selection	
	'1': set top probe height (no move)	
	'2': move probe to top of travel	
	'3': set top probe height and move there	
	'4': move probe to absorbance height	
2 – 5	top probe height (0.01 mm)	
	"1500" – "3250"	
<ETX>		
		status string (5)

If a probe height adjustment move is selected and the new height is lower than the current height, the reader will automatically move the carrier outside the reader before moving the top probe assembly.

CONFIGURE PROBE DIAMETERS ('u')

This command downloads a probe diameter configuration table to the reader. It does not actually change probe diameter – it merely informs the reader what is installed.

host:	**valid limits:**	**response:**
'u'		<ACK/NAK>
data bytes:		
bottom probe diameter (0.10 mm)	"010" – "250"	
top probe diameter (0.10 mm)	"010" – "250"	
<ETX>		
		status string (5)

SET FLUORESCENCE/LUMINESCENCE ASSAY DEFINITION ('v')

This command downloads to the reader a fluorescence/luminescence assay definition table to be used in subsequent plate reads, area scans, or wellset reads ('S', 'c', or 'd' commands) until a new definition replaces it. Only one assay – either absorbance or fluorescence/luminescence – can be in place, so this command will cause any assay definition currently loaded to be replaced. When a fluorescence/luminescence assay definition is in place, response data is always returned in the **FLx data mode**, and the **ELx status mode** is always used.

host:	**valid limits:**	**response:**
'v'		<ACK/NAK>
data bytes:		
1	read mode	
	'1': endpoint	
	'2': kinetic	
2	assay type	
	'2': fluorescence	
	'3': luminescence	
3 - 4	plate type (geometry)	
	"00": 24 wells (4x6)	
	"01": 48 wells (8x12)	
	"02": 96 wells (8x12)	
	"03": 384 wells (16x24)	
	"04": 72 well Terasaki (6x12)	
	"05": 60 well Terasaki (6x10)	
	"06": 96 well Terasaki (8x12)	
	"07": 96 well Hellma (8x12)	
	"08": 6 well (2x3)	
	"09": 12 well (3x4)	
	"10": 96 well Metric (8x12)	
	"11": previously defined custom geometry	
read boundaries, or auto-sensitivity block #1:		
5 – 6	first well row ("01" – "16")	
7 – 8	first well column ("01" – "24")	
9 – 10	last well row ("01" – "16")	
11 – 12	last well column ("01" – "24")	
end of read boundaries or auto-sensitivity block #1		

host:	valid limits:	response:
13	filterset count (‘1’ – ‘2’)	
filterset #1:		
14	probe position	
	‘0’: bottom	
	‘1’: top	
15 – 18	excitation wavelength	
	“0000”: filter plug	
	“0001”: open (air)	
	“0200” – “0999”: filter wavelength	
19 – 21	excitation bandpass (“001” - “099”)	
22 – 25	emission wavelength (“0000”, “0001”, “0200” - “0999”)	
26 – 28	emission bandpass (“001” - “099”)	
29 – 31	samples per well (“001” - “255”)	
32 – 34	delay before sampling (.010 seconds, “001” - “255”)	
35 – 37	delay between samples (.001 seconds, “000” - “255”)	
38	sensitivity	
	‘0’: fixed	
	‘1’: auto-sensitivity on-board	
	‘2’: auto-sensitivity via serial control	
39 – 41	fixed sensitivity (“000”, “025” - “255”)	
end of filterset #1		
42 - 43	on-board auto-sensitivity well row (“01” - “16”)	
44 – 45	on-board auto-sensitivity well column (“01” - “24”)	
46 – 50	on-board auto-sensitivity maximum value (“00100” - “90000”)	
51 – 53	kinetic reads (“002” – “300”)	
54 – 57	kinetic interval (seconds, “0000” – “9999”)	
58	shake mode	
	‘0’: none	
	‘1’: once before reading starts	
	‘2’: before each kinetic read	
59	shake speed	
	‘0’: slow	
	‘1’: medium	
	‘2’: fast	
	’3’: variable	
60	position in read series (endpoint only)	
	‘0’: single read	
	‘1’: first read in series	
	‘2’: next read in series	
	‘3’: final read in series	

host:	valid limits:	response:
61 eject plate between reads or filtersets		
	'0': no	
	'1': yes	
following auto-sensitivity blocks not available for use if more than one filterset defined		
auto-sensitivity block #2:		
62 – 63	first well row ("01" – "16")	
64 – 65	first well column ("01" – "24")	
66 – 67	last well row ("01" – "16")	
68 – 69	last well column ("01" – "24")	
auto-sensitivity block #3:		
70 – 71	first well row ("01" – "16")	
72 – 73	first well column ("01" – "24")	
74 – 75	last row well ("01" – "16")	
76 – 77	last row column ("01" – "24")	
end of auto-sensitivity blocks		
filterset #2:		
78	probe position	
	'0': bottom	
	'1': top	
79 – 82	excitation wavelength	
	"0000": filter plug	
	"0001": open (air)	
	"0200" – "0999": filter wavelength	
83– 85	excitation bandpass ("001" - "099")	
86 – 89	emission wavelength ("0000", "0001", "0200" - "0999")	
90 – 92	emission bandpass ("001" - "099")	
93 – 95	samples per well ("001" - "255")	
96 – 98	delay before sampling (.010 seconds, "001" - "255")	
99 – 101	delay between samples (.001 seconds, "000" - "255")	
102	sensitivity	
	'0': fixed	
	'1': auto-sensitivity on-board	
	'2': auto-sensitivity via serial control	
103 – 105	fixed sensitivity ("000", "025" - "255")	
end of filterset #2		
106 - 108	shake time (seconds, "000" – "999")	
109 – 111	checksum ("000" – "255")	
		status string (5)

After the assay definition is received, kinetic or endpoint plate reads may be initiated using the 'S' command. Area scans are initiated using the Run Area Scan ('c') command, which includes additional information concerning the region for each scanning pass across the plate. Wellset reads are initiated using the Read Well Set ('d') command, which selects individual wells instead of an entire plate.

The automatic sensitivity feature is not available with the area scanning function. However, sensitivities may be calculated in advance by performing endpoint reads using the Read Fluorescence/Luminescence ('S') command.

If auto-sensitivity via serial control is selected and the first well row is set to 0 ("00") for any auto-sensitivity block, that block will be assumed to be unused, and nothing will be returned for the block. Auto-sensitivity blocks must be selected for use in sequence, starting with block #1. Results data is returned for each block defined. If auto-sensitivity via serial control is desired for a plate read involving more than one filterset, only the first sensitivity block is available for use. Otherwise, the sensitivities must be calculated in advance, one filterset at a time.

A kinetic interval of zero will cause the plate to be read repeatedly with no pauses between reads.

Continuous shaking is available in the kinetic read mode by selecting a shake for every read and setting the shake time to zero. Shaking will then be performed using any extra time available in the selected kinetic interval while a plate read is not in progress.

If "time-resolved fluorescence" is specified as the assay type, and a read process is started without the special TR block installed in the excitation slot, an error will be flagged. The same will occur if a standard fluorescence read is started and the excitation filter wheel is not in place. Excitation wavelength configuration (see Configure Excitation/Emission Filters 'm' command) is not necessary for time-resolved assays. Wavelengths other than those found in the excitation wavelength table can be selected directly with this command.

If the incubator is enabled and on (setpoint > 0), incubator error trapping is available for running assays where accurate thermal control is critical. Once the trap is set at the beginning of a plate read (or wellset read or area scan), any event that may disrupt accurate incubation is recorded. Such events may include an incubator zone wandering out of range (± 2.0°C from setpoint) or a temperature sensor failure. Trapping is automatically turned off upon completion of the entire read process. Any errors detected are encoded into the final status string, as described in the **Status String Format** section.

GET EXCITATION/EMISSION FILTER CONFIGURATION ('w')

This command uploads the filter configuration table from the reader.

host:	**valid limits:**	**response:**
'w'	no arguments	<ACK/NAK>

reader response protocol:

for each excitation filter installed (four):	
wavelength (4)	"0000", "0001", "0200" – "0999"
bandpass (3)	"000" – "099"
for each emission filter installed (four):	
wavelength (4)	"0000", "0001", "0200" – "0999"
bandpass (3)	"000" – "099"
status string (5)	

A filter plug is indicated by "0000". No filter installed is indicated by "0001".

GET MINIMUM FLUORESCENCE/LUMINESCENCE KINETIC INTERVAL ('$')

This command provides a means for the user to determine the minimum kinetic read interval in seconds. This interval is calculated based on the current assay definition table, with plate geometry, wells to be read, and even baud rate taken into consideration. This is the same command as the Get Minimum Absorbance Kinetic Interval command; when an absorbance assay is in place, the response protocol is different to maintain compatibility with older absorbance readers.

host:	**valid limits:**	**response:**
'$'	no arguments	<ACK/NAK>

reader response protocol:

minimum kinetic interval (6)	"0000.0" – "9999.9"
status string (5)	

Minimum kinetic intervals are not available for wellset reads.

Common Commands

The following section describes computer control commands common to both absorbance and fluorescence/luminescence.

STORE PLATE CARRIER ('A')

This command causes the plate carrier to move inside the instrument to the incubation chamber. In this instrument, the incubation chamber for the carrier is back in the far corner, diagonal to the door opening.

host:	valid limits:	response:
'A'	no arguments	<ACK/NAK>
		status string (5)

GET SERIAL NUMBER ('C')

This command returns the factory-set serial number for the reader.

host:	valid limits:	response:
'C'		<ACK>
data bytes:		
1 – 8	serial number ("00000000" – "99999999")	
		status string (5)

RUN AREA SCAN ('c')

This command causes the reader to perform one pass of the X axis across a microplate, collecting data at the indicated locations. The parameters for the actual data collection are taken from the currently loaded assay definition table (see Set Assay Definition).

host:	valid limits:	response:
'c'		<ACK/NAK>
data bytes:		
1 - 5	Y start point (0.01 mm, "00558" – "08026")	
6 – 10	X start point ("00762" – "12065")	

host:	valid limits:	response:
11 – 15	X finish point (“00762” – “12065”)	
16 – 18	number of points to read (“001” – “255”)	
19	position in scan series (overrides assay definition series option)	
	‘0’: single scan	
	‘1’: first scan in series	
	‘2’: next scan in series	
	‘3’: final scan in series	
<ETX>		<ACK> if data valid

reader response protocol:

initial status string (5)		
for each filterset:		
data start code (1)		<CR>
for each point indicated:		
comma separator (1)		‘,’
data (5)		**see Data Modes**
row terminator (2)		<CR>, <LF>
temperature (3)	***(if specified)	“000” – “999”
data terminator (1)		<^Z>
checksum (3)		“000” – “255”
final data terminator (1)		<^Z>
final status string (5)	**(ELx status mode only)**	

*** Temperature response included only if requested. See the Include Temperature Response (‘i’) command for description, and the Get Temperature (‘h’ or ‘]’) command for data format.

Points for data collection are measured from the outer edge at the base of the upper left corner of a microplate. Units are 0.01 millimeters; i.e., “12345” represents 123.45 millimeters. An X start point greater than the X finish point will cause the plate to be processed from right to left. However, data is always returned as if the pass were performed from left to right.

The reader requires the dimensional specifications of the microplate selected for scanning to correctly calculate data points. Therefore, it is necessary that the Set Plate Geometry (‘{’), the Define Custom Geometry (‘y’), or the Set Fluorescence/Luminescence Assay Definition (‘v’) command be invoked to select a plate before scanning, to make that information available to the reader.

The automatic sensitivity feature is not available with the area scanning function. However, auto-sensitivity calculations may be performed prior to the area scan using endpoint reads with the Read Plate ('S') command.

Only endpoint reads may be specified.

Sweep mode is not available with the area scan.

See the Read Plate ('S') command description for details concerning results data format, fluorescence lamp control, and error handling and status string information.

READ WELL SET ('d')

This command causes the reader to return data collected at the center of each of the indicated wells, in the order specified. The parameters for the actual data collection are taken from the currently loaded assay definition table (see Set Assay Definition).

host:	**valid limits:**	**response:**
'd'		<ACK/NAK>
data bytes:		
1 – 2	well #1 row ("01" – "16")	
3 – 4	well #1 column ("01" – "24")	
5 – 8	well #2	
9 – 12	well #3	
13 – 16	well #4	
17 – 20	well #5	
21 – 24	well #6	
25 – 28	well #7	
29 – 32	well #8	
33	position in read series (overrides assay definition series option)	
	'0': single read	
	'1': first read in series	
	'2': next read in series	
	'3': final read in series	
<ETX>		<ACK> if data valid

reader response protocol:

initial status string (5)		
for each kinetic interval (one if endpoint read):		
for each filterset:		
data start code (1)		<CR>
timestamp (11)	***** (if specified)**	"hhh:mm:ss.s"
for each of eight wells:		
comma separator (1)		','
data (5)		**see Data Modes**
row terminator (2)		<CR>, <LF>
temperature (3)	*****(if specified)**	"000" – "999"
data terminator (1)		<^Z>
checksum (3)		"000" – "255"
final data terminator (1)		<^Z>
final status string (5)	**(ELx status mode only)**	

** Timestamp included only if requested. See the Include Timestamp ('Q') command for description. The timestamp indicates the time elapsed between the very first read and the current read for any given well on the microplate in a kinetic read series.

*** Temperature response included only if requested. See the Include Temperature Response command for description, and the Get Temperature ('h' or ']') command for data format.

Data is returned in the order wells are specified. If fewer than eight wells are to be read, each well's unused row and column must be replaced with "00". Eight wells' worth of data is always returned.

The on-board automatic sensitivity feature is available for use with this command. Using the well indicated for that purpose in the Assay Definition, auto-sensitivity data is collected and sensitivities calculated prior to the start of the read process for the indicated well set. The auto-sensitivity well may be included as part of the well set if desired.

Sweep mode is not available with the Read Well Set ('d') command.

See the Read Plate ('S') command description for details concerning results data format, fluorescence lamp control, and error handling and status string information.

GET BASECODE VERSION ('e')

This command returns a string containing the part number and version number for the instrument downloadable basecode software.

host:	**valid limits:**	**response:**
'e'	no arguments	<ACK/NAK>
reader response protocol:		
part number (7)		"0000000" – "9999999"
intermediate string (10)		"Version"
version code (7)		"x.xxyyy"
status string (5)		

The version number is indicated by the first four characters of the version code (x.xx). The remaining characters in the version code (yyy) may either be blank or represent a specific beta or prototype release code.

SET TEMPERATURE ('g')

This command sets the incubation chamber temperature setpoint. This command differs from the '[' command only in that it expects temperature data as an ASCII string.

host:	**valid limits:**	**response:**
'g'		<ACK/NAK>
data bytes:		
1 - 2	temperature setpoint ("00", "22" - "50")	
		status string (5)

If the instrument is not equipped with a working incubator, or if the indicated temperature setpoint is out of range, an incubator setpoint error will be returned with the standard status response string. A setpoint of zero will turn the incubator heaters off.

If the carrier is already inside the reader when incubation is turned on, the carrier will automatically move to the incubation chamber. Otherwise, for ideal incubation efficiency, the Store Plate Carrier ('A') command or door button should be used to move the carrier to the incubation chamber.

NOTE - If an incubator setpoint is selected that is more than two degrees above the current average incubator temperature, the incandescent lamp used for fluorescence reading is automatically shut off. Then, when all heating zones in the incubator are within two degrees of setpoint and nothing else has occurred to turn off the lamp, the lamp is automatically turned on again, and the lamp warmup timer is restarted at three minutes. See the Set Lamp ('L') command description for details.

GET TEMPERATURE (‘h’)

This command returns the current temperature in the incubation chamber. This command differs from the ‘]’ command only in that it returns temperature data as an ASCII string.

host:	valid limits:	response:
‘h’	no arguments	<ACK/NAK> temperature (3) status string (5)

The reader returns the current temperature as an ASCII representation of the mean incubator temperature scaled up by 10; i.e., “370” indicates a temperature of 37.0 degrees Celsius. Temperatures below the instrument minimum of 18 degrees are returned as “111”; temperatures above the instrument maximum of 55 degrees are returned as “999”.

If the instrument does not have incubation, or an incubation error has been detected, “000” will be returned as temperature data. An incubator temperature error will then be flagged and returned with the standard status response string. Otherwise, the current temperature (averaged over four thermal zones) is returned as defined above.

The reader will accept and process the Get Temperature command at any time, including during a read cycle. The temperature response will NOT interrupt a plate data response stream, however. If a Get Temperature command is received during a data transmission, it will not be processed until the transmission has completed; i.e., after the <^Z> checksum combination has been sent.

GET INCUBATION SETPOINT (‘H’)

This command returns the current setpoint for controlling incubation temperature. The temperature setpoint is returned as a two-digit ASCII representation. If incubation is not supported, the reader responds with <NAK> to the initial ‘H’ character. If incubation is off, the reader responds with a setpoint of “00”.

host:	valid limits:	response:
‘H’	no arguments	<ACK/NAK> setpoint (2) status string (5)

INCLUDE TEMPERATURE RESPONSE ('i')

This command causes the current temperature to be included in the results response any plate read using the 'S', 'c', or 'd' commands.

host:	**valid limits:**	**response:**
'i'		<ACK/NAK>
data bytes:	inclusion	
	'0': no temperature with plate data	
	'1': include temperature with plate data	

By default the instrument is initialized to not include a temperature response, and automatically reverts to this default when a new assay definition table is loaded.

PRESENT PLATE CARRIER ('J')

This command causes the plate carrier to move back outside the instrument where it can be loaded with a microplate.

host:	**valid limits:**	**response:**
'J'	no arguments	<ACK/NAK>
		status string (5)

SET DATA HANDSHAKING MODE ('N')

This command sets the data handshaking mode. When on, handshaking is used during all plate reads initiated using the 'c', 'd', or 'S' commands.

host:	**valid limits:**	**response:**
'N'		<ACK>
data bytes:	mode	
	'0': handshaking off	
	'1': handshaking on	
		status string (5)

When data handshaking is on, the reader expects the host to send an <ACK> after every wavelength in a plate read. See the Read Plate ('S') command description for details.

By default the instrument is initialized to not use data handshaking, and automatically reverts to this default when a new assay definition table is loaded.

Turning on handshaking also causes the reader to use the **ELx format** for data response.

GET CURRENT STATUS ('o')

This command returns the most recent error status set by the instrument. In the event of a reader timeout or <DLE> receipt, it can be used to determine the error code associated with the reader failure. It can also be used in place of pressing a key on the reader to stop the beeping after an error. The status is always returned in the **ELx status format**.

host:	**valid limits:**	**response:**
'o'	no arguments	<ACK> status string (5)

Requesting the current status through the use of this command will cause the status maintained on-board the instrument to be cleared except in the following situations:

1. A fatal error has been detected. The only way to clear this type of error is by recycling power on the instrument.
2. An error has been detected which requires a self-test to be executed before a read operation can be performed. In this case, only a successful self-test will cause the error to be cleared.

This command is most useful when invoked in pairs. The first try will yield the most recent error detected (or no error), which will then be cleared by the reader if possible. The next call will then yield either no error, or a previously detected error that may only be cleared by running a successful self-test.

Commands other than read commands may always be sent regardless of the current reader status; if there is a hardware issue preventing successful execution, a subsequent error status will be generated.

See **Status String Format** for error descriptions.

GET BASECODE CHECKSUM ('p')

This command returns a string containing the ASCII equivalent of a hexadecimal checksum calculated and saved for the instrument downloadable basecode.

host:	**valid limits:**	**response:**
'p'	no arguments	<ACK/NAK>
reader response protocol:		
checksum (4)		"0000" – "FFFF"
status string (5)		

INCLUDE TIMESTAMP ('Q')

This command causes the current temperature to be included in the results response for each plate read using the 'S' or 'd' commands.

host:	**valid limits:**	**response:**
'Q'		<ACK/NAK>
data bytes:	inclusion	
	'0': no timestamp with plate data	
	'1': include timestamp with plate data	

By default the instrument is initialized to not include a timestamp, and automatically reverts to this default when a new assay definition table is loaded.

SET QUIET MODE ('q')

This command allows the user to select a "quiet" mode. When this feature is off, the reader beeps when an error occurs, and will only stop when a Get Current Status ('o') command is sent. When quiet mode is selected, errors are automatically sent to the host if in **ELx status mode**, or immediately available via the 'o' command. This instrument is set to quiet mode automatically at power-up.

host:	**valid limits:**	**response:**
'q'		<ACK>
data bytes:	mode	
	'0': off	
	'1': on (quiet)	
		status string (5)

SET BAUD RATE ('R')

This command allows the user to select new baud rate, which will remain in effect until power is turned off or a new baud rate is selected. After the status string is returned at the previous baud rate, the serial port is re-initialized to operate at the new rate. Please allow a moment or so for this to take place before attempting communication at the new rate.

This instrument is set to 9600 baud by default at power-up.

host:	**valid limits:**	**response:**
'R'		<ACK>
data bytes:	baud rate	
	'0': 9600	
	'1': 19200	
	'2': 38400	
		status string (5)

DEFINE CUSTOM GEOMETRY ('y')

This command defines a custom geometry to be used for subsequent plate reads or spectral scans.

host:	**valid limits:**	**response:**
'y'		<ACK/NAK>
data bytes:		
1 - 2	rows ("01" – "16")	
3 – 4	columns ("01" – "24")	
5 – 9	first row ("00558" – "08026")	
10 – 14	last row ("00558" – "08026")	
15 – 19	first column ("00762" – "12065")	
20 – 24	last column ("00762" – "12065")	
25 – 29	external width ("08432" – "08611")	
30 – 34	external length("12573" – "12852")	
<ETX>		<ACK> if data valid
		status string (5)

Row and column values are measured from the outer edge at the base of the upper left corner of a microplate (near A1 on a standard 96-well microplate). Units are 0.01 millimeters; i.e., "12345" represents 123.45 millimeters. Once a custom geometry has been defined, it remains in place until it is either replaced by a new custom geometry, or the reader is switched off.

External width is defined as the distance between the upper left (near A1) and lower left (H1) corners on the outer edge at the base. External length is defined as the distance between the upper left (A1) and upper right (A12) corners.

The new geometry is automatically selected for future plate reads until a new assay definition is sent. The defined custom geometry can then be re-selected in one of two ways: by selecting geometry #11 via the Set Plate Geometry ('{') command (after a new assay definition has been sent), or as geometry #11 in the Set Fluorescence/Luminescence Assay Definition ('v') command. In either case, the custom geometry must be defined using this command before it can be selected.

HALT ('X')

This command causes any read or spectral scan in progress to be halted.

host:	**valid limits:**	**response:**
'X'	no arguments	none, or <DLE>

The following events occur when this command is invoked:

1. The scan or read process is halted, and all axes are returned to their home positions (the plate is moved back outside where it can be accessed).
2. The reader transmits a <DLE> character to the computer when the above process is completed.
3. No more data is transmitted by the read or scan in progress. An error response ("0100") may be generated (see 'S' command details).
4. If no read is in progress, the reader transmits a <DLE> character to the computer.

TERMINATE READ SERIES ('x')

This command ends a read sequence early, aborting the current plate in progress, but performing all error-checking necessary to validate previous data.

host:	**valid limits:**	**response:**
'x'	no arguments	none, or <DLE>

The following events occur when this command is invoked:

1. The scan or read process is halted, final error-checking is performed in order to validate data results previously transmitted, and all axes are returned to their home positions (the plate is moved back outside where it can be accessed).
2. The reader transmits a <DLE> character to the computer when the above process is completed.
3. No more data is transmitted by the read or scan in progress. An error response may be generated (see 'S' command details).
4. If no read is in progress, the reader transmits a <DLE> character to the computer.

SELF-TEST ('*')

This command causes the reader to perform a system self-test and calibration. This should be performed any time the filter configuration is changed on-board the instrument.

host:	**valid limits:**	**response:**
'*'		<ACK/NAK>

reader response protocol:
data stream (?)
status string (5)

The reader responds by sending a variable-sized stream of ASCII character data representing various calibration and test results. This data stream is followed by the standard status response string.

SET TEMPERATURE ('[')

This command sets the incubation chamber temperature setpoint. This command differs from the 'g' command only in that it expects temperature data as a two-byte integer value.

host:	**valid limits:**	**response:**
'['		<ACK/NAK>
data bytes:		
1 - 2	temperature setpoint (0, 22 - 50)	
		status string (5)

If the instrument is not equipped with a working incubator, or if the indicated temperature setpoint is out of range, an incubator setpoint error will be returned with the standard status response string. A setpoint of zero will turn the incubator heaters off.

If the carrier is already inside the reader when incubation is turned on, the carrier will automatically move to the incubation chamber. Otherwise, for ideal incubation efficiency, the Store Plate Carrier ('A') command or door button should be used to move the carrier to the incubation chamber.

NOTE - If an incubator setpoint is selected that is more than two degrees above the current average incubator temperature, the incandescent lamp used for fluorescence reading is automatically shut off. Then, when all heating zones in the incubator are within two degrees of setpoint and nothing else has occurred to turn off the lamp, the lamp is automatically turned on again, and the lamp warmup timer is restarted at three minutes. See the Set Lamp ('L') command description for details.

GET TEMPERATURE (']')

This command returns the current temperature in the incubation chamber. This command differs from the 'h' command only in that it returns temperature data as a two-byte integer value.

host:	**valid limits:**	**response:**
']'	no arguments	<ACK/NAK> temperature (2) status string (5)

The reader returns the current temperature as a 2-byte integer value (not ASCII, so low byte first).

Temperature is returned scaled up by 10; i.e., 370 indicates a temperature of 37.0 degrees Celsius. Temperatures below the instrument minimum of 18º are returned as 111 (0x006F); temperatures above the instrument maximum of 70º are returned as 999 (0x03E7).

If the instrument does not have incubation, or an incubation error has been detected, 0x0000 will be returned as temperature data. An incubator temperature error will then be flagged and returned with the standard status response string. Otherwise, the current temperature (averaged over four thermal zones) is returned as defined above.

The reader will accept and process the Get Temperature command at any time, including during a read cycle. The temperature response will NOT interrupt a plate data response stream, however. If a Get Temperature command is received during a data transmission, it will not be processed until the transmission has completed; i.e., after the <^Z> checksum combination has been sent.

GET INSTRUMENT CONFIGURATION ('}')

This command returns a 16-bit binary-encoded word defining the instrument configuration.

host:	**valid limits:**	**response:**
'}'	no arguments	<ACK/NAK>
reader response protocol:		
configuration (2)		0x0000 – 0xFFFF
no status string returned		

Instrument configuration data is returned low byte first. Encoding for the *Synergy HT* is defined as follows:

code:	description:
0x0376	Synergy HT w/ Bottom Probe
0x037E	Synergy HT w/ Bottom Probe and Incubator
0x0776	Synergy HT w/ Top Probe
0x077E	Synergy HT w/ Top Probe and Incubator
0x0B76	Synergy HT w/ Dual Probes
0x0B7E	Synergy HT w/ Dual Probes and Incubator
0x1776	Synergy HTTR w/ Top Probe
0x177E	Synergy HTTR w/ Top Probe and Incubator
0x1B76	Synergy HTTR w/ Dual Probes
0x1B7E	Synergy HTTR w/ Dual Probes and Incubator

General encoding for all readers, per bit, is defined as follows:

bit:	mask:	description:	
0	0x0001	0: extended UV range	1: standard UV range
1	0x0002	0: standard IR range	1: extended IR range
2	0x0004	0: eight-channel reads	1: single-channel reads
3	0x0008	0: no incubation	1: incubation available
4	0x0010	0: incandescent lamp	1: xenon flash lamp
5	0x0020	0: replaceable filters	1: monochromator
6	0x0040	0: standard beam	1: narrow beam (384-capable)
7	0x0080	0: standard (or no) door	1: robotic-controlled door
8	0x0100	0: absorbance only	1: fluorescence available
9	0x0200	0: keypad interface	1: serial control only
10	0x0400	0: bottom probe only	1: top probe only
11	0x0800	0: single probe	1: dual probe control
12	0x1000	0: no TR hardware	1: time-resolved hardware
13	0x2000	currently unused	
14	0x4000	currently unused	
15	0x8000	currently unused	

CHECK FEATURE (‘z’)

This command answers queries regarding features that may have been added after the initial release of an instrument.

host:	**valid limits:**	**response:**
‘z’	no arguments	<ACK/NAK>
data bytes:		
1 - 4	updated feature:	associated commands:
	“1001”: well set reads	‘d’
	“1002”: reads using two wavelength sets	‘V’, ‘v’
	“1003”: area scanning	‘c’
	“1004”: serial wavelength configuration	‘M’, ‘m’
	“1005”: shaking	‘V’, ‘v’, ‘&’
	“1006”: absorbance reads (*)	‘V’
	“1007”: high-speed sweep reads (*)	‘S’, ‘&’, ‘k’
	“1008”: partial plate reads	‘v’, ‘S’, ‘r’, ‘%’, ‘$’
	“1009”: incubation	‘g’, ’h’, ‘[’, ‘]’, ‘i’
	“1010”: sensitivity response	‘f’
	“1011”: multi-wavelength read format	‘V’
	“1012”: serial read mode selection (*)	‘k’
	“1013”: ELx format status response (*)	‘n’, ‘o’
	“1014”: serial-only instrument	
	“1015”: basecode checksum	‘p’
	“1016”: barcode scanning	‘s’
	“1017”: terminate read series	‘x’
	“1018”: custom geometry	‘y’
	“1019”: alignment test	‘v’
	“1020”: timestamp (*)	‘Q’
	“1021”: serial number	‘C’
	“1022”: incubation setpoint	‘H’
	“1023”: re-calibrate on wavelength change (*)	‘M’, ‘m’
	“1024”: dispensing	(not available)
	“1025”: setpoint to 70 degrees	‘g’, ‘[’
	“1027”: handshaking mode (*)	‘N’
	“1028”: high baud rate	‘R’
	“1029”: time-resolved fluorescence (*)	‘v’, ‘T’

<ETX>

reader response protocol:

feature available (1)	‘0’ / ‘1’
feature state if applicable (1)	‘0’ – ‘9’
miscellaneous additional data (15)	
status string (5)	

If the ‘z’ is acknowledged (ACK), the instrument will then return ‘1’ if the feature is available, ‘0’ if not.

If applicable, indicated by (*), the current state of the requested feature is returned in the response. This can occur when a serial command defined above may be used to modify a value associated with the feature, or if the instrument itself can modify the state of the feature.

Miscellaneous data TBD.

Data Modes

Depending on the assay format currently in place, data may be returned by the reader in one of two modes.

The **312 data mode** is used when the most recent assay definition received by the reader is set using the Set Absorbance Assay Definition ('V') command. Actual absorbance data is returned in the **312 data format** – five characters consisting of a '+' or '-', followed by a four-character ASCII absorbance string ("1234"). The implied decimal point must be inserted by the host after the first digit.

The **FLx data mode** is used when the assay currently in place was set using the Set Fluorescence/Luminescence Assay Definition ('v') command. Fluorescence or luminescence data is returned in Relative Fluorescence Units in the **FLx data format** – a five-character ASCII string ("12345").

Overrange Data: If the data is determined to be overrange by the reader in either format, the data is returned as a five-character ASCII string "*****".

Status String Format

Following execution of each command (except as noted), the *Synergy HT* sends a status string back to the computer.

NOTE – Commands applicable specifically to the use of fluorescent or luminescent chemistries ('f','L', 'l', 'm', 'P', 't', 'u', 'v', and 'w') **always** return a final status string in the **ELx status format**, as is the case when a fluorescence or luminescence assay is currently in place on the reader. This is done to maintain consistency with previous instruments using similar communication protocols. While being used as an absorbance reader, the *Synergy HT* conforms as much as possible to the customary absorbance protocol. The fluorometric protocol is followed otherwise.

312 Status Mode Format:

This status string consists of 5 successive ASCII characters – RS, S1, S2, S3, and ETX:

⇒ **RS** A record separator that marks the beginning of the status string.

⇒ **S1** Always ASCII zero ('0')

⇒ **S2** A single digit, used as a reader fault or error code number.

ERROR CODES

Error codes indicate the following:

'0': no fault or error

'8': instrument failure - perform self-test

'9': error in assay, scan, or table definition

'A': error in well range selection

'B': incubator setpoint error

'C': incubator temperature error

'E': barcode error

⇒ **S3** Always ASCII zero ('0')

⇒ **ETX** End Of Text – marks the end of the status block.

ELx Status Mode Format:

This status string consists of 5 successive ASCII characters – a four-byte string representing a hexadecimal status code, and then ETX.

Items described in angle brackets (<>) are indicated by an ASCII digit replacing the last '0' character in the status code. Look in the list associated with that type of error for the particular character.

Items described in curly braces ({}) are indicated by an ASCII digit replacing the next-to-last '0' character in the status code. Look in the list associated with that type of error for the particular character.

Fatal errors indicate a hardware failure, also shown on the instrument display screen, and require recycling of instrument power.

NOTE – Errors listed below are common to all reader instruments, and may not all be applicable to any single given reader.

Fatal Errors

TCB NOT AVAIL ERR	"A100"	// task control block not available
READ NOT AVAIL ERR	"A200"	// read already in progress
NOT AVAIL ERR	"A300"	// <device> not available
CHECKSUM ERR	"A400"	// failed code checksum test on power-up
POWER ERR	"A500"	// 24V power failure <power test>
DFLASH TIMEOUT ERR	"A600"	// data flash write timed out
DFLASH ERR	"A700"	// data flash readback didn't match write {test}<chip>
CFLASH TIMEOUT ERR	"A800"	// code flash write timed out
HEAP CORRUPTION ERR	"A900"	// memory allocation heap corrupted
ATOD ERR	"AA00"	// <device> A/D converter never saw ready transition

Non-Fatal Errors

NO ERR	"0000"	// no errors detected
ABORT ERR	"0100"	// read function aborted
NO SENSOR ERR	"0200"	// <motor> didn't find opto-sensor transition
NO BEAM ERR	"0300"	// <motor> didn't find saturation transition
MOTOR VERIFY ERR	"0400"	// <motor> failed positional verify
SATURATION ERR	"0500"	// A/D signal saturated <test type>
FILTER GAIN ERR	"0600"	// <filter> gain out of range
NOISE TEST ERR	"0700"	// reader {channel} failed noise test
OFFSET TEST ERR	"0800"	// reader {channel} failed offset test
DARK RANGE ERR	"0900"	// read-time {channel}<filter> dark out of range
AIR RANGE ERR	"0A00"	// read-time {channel}<filter> air/blank out

Non-Fatal Errors (cont.)

ASSAY NUM ERR	“0B00”	// invalid <assay number>
PRINT TIMEOUT ERR	“0C00”	// printer timed out
CAL CHECKSUM ERR	“0D00”	// failed calibration checksum test
WAVE NOT FOUND ERR	“0E00”	// wavelength not found in table <read filter>
FILTER SIGNAL ERR	“0F00”	// {channel}<filter> signal out of range
CNFG DATA ERR	“1000”	// necessary configuration data missing
CNFG CHECKSUM ERR	“1100”	// failed configuration checksum test
CAL DATA ERR	“1200”	// necessary calibration data missing
MOTOR NOT HOMED ERR	“1300”	// <motor> not homed successfully
ASSAY INCUBATE ERR	“1400”	// assay expects incubation when none available
INCUBATOR FAILURE	“1500”	// incubator failure {error code}<zone(s)>
SC ASSAY DEF ERR	“1600”	// computer control assay definition error
KIN INTERVAL ERR	“1700”	// interval too short for selected options
KIN COUNT ERR	“1800”	// too many kinetic intervals
MALLOC ERR	“1900”	// malloc failed
STORE CURVE ERR	“1A00”	// store curve failure
GET CURVE ERR	“1B00”	// get curve failure
ATOD INIT ERR	“1C00”	// A/D calib STBY transition not detected
RESULTS DATA ERR	“1D00”	// results data error
CLOCK ERR	“1E00”	// error in clock communications
OVERLAP ERR	“1F00”	// bandpass overlap in filterset
BARCODE ERR	“2000”	// no valid barcode detected
INVALID PARAM ERR	“2100”	// invalid parameter value selected
PMT ERR	“2200”	// PMT test signal too high <test type>
LAMP ERR	“2300”	// lamp control failure <test type>
XY POS ERR	“2400”	// X/Y test sensor position incorrect <motor>
FLASH MISS ERR	“2500”	// <motor> missed sweep mode flash location
XY LIMIT ERR	“2600”	// limit exceeded for area scan or custom plate
PANEL METHOD ERR	“2700”	// <assay> method doesn’t match first panel assay
MOTOR IN USE ERR	“2800”	// <motor> already in use
VREF ERR	“2900”	// voltage reference failed <test type>
PLATE JAM ERR	“2A00”	// <motor> didn’t find middle sensor
PROBE Z CAL ERR	“2E00”	// probe Z calibration failure <cal code>
SPOOL TIMEOUT ERR	“2F00”	// data handshaking timeout
EXCITATION BLOCK ERR	“3000”	// wrong block in excitation slot <1=TR block>

***Test Type Codes* <lowest digit in returned error code>**

FAIL VTEST HI	'1'	// higher voltage level incorrect
FAIL VTEST LO	'2'	// lower voltage level incorrect
FAIL VTEST RATIO	'3'	// voltage ratio too low
FAIL VTEST NOISE	'4'	// noise too high
FAIL WELL TEST	'5'	// saturation at well
FAIL LED PMT	'6'	// PMT saturation collecting LED reference data
FAIL LED DIODE	'7'	// diode saturation collecting LED reference data
FAIL POWER 24V	'2'	// 24V power failure
TEST LAMP	'1'	// lamp current test
TEST 24V	'2'	// 24V power drive test
TEST MOTOR	'3'	// motor drive test
TEST XF MIN	'4'	// xenon flash min power test
TEST XF LOW	'5'	// xenon flash low power test
TEST XF HIGH	'6'	// xenon flash high power test
TEST XF MAX	'7'	// xenon flash max power test
TEST XF HTRF	'8'	// xenon flash TR power test

***Motor Codes* <lowest digit in returned error code>**

Carrier X Axis	'0'
Carrier Y Axis	'1'
Excitation Filter Wheel '2'	
Emission Filter Wheel	'3'
Monochromator Filter Wheel	'4'
Monochromator	'5'
Probe Height Axis	'6'
Probe Changer	'7'

***Incubator Codes* {second lowest digit in returned error code}**

Range Error	'0'
Thermistor Error	'1'
A/D Error	'2'

Affected zones are encoded in the lowest digit returned – one bit per zone, starting from bit 0.

***Data Flash Codes* {second lowest digit in returned error code}**

Readback Error	'0'	// data readback didn't match data written
Copy Error	'1'	// final data readback didn't match original passed in

***A/D Device Codes* <lowest digit in returned error code>**

Absorbance measurement	'1'
Fluorescence measurement	'2'
Incubation measurement	'3'
Voltage reference	'4'

***Probe Z Calibration Codes* <lowest digit in returned error code>**

Jig Windup	'2'	// not enough windup at jig

Appendix C
Error Codes

This chapter describes Error Codes that may appear.
If an error is displayed, call Bio-Tek's Technical Assistance Center.
See Chapter 1, Technical Support for contact information.

Error Codes

An error code is reported on a PC-based system using compatible software as a four-digit identifier. The first digit is usually "0" or "A." Digits 0, 1, or 2 denote a non-critical error, which means the instrument will still respond to input. An A denotes a serious error, which requires that the reader be powered down before additional input will be accepted. Additional error codes can be found in Appendix B, pages 47-50.

Error Code Structure

Error codes will be displayed on the controlling PC as a four-digit identifier. The first character will be 0, 1, 2, or A.

- **0, 1 or 2 (non-critical error):** These are outlined on the following pages.
- **A (serious error):** If these occur, turn off the instrument and repower. If the error reappears, call Bio-Tek TAC for troubleshooting assistance (see Chapter 1, page 1-9). These types of errors are listed as "Fatal Errors" on page B-47 of *Appendix B*.

Note: If any error code is displayed, a System Self Test should be conducted for diagnostic purposes. If using KC4, run an Optics Test.

The last digit of some error codes gives specific information related to the type of error. For example, in the Home Sensor Initial Find Errors table on the following page, the last digit indicates which sensor was in error.

Home Sensor Initial Find Errors

Home Sensor initial find errors: These errors occur when the optosensor for the axis in question never electrically transitions from a high state to a low state. The causes can range from a simple disconnected cable, obstructed axis (plate or shipping screw limiting travel) or a defective sensor. The last digit of the error will identify the axis.

Displayed Error	Potential Cause
ERROR 0200	X motor axis did not home correctly
ERROR 0201	Y motor axis did not home correctly
ERROR 0202	EX filter wheel did not home correctly
ERROR 0203	EM filter wheel did not home correctly
ERROR 0204	Monochromator filter wheel did not home correctly
ERROR 0205	Monochromator did not home correctly
ERROR 0206	Z axis (top probe) did not home correctly
ERROR 0207	Probe changer did not home correctly

¡¡¡	In cases where a sensor is not functioning, the motor may drive the axis to its mechanical stop and generate substantial noise.

Displayed Error	Potential Cause
ERROR 0300	Carrier failed to find light beam
ERROR 0301	Filter wheel did not find home
ERROR 0303	Monochromator did not find home

Errors 0300, 0301, and 0303 indicate that a particular axis has moved to a point where the light beam from the optics is no longer detectable by the measurement electronics.

PROBABLE CAUSE:

- **Carrier -** A loose belt, loose motor pulley, or defective motor drive may cause the carrier to ignore movement instructions.
- **Filter Wheel Motor -** The filter wheel drive gear is loose or motor drive failure is impeding filter wheel movement.

Home Sensor Verification Errors

Home Sensor Verification Errors: These errors occur when the optosensor is not found again with in a range of where it was last found. The causes can range from loose transmission components to marginal adjustments. The last digit of the error identifies the axis in question.

Displayed Error	Potential Cause
ERROR 0400	Carrier axis failed position verify
ERROR 0401	Filter wheel motor failed position verify
ERROR 0402	Robot failed position verify
ERROR 0403	Monochromator failed position verify
ERROR 0404	Monochromator filter wheel did not home verify correctly
ERROR 0405	Monochromator did not home verify correctly
ERROR 0406	Z axis (top probe) did not home verify correctly
ERROR 0407	Probe changer did not home verify correctly

PROBABLE CAUSE:

- The belt is slipping due to incorrect tension, a loose motor pulley, or a loose belt clamp. This can also be caused by a defective motor drive circuit.
- A set screw on the motor shaft is loose.
- A flash lamp not flashing consistently has caused the monochromator to fail its homing.

Saturation Errors

These errors occur in several places when the measurement channel in use is found to be in an unexpected saturation condition. The error decoding is complex and requires some orientation in terms of where the failure occurs. The correct identification will depend on listening to the unit function prior to the reported error. For example, if the monochromator motor is in motion during a self-check, a 0500 error would indicate an ABS measurement channel error. 0501 would be an absorbance reference channel. If the fluorometric measurement system is in use (filter wheels homing) a 0500 error could indicate a missing filter. In this case, a 0501 would indicate a missing filter in position 1 of the wheel, which was last homing prior to the error condition being detected.

Displayed Error	Potential Cause
ERROR 0500	Channel 0 Saturation
ERROR 0510	Channel 1 Saturation
ERROR 050X	Filter 1-4 not installed

PROBABLE CAUSE:

- All wavelength locations must have either a bandpass or a filter blank installed, or this error will occur. If the entire wheel is not installed, the reader will also display this error.

Displayed Error	Potential Cause
ERROR 0601	LAMBDA #1 Gain out of range
ERROR 0602	LAMBDA #2 Gain out of range
ERROR 0603	LAMBDA #3 Gain out of range
ERROR 0604	LAMBDA #4 Gain out of range
ERROR 0605	LAMBDA #5 Gain out of range
ERROR 0606	LAMBDA #6 Gain out of range

Errors 0601-0606 indicate that the gain for a specific wavelength is out of the range necessary to ensure the filter's performance to specifications.

PROBABLE CAUSE:

- A defective lamp could provide enough reduction in signal to cause this error. Misaligned optics could also cause this error.

Absorbance Reader Noise Errors

Typical noise data during the system self check is under 5 counts in both measurement and reference channels. Failure of this test indicates basic instability. The instrument should be returned for service to fix the problem.

Displayed Error	Potential Cause
ERROR 0700	Reader failed noise test

Error 0700 indicates that the measurement electronics do not read repeatably at a maximum gain of 10, with the light blocked.

Displayed Error	Potential Cause
ERROR 0800	Reader failed offset test

Error 0800 indicates that the measurement electronics' dark current offset is outside of acceptable limits at maximum gain.

PROBABLE CAUSE:

- Ambient light leak or faulty printed circuit board.

Displayed Error	Potential Cause
ERROR 0900	Read time dark value out of range

Error 0900 indicates that the dark current value taken during the current read is significantly different than the same reading taken during the power-up self-check.

PROBABLE CAUSE:

- The measurement electronics background noise has changed since the last power-up self-check.

Displayed Error	Potential Cause
ERROR 0A00	Read time air blank out of range

Error 0A00 indicates that the blank (full signal) reading taken during the current read has changed significantly from the same reading taken during the power-up self-check.

PROBABLE CAUSE:

- The measurement electronics full signal level has changed since the power-up self-check was last run.

Internal Self-Test Errors

Displayed Error	Potential Cause
ERROR 0D00	Reader failed calibration checksum test
ERROR 0E00	Wavelength not detected in reader's wavelength table (1: Meas; 2: Ref; 3: Sec)
ERROR 0F00	Reader's wavelength or channel signal out of specified range
ERROR 1000	Required reader configuration data missing
ERROR 1100	Failed configuration checksum test
ERROR 1200	Calibration data missing
ERROR 1300	Motor not correctly homed
ERROR 1400	Assay incubation error. Assay requires incubation but the instrument does not appear to have an incubator.
ERROR 1500	Incubator failed to hold temperature within tolerances during the assay
ERROR 1600	Computer control assay definition error
ERROR 1700	Kinetic interval too short for selected options
ERROR 1800	Too many kinetic intervals selected
ERROR 1900	Memory allocation failure
ERROR 2400	Test (mid-track) sensor position incorrect
ERROR 2500	Motor went by flash location too soon

These errors indicate the reader has failed one or more internal self-tests. Contact Bio-Tek's Technical Assistance Center for instructions.

Additional errors are listed on pages B-47 through B-50 of *Appendix B*.

Appendix D
Microplate Location Dimensions

This appendix contains information concerning the location of the microplate and mounting holes of the instrument.

Microplate Carrier Position

Figure D-1 shows the location of the microplate carrier position in reference to the exterior surfaces of the *Synergy HT* and mounting poles on the bottom. The illustration should facilitate system setup with a robotic unit.

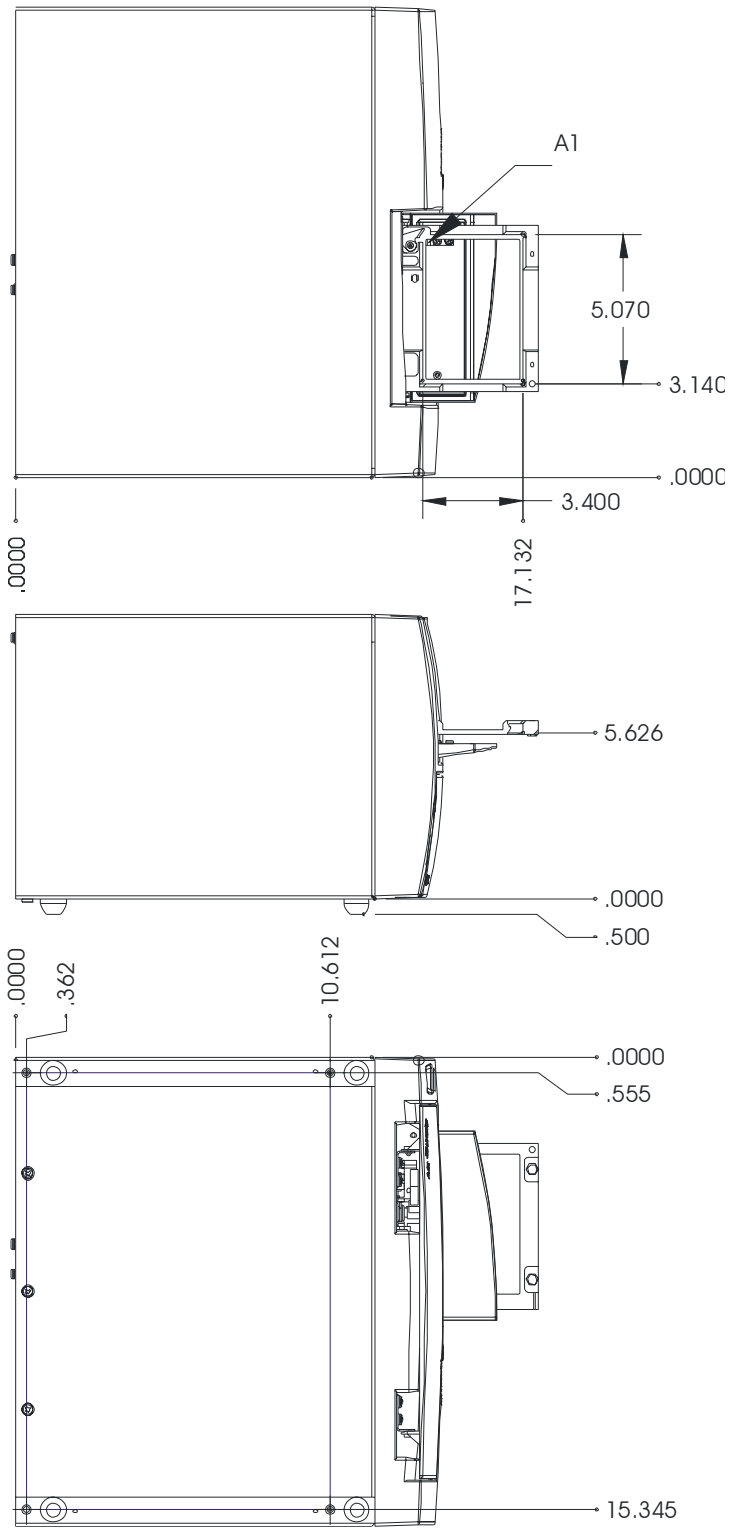


Figure D-1: *Dimensions of microplate carrier position and mounting holes*