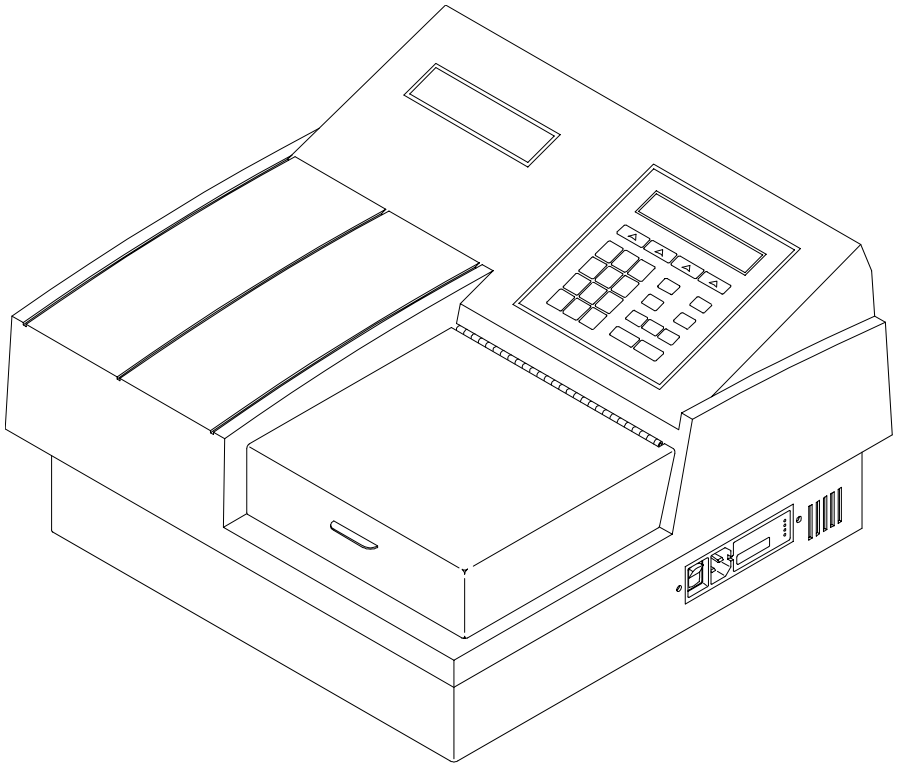
PowerWave x

BIO-TEK INSTRUMENTS, INC.



# Microplate Scanning Spectrophotometer

# Operator's Manual

This **Manual Update** contains changes to the PowerWaveX™ Operator's Manual. These changes will be incorporated in the next full revision (Rev E) of the Manual.

## Page 4-17, Liquid Test 1

***Recommendation:***

After pipetting the diluted test solution into the microplate (step 3) and *before* reading the plate, we strongly recommend shaking the plate at Variable speed for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. Alternatively, wait 20 minutes after pipetting the diluted test solution before reading the plate.

## Pages 4-7 through 4-13, Universal Plate Test

***Clarification:***

Bio-Tek's "Absorbance Test Plate" (part number 7260522) can be used to test the alignment, accuracy, repeatability, and linearity of your PowerWaveX Microplate Spectrophotometer. Revision D of the Operator's Manual refers to this test plate by its *former* name, the "Universal Test Plate."

BIO-TEK®

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## Page 4-2, Recommended Test Schedule

***Correction:***

The schedule shown in *Table 4-1* defines the factory-recommended intervals for performance testing for a microplate reader used for one shift seven days a week. **Note:** The risk factors associated with your tests may require that the Operational and Performance Qualification procedures be performed more or less frequently than shown below.

**Table 4-1 Recommended Test Schedule**

| | Installation Qualification | Operational Qualification | Performance Qualification | Performance Qualification |
|---|---|---|---|---|
| | | *Initially & Annually* | *Monthly* | *Quarterly* |
| System Self-Test, p. 4-4 | ✓ | ✓ | ✓ | |
| Absorbance Plate Test, p. 4-7 | ✓ | ✓ | ✓ | |
| Liquid Test 1*, p. 4-17 **or** Liquid Test 2*, p. 4-19 | | ✓ | | ✓ |
| Liquid Test 3**, p. 4-22 | | ✓ | | |
| Robotic Lube, p. 4-25 | Every six months, or after 10,000 cycles. | | | |

* If you have an Absorbance Test Plate, run Liquid Test 1. If you do not have an Absorbance Test Plate, run Liquid Test 2.

** Liquid Test 3 is optional; it is provided for sites requiring verification at wavelengths lower than those attainable with the Absorbance Test Plate.

*Liquid Tests should be performed as part of the **Operational Qualification**, during initial installation and annually, as shown in this revised chart.*

# *PowerWaveX™*

## Microplate Scanning Spectrophotometer

## Operator's Manual

**For R & D Use**

**and**

***in vitro* Diagnostic Use**

**OCTOBER 2002**



**PART NUMBER 7261014**

**REVISION D**

**BIO-TEK® INSTRUMENTS, INC.**

## Notices

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| | 802-655-4040 (outside the U.S.) |
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## REVISION HISTORY

| Revision | Date | Change |
|---|---|---|
| A | 8/98 | First Release. |
| B | 2/99 | Incorporated Manual Updates. |
| C | 8/99 | Added support for *PowerWave$_x$ Select* model.<br>Enhanced *Chapter 4, Performance Verification & Qualification*. Assigned each of the recommended maintenance tasks the Installation, Performance, and/or Operation Qualification classification. |
| D | 10/02 | Changed *PowerWave$_x$* to *PowerWaveX* throughout. Updated Notices, Chapters 1 through 4, and Appendices B and C. Incorporated manual update for periodic mantenance of robotic units in Ch 4. Added Cleaning section to Appendix A. |

## 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. |
| `DEFINE` | Text in `COURIER` font represents menu options as they appear in the instrument's display. |
| **Note:** | Bold text is primarily used for emphasis. |
|  | This icon calls attention to important information. |

## Warnings

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

**Please read the following hazards and cautions before operating the instrument!**

### HAZARDS

**Warning! Power Rating.** The *PowerWaveX* 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.

**Warning! Software.** The microplate reader operator must follow the manufacturer's assay package insert when modifying software parameters and establishing result calculation methods, using the reader's on-board software.

**Warning! Data Reduction Protocol.** The reader's software will flag properly defined controls when they are out of range. It will present all the data with the appropriate error flags in order for the user to determine their validity. Because there have been no limits applied to the raw absorbance data, all information exported via computer control must be analyzed completely.

**Warning! Unspecified Use.** Failure to operate this equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.

### 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 18-40°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. A four-position line voltage select switch is used to adjust for different line voltages. This switch is located on the power input module. See the section *Adjusting Line Voltage Input Range* in Chapter 2 for more details.

**Caution: Carrier Retention Screw.** The carrier retention screw 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:

- **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".

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

## EMC EC DIRECTIVE 89/336/EEC

- **EN 50081-1, CLASS A-Emissions**

  The system has been type tested by an independent testing laboratory and found to meet the requirements of EC Directive 89/336/EEC for Radiated Emissions and Line Conducted Emissions. Verification was to the limits and methods of EN 55022. The device is classified as EN 55022, Class A.

- **EN 50082-1 Immunity**

  The system was also tested and found to meet requirements for Electrostatic Discharge Susceptibility, Radiated Susceptibility, and Electrical Fast Transient/Burst Susceptibility. Verification of compliance was conducted to the limits and methods of EN 50082-1:1992; IEC 1000-4-2:1995; IEC 1000-4-3:1995; IEC 1000-4-4:1995; EN 61000-4-6:1996; EN 61000-4-11: 1994.

## 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: 1993**

  "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements" (including amendment No. 2: 1995).

## Safety Symbols

The following warning and informational symbols may be found in various locations on the *PowerWaveX*. 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, 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)

**Caution (refer to accompanying documents)**

Attention (voir documents d'accompanement)

Achtung siehe Begleitpapiere

Atención (vease los documentos incluidos)

Attenzione, consultare la doc annessa

## Intended Use Statement

The *PowerWaveX* is an eight-channel, automated, benchtop, general-purpose, Microplate Spectrophotometer that performs *in vitro* diagnostic 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 software in conjunction with the specific laboratory diagnostic assay. This evaluation must include the confirmation that new performance characteristics for the specific assay are met.

This system is designed for use with a variety of microplate-based assays. A versatile curve-fitting and statistical software program is preloaded on every *PowerWaveX*; plate templates and formulas are automatically combined with the protocol assay setup. Data results may be printed out, or sent to a computer running a Bio-Tek software package, such as KCjunior™ or KC4™ for Windows®.

The on-board software provides:

- An easy-to-use, menu-driven interface
- Endpoint curvilinear regressional and statistical calculations
- Curve fitting, with 4-parameter, cubic, quadratic, linear, cubic-spline, point-to-point and 2-P (Logit) methods
- Formula calculations for more complex mathematical operations
- Ability to define controls and positive and negative cutoffs
- Kinetic read capability, with maximum slope, R-squared at maximum slope and Onset OD Time analysis
- Ability to perform a spectral scan reading from 200 to 999 nm or 340 to 999 nm, depending on the model
- Scanning read mode to provide area under the curve calculations
- Optional preprogrammed common biological assays

### 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 *PowerWaveX*. 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 *PowerWaveX* 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.

# Table of Contents

## List of Ilustrations

## List of Tables

# Chapter 1
# *Introduction*

*This chapter introduces the PowerWaveX and describes its hardware and software features and technical specifications. Instructions on how to contact Bio-Tek for Technical Assistance are included on page 1-8.*

## Introducing the *PowerWaveX*

Bio-Tek's *PowerWaveX* is an eight-channel reader-assay system for research and development and *in vitro* diagnostic use. The reader can serve as a stand-alone system, or as an integral part of a PC-based system, using Bio-Tek's KC4 or KCjunior software packages.

The *PowerWaveX* is a spectrophotometer that has the capability of spectral scanning using a monochromator. This allows the user to perform endpoint and kinetic analysis, in 96-well microplates, using any wavelength between 200 and 999 nm, or 340 and 999 (depending on the model), thereby achieving the maximum absorbance for any sample.

The reader features superior optical specifications, with an extended dynamic range of up to 4.000 absorbance units.

The instrument's on-board processor, 2 X 24 character LCD screen, and membrane keypad allow easy definition and management of assay protocols, templates, formulas, and data. Results can be output in a printed report format, or exported for use in a variety of microplate-based data manipulation applications.

## Variations

Bio-Tek's *PowerWaveX* may be configured with all or selected options:

- *PowerWaveX Select* models support the reading of 96- *and* 384-well plates.
- "I" model instruments have a 4-Zone™ incubation chamber that controls the temperature from 4° above ambient to 50°C.
- "R" model instruments are robotic compatible.
- An internal bar code scanner option is also available.

| | |
|---|---|
| **iii** | **Note:** 384-well plate reading must be performed through computer control or with KC4 or KCjunior software. See *Appendix B* for more information. |

## Hardware Features

- Xenon flash light source
- Eight optics channels, with an additional reference channel
- Monochromator with λ range of 200 to 999 nm or 340 to 999 nm, depending on reader model
- A 2 X 24 character LCD display
- A membrane keypad with alphanumeric keys
- Adjustable plate shake frequency and durations
- Reads 96-well microplates with 0.355" / 9-mm well centers
- Reads 384-well microplates with 0.177" / 4.5-mm centers (*PowerWaveX Select* model only)
- Operates from 100, 120, 230, or 240 VAC @ 50-60 Hz (with external switching)
- One serial COM port (25-pin male connector)
- One parallel port (25-pin female connector)
- Internal bar code option
- 4-Zone™ incubation chamber option
- Robotics interface option

## Software Features

- Easy-to-use menu-driven interface
- Endpoint, Kinetic and Scanning calculations
- Curve fitting, with 4-parameter, cubic, quadratic, linear, 2-P, cubic-spline and point-to-point methods
- Transformation and formula calculations for more complex mathematical operations, including validations
- Up to 55 assays are available on-board (27 open assays, plus preprogrammed common biological assays)
- Automatically stores results for the last 8 plates
- Spectral scanning using on-board software with report of peak OD and wavelength at peak OD

## Package Contents

- Microplate scanning spectrophotometer
- Power cord
- Operator's Manual and Warranty Registration Card (PN 7261014)
- Assay Reference Guide, depending on the model (PN 7271006)
- Dust cover (PN 7342066)
- Parallel cable (PN 71072)
- Serial cable (PN 75053)
- *PowerWaveX Select* model only: Allen wrench (PN 49329)

## Optional Accessories

- Service Manual for all *PowerWaveX* models (PN 7261016)
- Universal Test Plate (PN 7260522)
- Bio-Cell™ for 1-cm wavelength readings (PN 7272051)
- Bio-Cell adaptor plate for containing up to eight Bio-Cells (PN 7270512)
- Installation-Operational-Performance (IQ-OQ-PQ) package (PN 7260219)

## Specifications

- **Microplates**

  All models accommodate standard 96-well microplates. The *Select* model also accommodates 384-well microplates.

  | | |
  |---|---|
  | iii | **Note:** Testing is recommended with 384-well plate types to determine compatibility. The *Select* was tested with Greiner and Costar 384-well plates, and with Nunc black-sided clear-bottom 384-well plates, and results meet specifications. |

- **Speed of Reading**

  The actual plate read time is dependent on the method of reading:

  - In **Normal** read mode, the reading speed is variable with the absorbance of the samples. If a sample measurement is less than 1% of the channel blank signal, the reader will automatically go into an enhanced read mode where longer read intervals are applied for increased performance.
  - In **Rapid** read mode, enhanced readings are not used; therefore, the reading speed is not variable.

  Wavelength choice will also change read times slightly. Each wavelength has a unique location within the monochromator, and the different locations require varying amounts of time to position. This effect is more noticeable in dual-wavelength assays.

| **96-Well Read Timing** | 630 nm | 630/450 nm |
|---|---|---|
| **Normal Read Mode** | Single | Dual |
| Endpoint | 23 to 31 sec. | 35 to 53 sec. |
| **Rapid Read Mode** | Single | Dual |
| Endpoint | 23 sec. | 35 sec. |

**Kinetics:** 14 seconds from A1 to A1 in rapid mode, single wavelength.

| **384-Well Read Timing** | 630 nm | 630/450 nm |
|---|---|---|
| **Normal Read Mode** | Single | Dual |
| Endpoint | 41 to 86 sec. | 71 to 141 sec. |
| **Rapid Read Mode** | Single | Dual |
| Endpoint | 37 sec. | 64 sec. |

**Kinetics:** 28 seconds from A1 to A1 in rapid mode, single wavelength.

- **Optical Specifications**

| | |
|---|---|
| λ range: | 200 to 999 nm or 340 to 999 nm (model-dependent) |
| λ accuracy: | ± 2 nm |
| λ repeatability: | ± 0.2 nm |
| λ bandpass: | 5 nm |

- **Optical Performance**

### 96-Well Plate Reading

The following specifications apply to 96-well, flat- or round-bottom plates, single wavelength endpoint or kinetic (with intervals of at least 20 seconds) readings. **Note:** For the following performance, the Gain on the Optics test should be below 6, in normal read mode.

### Endpoint or Kinetic with an interval ≤ 20 seconds:

**Absorbance**
**Measurement Range:** 0.000 to 4.000 Abs

| | |
|---|---|
| **Accuracy:** | 0.000 to 2.000 Abs ± 1% ± 0.010 Abs |
| | 2.000 to 3.000 Abs ± 3% ± 0.010 Abs |
| **Linearity:** | 0.000 to 2.000 Abs ± 1% |
| | 2.000 to 3.000 Abs ± 3% |
| **Repeatability:** | 0.000 to 2.000 Abs ± 1% ± 0.005 Abs |
| | 2.000 to 3.000 Abs ± 3% ± 0.005 Abs |

### Spectral Scanning or Kinetics with short intervals:

When read intervals from 8 seconds to 20 seconds are selected, or spectral scanning is performed, the specifications are as follows:

**Absorbance**
**Measurement Range:** 0.000 to 3.000 Abs

| | |
|---|---|
| **Accuracy:** | 0.000 to 2.000 Abs ± 1% ± 0.010 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.010 Abs |
| **Linearity:** | 0.000 to 2.000 Abs ± 1% |
| | 2.000 to 2.500 Abs ± 3% |
| **Repeatability:** | 0.000 to 2.000 Abs ± 1% ± 0.005 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.005 Abs |

### 384-Well Plate Reading

The following specifications apply to 384-well, flat- or round-bottom plates, single-wavelength endpoint or kinetic (with intervals of at least 20 seconds) readings.
**Note:** For the following performance, the Gain on the Optics Test should be below 6, in normal read mode.

#### Endpoint or Kinetic with an interval ≤ 20 seconds:

**Absorbance**
**Measurement Range:** 0.000 to 4.000 Abs

| | |
|---|---|
| **Accuracy:** | 0.000 to 2.000 Abs ± 1% ± 0.010 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.010 Abs |
| **Linearity:** | 0.000 to 2.000 Abs ± 2% |
| | 2.000 to 2.500 Abs ± 3% |
| **Repeatability:** | 0.000 to 2.000 Abs ± 2% ± 0.010 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.010 Abs |

#### Spectral Scanning or Kinetics with short intervals:

When read intervals from 32 seconds to 80 seconds are selected, or spectral scanning is performed, the specifications are as follows:

**Absorbance**
**Measurement Range:** 0.000 to 4.000 Abs

| | |
|---|---|
| **Accuracy:** | 0.000 to 2.000 Abs ± 1% ± 0.010 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.010 Abs |
| **Linearity:** | 0.000 to 2.000 Abs ± 2% |
| | 2.000 to 2.500 Abs ± 3% |
| **Repeatability:** | 0.000 to 2.000 Abs ± 2% ± 0.010 Abs |
| | 2.000 to 2.500 Abs ± 3% ± 0.010 Abs |

- **Hardware and Environmental Specifications**

| | |
|---|---|
| **Display:** | 2 X 24 Character LCD |
| **Light Source:** | Xenon flash light source<br>- 10 W max. average power<br>- Life: 1 billion flashes |
| **Dimensions:** | 16.0*"* deep X 15.5*"* wide X 9.75*"* high<br>40.1 cc deep X 39.3 cc wide X 24.8 cc high |
| **Weight:** | 40 lb. maximum (18.2 kg) |
| **Environment:** | Operational temperature 18-40°C |
| **Humidity:** | 10% to 85%, non-condensing |
| **Power Consumption:** | 100 VA<br>Four Voltage Ranges accommodated by voltage selection switch:<br>Range 1 100 VAC 90-110 VAC, 50-60 Hz<br>Range 2 115 VAC 103-127 VAC, 50-60 Hz<br>Range 3 230 VAC 207-253 VAC, 50-60 Hz<br>Range 4 240 VAC 216-264 VAC, 50-60 Hz |
| **Incubation option:** | Temperature Control: 4° over ambient to 50°C<br>Temperature Variation: ± 0.5°C across the plate @ 37°C (with the plate sealed) |
| **Internal bar code reader option:** | If enabled**,** the internal bar code reader recognizes a number of common bar code types during the plate read operation. Specific information about each bar code type is available from Bio-Tek Technical Services. The reader's bar code option is compatible with the following bar code types:<br>CODABAR UPC<br>CODE 39 EAN<br>INTERLEAVED 2 of 5 MSI<br>CODE 11 PLESSEY<br>CODE 93 CODE 128 |
| **Robotic interface option:** | The Robotic interface option allows the reader to function with an autoloading robot. Using computer control commands from a host PC, the reader's functions can be controlled in conjunction with the robotic system. The Robotic interface model can be configured with all options available for the standard *PowerWaveX.* |

## Technical Support

Bio-Tek's *PowerWaveX* is backed by a superior support staff. If the *PowerWaveX* 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 reader's on-board software configuration information. To see this, start at the reader main menu and press `UTIL` ➔ `TESTS` ➔ `CHKSUM`.
- The specific steps that produce your problem.
- Any error codes displayed on the screen (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 2*, *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

If you prefer, 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
# *Installation*

*This chapter includes instructions for unpacking and setting up the PowerWaveX, instructions for connecting to printers and/or serial devices, and descriptions of some of the reader's components.*

## Operating Environment

For optimal operation, install the *PowerWaveX* on a level surface in an area where ambient temperatures between 18°C (65°F) and 40°C (104°F) 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:** Optical density 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

| | |
|---|---|
| **iii** | **Important!** Keep the shipping cartons and the packaging 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 packaging materials have been damaged, replacements are available from Bio-Tek. |

1. Carefully open the top of the shipping container, and remove the dust cover. See *Figure 2-1*. The box will include a power cord, Operator's Manual, serial cord, and optionally, a Service Manual.
2. Remove the top foam from the reader.
3. Lift the reader out of the bottom foam, and place it on a level surface. Remove the reader from the plastic bag.

4. Place all shipping material back into the shipping box for reuse if the instrument needs to be shipped again.
5. Inspect the packaging and instrument for shipping damage such as visible dents or scratches.
6. If the reader is damaged, notify the carrier and your manufacturer's representative.
7. Contact Bio-Tek's Technical Assistance Center for an RMA (Return Materials Authorization) number before returning equipment for service. Mark the RMA number on the outside of the shipping container. See page 1-8 for contact information.

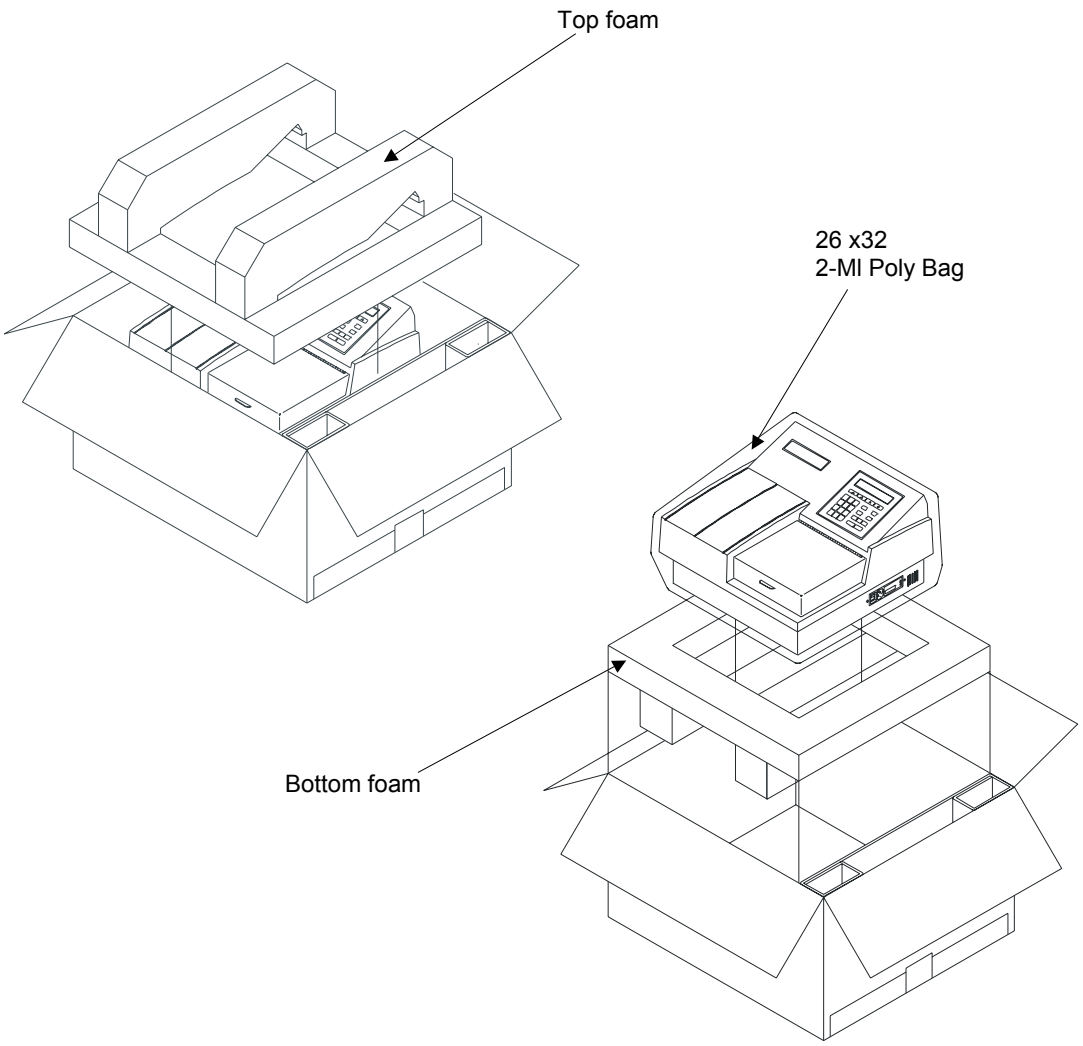



***Figure 2-1**: Unpacking and repackaging the reader*

## Removal of the Carrier Retention Screw in PowerWaveX Select Models

| | |
|---|---|
| iii | **Note:** The *PowerWaveX **Select*** model is shipped with a **carrier retention screw** that must be removed before the reader is used. An allen wrench (PN 49329) is supplied for this task. See *Figure 2-2* below. |

Lift up the front door. Using the allen wrench provided, remove the carrier retention screw, wave washer and flat washer. Save all of the shipping hardware.

| | |
|---|---|
| iii | **Important!** Replace the carrier retention screw and washers prior to shipment, or you will void the manufacturer's warranty. |

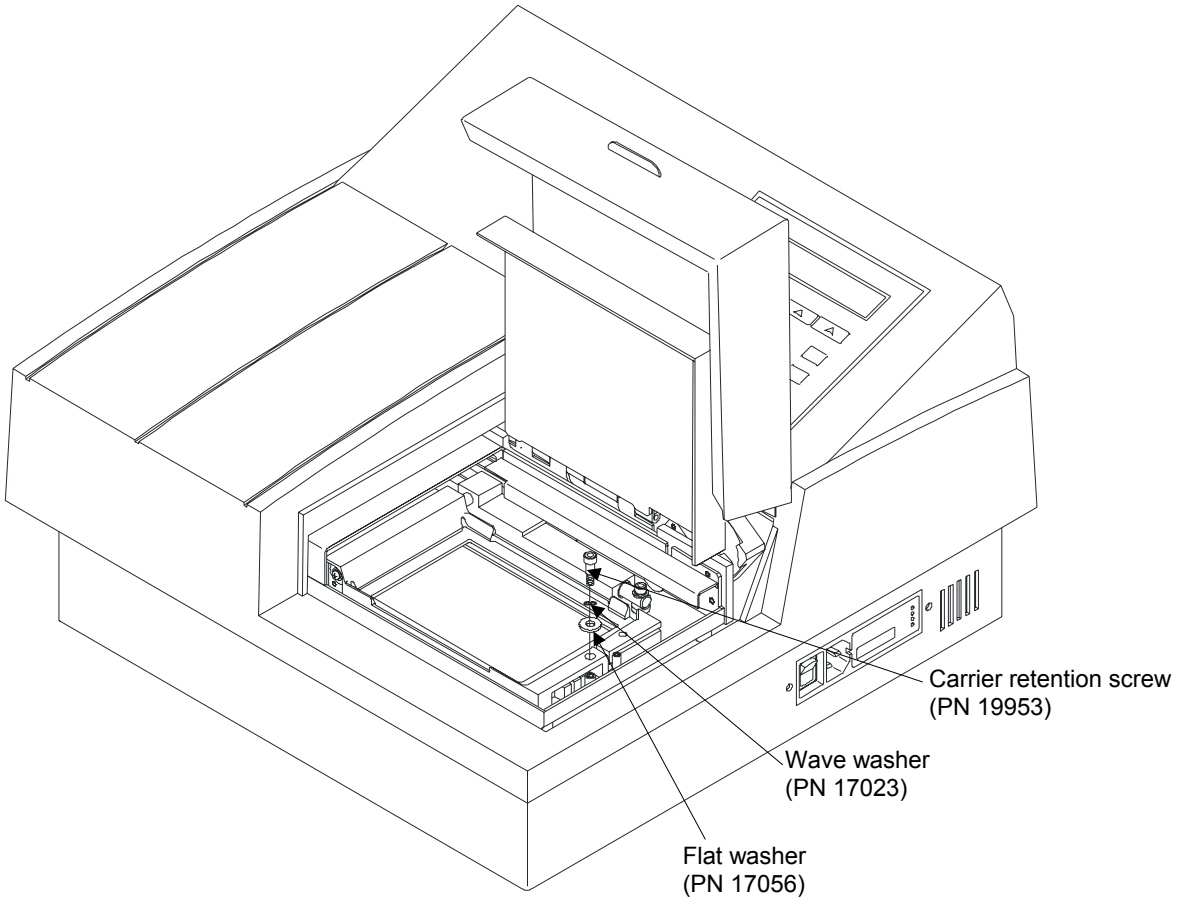



***Figure 2-2**: Removal of the carrier retention screw and accompanying washers from the PowerWaveX Select*

### AFTER UNPACKING, VERIFY PERFORMANCE

Before using the *PowerWaveX* for the first time, verify that it is operating properly by turning it on. 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.

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

### BEFORE REPACKAGING THE INSTRUMENT

1. Decontaminate the reader prior to shipping (see *Appendix A, Decontamination and Cleaning*).
2. For *PowerWaveX Select* models, replace the carrier retention screw and its accompanying washers. See *Figure 2-2* for an illustration.
3. Once the reader is clean, pack it 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 (PN 7263004).

## Adjusting the Reader's Wavelength Table Settings

The *PowerWaveX* has an internal wavelength table that stores six user-definable wavelength values. When defining an assay, these six wavelengths are presented as selectable options. These values can be easily viewed or changed:

- From the Main Menu, select `UTIL`.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | A | D | Y | | | | 9 | : | 4 | 5 | P | M | | | 0 | 5 | / | 0 | 9 | / | 9 | 9 |
| R | E | A | D | | | D | E | F | I | N | E | | R | E | P | O | R | T | | U | T | I | L |

*The Main Menu*

- The Select Utility Option menu appears.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | U | T | I | L | I | T | Y | | O | P | T | I | O | N | ? | | |
| T | E | S | T | S | | S | E | T | U | P | | O | U | T | P | U | T | | R | E | A | D | |

*Selecting SETUP from the Utility Option menu*

- From the Select Utility Option menu, select SETUP. The Edit Setup Information menu appears.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | ? | |
| | D | A | T | E | | | T | I | M | E | | L | A | M | B | D | A | | * | M | O | R | E |

*Selecting LAMBDA from the Edit Setup Information menu*

- From the Edit Setup Information menu, select LAMBDA.
- The Enter Lambda #1 screen appears, showing the first value in the wavelength table.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| L | A | M | B | D | A | # | 1 | | W | A | V | E | L | E | N | G | T | H | : | 2 | 0 | 0 | |

*Viewing or changing the reader's internal wavelength table*

- To advance to the next wavelength, press **ENTER.**
- To change the current wavelength value, use the reader's numeric keypad to enter a number at the current cursor location. Once a selection is made, the cursor automatically advances to the next editable location. To save the entry and advance to the next wavelength, press the **ENTER** key.
- Any wavelength in the range 200 to 999 nm may be entered (or 340 to 999 nm on the *PowerWaveX 340)*.
- When the last (sixth) wavelength has been entered or viewed, the software returns to the Edit Setup Information menu:

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | ? | |
| | D | A | T | E | | | T | I | M | E | | L | A | M | B | D | A | | * | M | O | R | E |

*Edit Setup Information menu*

- Press the **Main Menu** key to return to the Main Menu.

| | |
|---|---|
| iii | **Note:** After selecting new wavelengths, the reader system test must be run. From the Main Menu, press UTIL ➔ TESTS ➔ SYSTEM to start the test. |

## Printing and Data Communications

### INSTALLING A PRINTER

The parallel port on the *PowerWaveX* (see *Figure 2-3* below) allows connection to Epson- and HP-compatible deskjet printers. See *Compatible Printers* on page 2-17 for information on specifying a printer. The reader's parallel port requires a 25-pin D-sub-connector.

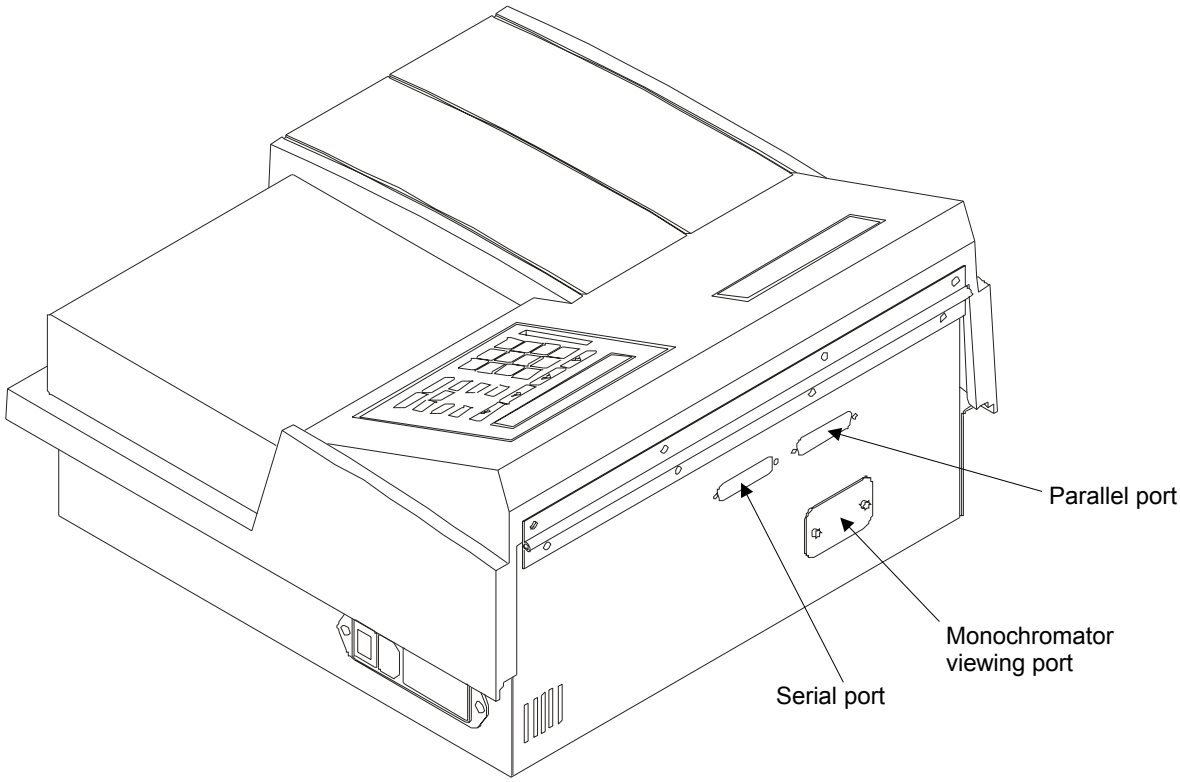



***Figure 2-3**: Serial and parallel connectors*

The parallel port's pinout definition is described in *Table 2-1*. A printer cable (PN 71072) is supplied with the reader. If the cable that came with the reader gets lost or damaged, Bio-Tek offers replacement printer cables.

Contact your authorized Bio-Tek dealer for information on cable prices and availability.

To attach a printer to the *PowerWaveX*:

1. Power down the printer and place it in a location adjacent to the *PowerWaveX*.
2. Attach one end of the parallel cable to the printer's parallel port.
3. Attach the other end of the cable to the reader's parallel port, located on the instrument's rear panel (see *Figure 2-3*).
4. Ensure that the securing screws on both ends of the cable are tightened, and power up the reader and printer.

| | |
|---|---|
| iii | **Important:** To avoid system instability, connect the printer to the reader *before* powering up the reader. Turn on the *PowerWaveX* first, then the printer. |

## PARALLEL PORT PIN DEFINITION

*Table 2-1* illustrates the pin definitions for the reader's 25-pin (socket-female) D-sub Parallel connector.

**Table 2-1**
**Parallel Connector Pinouts**

| Parallel Port Pinout | | | |
|---|---|---|---|
| **Pin** | **Signal** | **Pin** | **Signal** |
| 1 | PSTROBE | 14 | NC |
| 2 | D0 | 15 | NC |
| 3 | D1 | 16 | RESET |
| 4 | D2 | 17 | NC |
| 5 | D3 | 18 | GND |
| 6 | D4 | 19 | GND |
| 7 | D5 | 20 | GND |
| 8 | D6 | 21 | GND |
| 9 | D7 | 22 | GND |
| 10 | NC | 23 | GND |
| 11 | BUSY | 24 | GND |
| 12 | NC | 25 | GND |
| 13 | NC | | |

## SERIAL PORT FOR COMMUNICATION WITH OTHER DEVICES

The *PowerWaveX* has a 25-pin serial (RS-232) port located on the rear panel of the instrument (see *Figure 2-3* for an illustration of the serial cable connection). 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.

## CONNECTING TO THE PC AND ESTABLISHING COMMUNICATION

1. Power down the computer and the reader.
2. Connect the appropriate serial cable to both machines (see *Figure 2-3*). The serial port on the reader is a DTE configuration with a 25-pin (pin-male) D-sub connector. The connector's pinout is illustrated in *Table 2-2*.
3. Power up the reader and the computer. 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.
4. Ensure that the *PowerWaveX* and the computer are operating with the same communications parameters as described on the following page.

## SERIAL PORT PINOUT DESCRIPTION

*Table 2-2* describes the reader's serial /RS-232 pin connection.

**Table 2-2**
**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 | | |

Before serial communication can be initiated between the *PowerWaveX* and another device (such as a host PC running Bio-Tek's KC4 or KCjunior software packages), the communication parameters must match on both devices. The reader's default communication parameters are:

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

The reader's Baud Rate can be changed to 1200 or 2400, if necessary. The reader's Data Bits, Parity, and Stop Bits settings cannot be changed.

To change the Baud Rate:

- At the Main Menu, select `UTIL.`

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | A | D | Y | | | | 9 | : | 4 | 5 | P | M | | | 0 | 5 | / | 0 | 9 | / | 9 | 9 |
| R | E | A | D | | | D | E | F | I | N | E | | R | E | P | O | R | T | | U | T | I | L |

*Main Menu screen*

- At the Select Utility Option menu, select `SETUP`.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | U | T | I | L | I | T | Y | | O | P | T | I | O | N | ? | | |
| T | E | S | T | S | | S | E | T | U | P | | O | U | T | P | U | T | | R | E | A | D | |

*Selecting Setup*

- At the Edit Setup Information screen, select `*MORE` to see more options, including `RS232`.

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | | |
| | D | A | T | E | | | T | I | M | E | | L | A | M | B | D | A | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | ? | |
| R | S | 2 | 3 | 2 | | | C | A | L | | P | L | A | T | E | | | | * | M | O | R | E |

- Select `RS232` to advance to the Select Baud Rate screen.

- The top line of the display shows the currently selected Baud Rate.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | B | A | U | D | | R | A | T | E | : | | 9 | 6 | 0 | 0 | | |
| | 1 | 2 | 0 | 0 | | | 2 | 4 | 0 | 0 | | | 9 | 6 | 0 | 0 | | | V | I | E | W | |

*Selecting the Baud Rate*

- To **change** the Baud Rate, press the soft key corresponding to the desired setting. The top line of the display automatically updates to reflect the new setting.
- To **view** the reader's other communication settings, select `VIEW`.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | S | 2 | 3 | 2 | | S | E | T | T | I | N | G | S | : | N | O | | P | A | R | I | T | Y |
| 2 | | S | T | O | P | - | B | I | T | S | | | 8 | | D | A | T | A | - | B | I | T | S |

*Viewing Other Communication Settings*

## Components

### LAMP

The 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, contact Bio-Tek for bulb replacement information.

### COOLING FAN

Located on the bottom right edge of the instrument is a switch that activates an internal **cooling fan.** When the *PowerWaveX* is on, the temperature in the measurement chamber will be approximately 7°C above ambient temperature.

For users that need the measurement chamber to be close to **ambient,** Bio-Tek recommends using the cooling fan. The fan will bring the temperature within 4°C of ambient. This would be important (for example) for kinetic measurements that need to be made close to room temperature.

The fan should be off when non-temperature-sensitive testing is being completed, as prolonged use may introduce dust into the internal mechanisms of the instrument.

## Adjusting the Line Voltage Input Range

The *PowerWaveX* is equipped with a four-voltage range power input module. This power input module, located on the right side of the instrument, can be adjusted for 100 VAC, 115 VAC, 230 VAC and 240 VAC voltage inputs. The setting can be determined visually by observing which indicator hole on the power input module has a peg within it. The ON/OFF switch and fuses are also housed within the power input module. The following instructions can be used to change the input voltage range. *Figures 2-4A* and *B* illustrate the power input module.

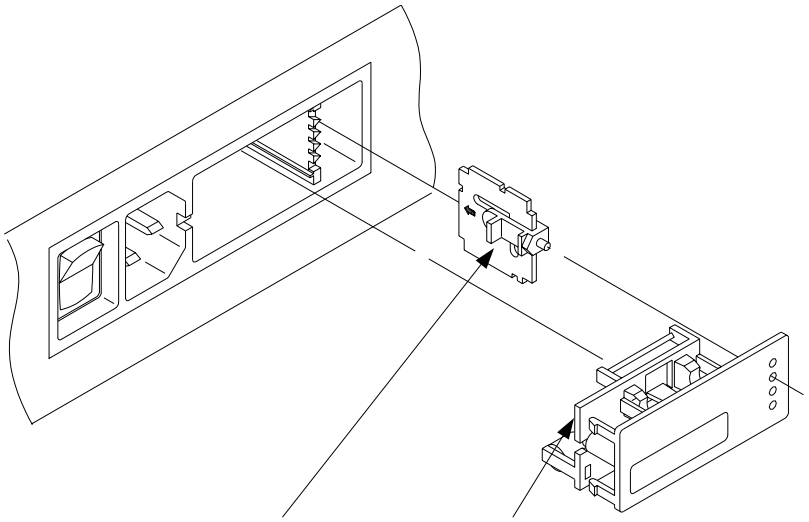


***Figure 2-4A:*** *Voltage selector board and fuse holder*

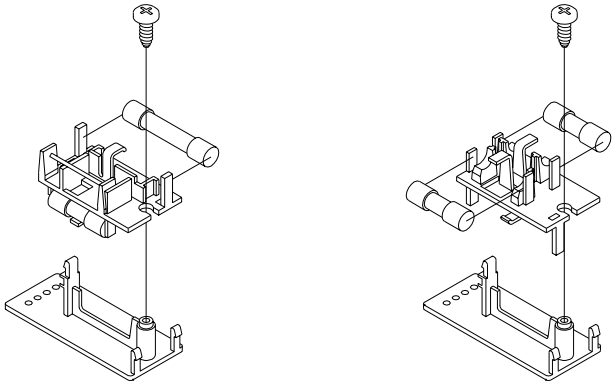


***Figure 2-4B:*** *Selecting fuse position*

1. Unplug the reader and remove the power cord.
2. Use a small flat-blade screwdriver to pop the fuse holder out of the power input module.

3. A small voltage select switchboard is located on the right of the power input module. The switchboard, which can be removed with needle-nose pliers, can be oriented four ways to select four different voltage input ranges.
4. Once the switchboard is removed, text can be read on one side of the board. This text identifies the switch positions. The text is always facing the ON/OFF switch when installed in the power input module. The text matching the desired voltage range should be on the edge of the switchboard, which goes into the power input module as the arrow on the board indicates. The white plastic indicator will need to rotate around so that it fits into the correct groove on the switchboard.
5. Verify proper fusing before reinserting the switchboard and fuse holder.
6. Once the switchboard is reinstalled, the white plastic indicator peg should line up with the fuse holder voltage indicator holes. If the peg does not fit in the hole that indicates the voltage intended, the switchboard is not installed correctly. Do not power up the instrument until the voltage input range to be used is indicated correctly by the peg.

## Adjusting the Fuse Configuration or Fuse Replacement

Both U.S. and European fuses are installed in the reader's power input fuse module. The reader's fuses are configured at the factory prior to shipping. Use the following procedure if you need to change the fuse configuration, or replace fuses. A failed fuse is usually an indication of another problem, which a new fuse is not likely to fix. Contact Bio-Tek Technical Services if the fuse replacement fails to rectify the problem.

1. Use a small, flat-blade screwdriver to remove the fuse module from the power input module.
2. The fuse module has two fuse configurations:
    - The U.S. configuration has a Hot fused .75-amp slo blo (PN 46023).
    - The European configuration has both Hot and Neutral fused .315-amp slo blo T 5 X 20 mm (PN 46051).
3. To replace a defective fuse, pop out the old fuse and replace it with the correct new one.
4. The configuration of the fuse module (U.S. or European) is determined by which fusing network is facing the inside of the power input module (refer to *Figure 2-4B*). To change the configuration, remove the Phillips-head screw that anchors the fuse holder. Remove the fuse holder and turn it over. Replace the screw and reinstall the fuse module in the power input module.

## Other Utility Options

The *PowerWaveX* contains several global configurable options, such as date and time, report output, and plate reading preferences. These options are accessed via the Select Utility Option menu (see below), and include:

- **TESTS:** Run a SYSTEM test to check reader optics or the CALPLATE test to check alignment and calibration. Select CHKSUM to perform an internal checksum test, and to display the version and configuration information for the currently installed software.
- **SETUP:** Set the current date and time, as well as the date and time formats.
- **OUTPUT:** Specify where plate data should be sent, to a printer, a computer, or to both. Additional options include report format (Column and/or Matrix), and whether or not to print standard curves.
- **READ:** Enable or disable read-time prompting for Plate ID, Sample ID, and Sample Count. Specify whether or not to read in Rapid mode.

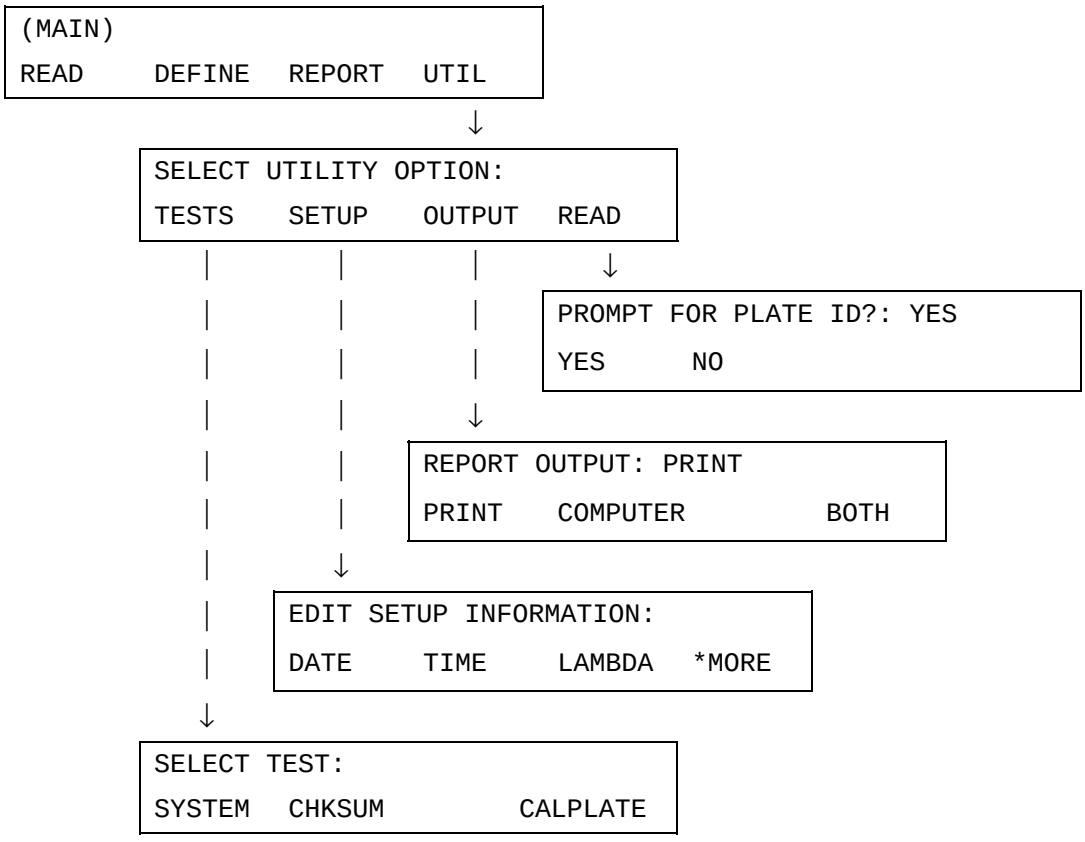



***Figure 2-5:*** *Options available under UTIL*

To set the current Date and Time, and/or to change their formats:

- From the Main Menu, select `UTIL` ➔ `SETUP`.

  The Edit Setup Information menu will appear.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | | |
| | D | A | T | E | | | T | I | M | E | | L | A | M | B | D | A | | * | M | O | R | E |

*Selecting DATE from the Edit Setup Information menu*

- Select `DATE`. The DATE entry screen will appear.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | A | T | E | : | | | 0 | 3 | / | 1 | 6 | / | 9 | 9 | | | | | | | M | D | Y |
| M | M | D | D | Y | Y | | | | | | | D | D | M | M | Y | Y | | | | | | |

*Entering the Date and selecting a format*

- Enter the date using the numeric keys on the keypad. The cursor is positioned under the first editable field, and advances automatically.
- To change the date format, select `MMDDYY` or `DDMMYY`. The display automatically updates to reflect the new format.
- Press **ENTER** to return to the Edit Setup Information menu.
- To change the current time and/or the time format, select `TIME` from the Edit Setup Information menu. The Time entry screen will appear.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | I | M | E | : | | | | 0 | 3 | : | 1 | 1 | P | M | | | | | | 1 | 2 | H | R |
| 1 | 2 | H | O | U | R | | | | | | 2 | 4 | H | O | U | R | | | A | M | / | P | M |

*Entering the Time and selecting a format*

- Enter the time using the numeric keys on the keypad. The cursor is positioned under the first editable field, and advances automatically.
- To change the time format, select `12HOUR` or `24HOUR`, then `AM` or `PM`. The display automatically updates to reflect the new format.
- Press **ENTER** to return to the Select Utility Options menu.

To specify data output options:

- From the Main Menu, select UTIL ➔ SETUP ➔ OUTPUT. The Report Output screen will appear.

### Data Output

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | P | O | R | T | | O | U | T | P | U | T | ? | | P | R | I | N | T | |
| P | R | I | N | T | | | | C | O | M | P | U | T | E | R | | | B | O | T | H | |

*Selecting the Report Output*

- The current output option is displayed on the top line. Select PRINT to send reports directly to a printer, COMPUTER to send data out through the serial port, or BOTH.
- **Note:** These options have no effect on data output if the reader is being controlled by software (such as KC4 or KCjunior) running on a host PC.
- Press **ENTER** to continue. The Select Printer screen will appear.

### Compatible printers

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | P | R | I | N | T | E | R | : | | E | P | S | O | N | | |
| E | P | S | O | N | | | | H | P | | | | | | | | | | | | | |

*Selecting a printer*

- BTI readers support printers using either HP's PCL3 language, such as the HP DeskJet series, or Epson's LQ language. For the latest list of compatible printers, contact Bio-Tek Instruments' Technical Assistance Center (see page 1-8 for details), or visit our website, www.biotek.com.
- Select EPSON or HP as appropriate.
- Press **ENTER** to continue. The Report Type screen will appear.

## Report Types

| R | E | P | O | R | T |  | T | Y | P | E | ? |  | M | A | T | R | I | X |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | L | U | M | N |  |  |  |  |  |  | M | A | T | R | I | X |  |  | B | O | T | H |

*Selecting the Report Type*

- **Note:** *Appendix D* contains sample reports.
- The currently selected report type is displayed in the top line. Select COLUMN to print information in a list (columnar) format, MATRIX to print in a format that resembles the plate type (e.g., 8 x 12 matrix), or BOTH.
- Press **ENTER** to continue. The Samples in Col Rpt screen will appear.

| S | A | M | P | L | E | S |  | I | N |  | C | O | L |  | R | P | T | ? |  |  | N | O |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Y | E | S |  |  |  |  | N | O |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

*Specifying whether or not to include samples on the Column Report*

- Select YES to print results for all wells on the plate, including samples.
- Select NO to limit the results information to blanks, controls, and standards.
- Press **ENTER** to continue. The Print Curve-Fit screen will appear.

| P | R | I | N | T |  | C | U | R | V | E | - | F | I | T | ? |  | N | O |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Y | E | S |  |  |  |  | N | O |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

*Specifying whether or not to print the standard curve*

- Select YES to print the standard curve (if the assay generates one), or NO to suppress this report.
- Press **ENTER** to return to the Select Utility Option menu.

To specify various read-time options:

- From the Main Menu, select `UTIL` ➔ `READ`.

  The Prompt for Plate ID screen will appear. Press **ENTER** to cycle through the prompt screens.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P** | **R** | **O** | **M** | **P** | **T** | | **F** | **O** | **R** | | **P** | **L** | **A** | **T** | **E** | | **I** | **D** | **?** | | **Y** | **E** | **S** |
| | **Y** | **E** | **S** | | | | | **N** | **O** | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P** | **R** | **O** | **M** | **P** | **T** | | **F** | **O** | **R** | | **S** | **A** | **M** | **P** | **L** | **E** | | **I** | **D** | **?** | | **N** | **O** |
| | **Y** | **E** | **S** | | | | | **N** | **O** | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P** | **R** | **O** | **M** | **P** | **T** | | **S** | **A** | **M** | **P** | **L** | **E** | | **C** | **O** | **U** | **N** | **T** | **?** | | **Y** | **E** | **S** |
| | **Y** | **E** | **S** | | | | | **N** | **O** | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R** | **E** | **A** | **D** | | **I** | **N** | | **R** | **A** | **P** | **I** | **D** | | **M** | **O** | **D** | **E** | **?** | | **N** | **O** | | |
| | **Y** | **E** | **S** | | | | | **N** | **O** | | | | | | | | | | | | | | |

*Selecting Read Preferences*

- If selected, at read-time:

  `PLATE ID` prompts for microplate identification.

  `SAMPLE ID` prompts for an identification for each sample group.

  `SAMPLE COUNT` prompts for the number of samples on the plate.

- If `RAPID MODE` is disabled, the plate reads at a speed designed to ensure low CVs and precise results. If Rapid Mode is enabled, the plate reads more quickly but may possibly result in higher CVs. If an assay favors speed over precision, consider reading in Rapid Mode.
- Pressing **ENTER** after each selection advances the display.
- When selections are completed, the display returns to the Select Utility Option menu.

# Chapter 3
# *Operation*

*This chapter includes instructions for operating the PowerWaveX and its software.*

## Overview

The *PowerWaveX* features a 25-key front panel with a 2 X 24 character LCD (Liquid Crystal Display), allowing access to the reader's program menus. Test results can be sent to an attached printer and/or through the reader's bi-directional serial port to a host computer. The serial port also allows for computer control of the instrument, and provides the means for downloading additional assay definition files to the instrument.

The *PowerWaveX* may be loaded with custom pre-programmed assays and/or with "Open" assays.

- The pre-programmed assays allow you to easily select, modify, and run (for example), several common ELISA and important nucleic acid applications directly from the front panel.
- The Open assays contain standard default settings, upon which assays can be programmed.

This chapter describes how to program Open assays on the *PowerWaveX*. The custom pre-programmed assays are described in Bio-Tek's ***Assay Reference Guide*** (PN 7271006).

| | |
|---|---|
| iii | **Important:** Screens and options throughout this chapter pertain to "Open" assays. Pre-programmed assays may not show all possible screens or options, or may show custom screens not described here. Please refer to the Assay Reference Guide for details on pre-programmed assays. |

## Front Panel

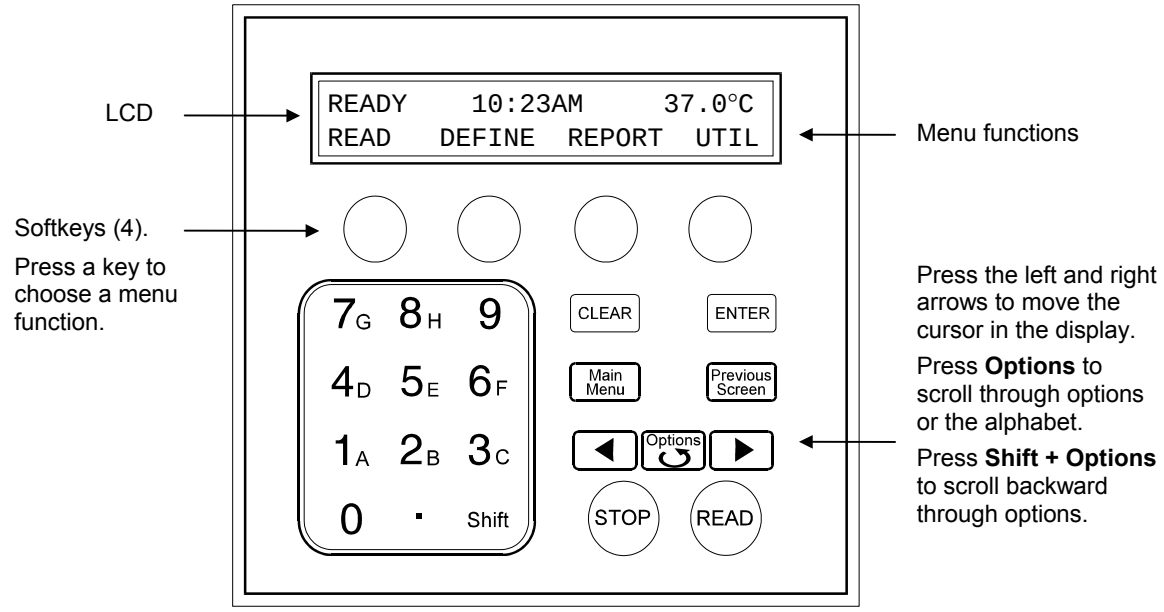



***Figure 3-1:*** *PowerWaveX Front Panel*

### SELECTING MENU FUNCTIONS

The menu functions appear in the bottom (second) line of the front panel's LCD. To make a selection, press the SoftKey below the menu function.

### SCROLLING THROUGH OPTIONS

Certain functions, such as entering an assay name or selecting an assay, offer a set of options from which to make a selection. To view the different options, press the **Options** key or the **Shift + Options** key combination. Press the **ENTER** key to select the current option.

### ENTERING ASSAY NAMES

```
NAME:  DNA Quant
  -      /      :      _
```

Each *PowerWaveX* assay requires the entry of a name, using up to 16 alphanumeric characters. To enter an assay name via the keypad, press Shift + key A-H, or scroll through the alphabet with the **Options** key for A-Z. Use the forward or reverse arrows to move the cursor within the display. To delete an entry, press the **CLEAR** key. Press the **ENTER** key to store the completed assay name and continue.

The NAME display offers four symbols that can be used in an assay name: dash (hyphen), forward slash, colon, and underscore. These symbols appear in the LCD as SoftKey choices (see above). To include a symbol within an assay name, press its corresponding SoftKey.

### MOVING BACKWARD THROUGH THE MENUS

To move to a previous menu, press the **Previous Screen** key.

### SAVING ASSAYS

After defining an assay, press the **Main Menu** key. The changes are saved automatically.

### STARTING OR STOPPING A READ

To begin a plate read, press the **READ** key. To abort the read, press the **STOP** key.

## Startup Screen

The *PowerWaveX* performs a self-test when powered on, displaying the Startup screen until initialization is complete. During this period, no keys are active. If the instrument fails to pass the self-test, a beep will sound, and an error code will display. Refer to *Appendix C* to interpret error codes. Contact Bio-Tek Instrument's Technical Assistance Center for assistance on troubleshooting errors (see page 1-8 for contact information).

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | N | S | T | R | U | M | E | N | T | | I | D | | | | | | | | | |
| S | E | L | F | - | T | E | S | T | . | . | . | | | | | | | | | | |

*PowerWaveX startup screen*

## Main Menu

Once the system is initialized, the **Main Menu** is displayed. The keypad's four SoftKeys, located below the on-screen menu options (READ, DEFINE, REPORT and UTIL), are activated, and may be selected.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | A | D | Y | | | | 9 | : | 4 | 5 | A | M | | | 0 | 5 | / | 0 | 9 | / | 9 | 9 |
| R | E | A | D | | | D | E | F | I | N | E | | R | E | P | O | R | T | | U | T | I | L |

*Main Menu screen*

Press the SoftKey that corresponds to a displayed menu option to activate that option:

- **READ:** Choose an assay for plate reading. Alternatively, press the key labeled READ on the keypad.
- **DEFINE:** Define the data reduction parameters for a new assay, or modify an existing one.
- **REPORT:** Print a Result, Map, Assay, or List report.
- **UTIL:** Run a System Test or Calibration Plate Test. Set up various global configuration options such as date and time, report output, and plate reading preferences.

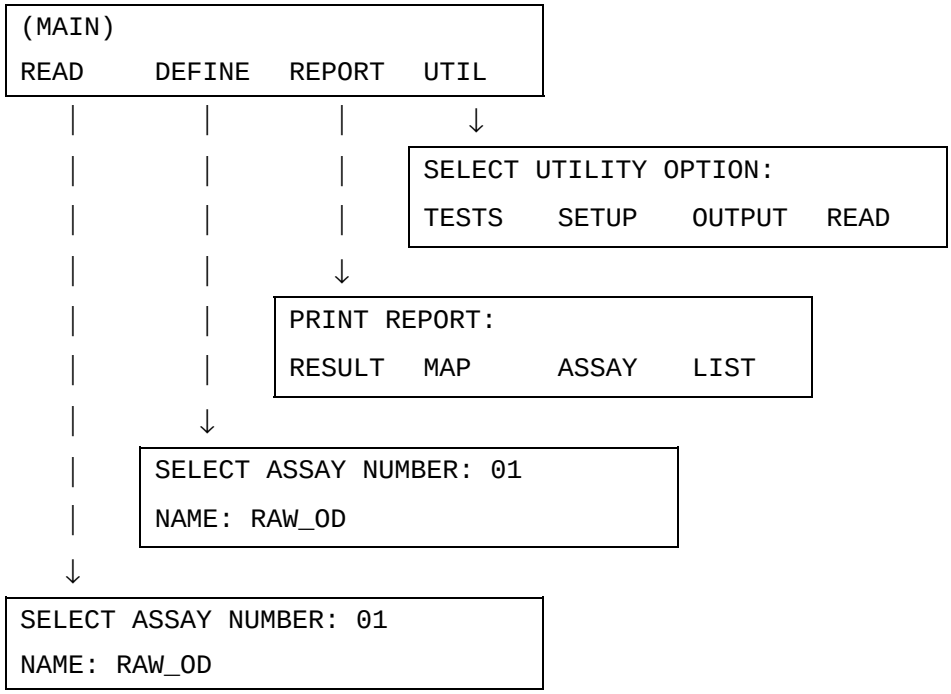
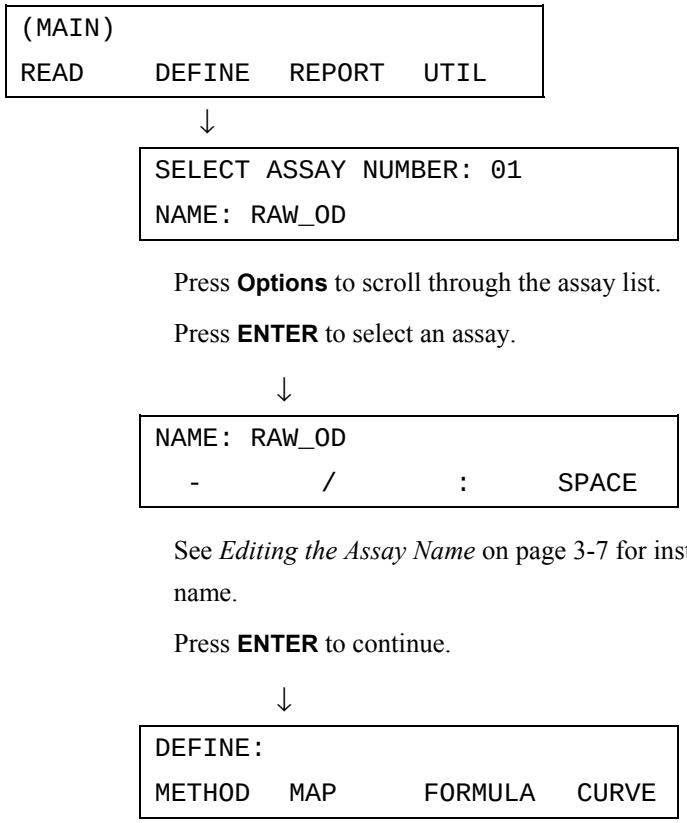



***Figure 3-2:*** *Options available from the Main Menu*

## DEFINE

The Main Menu option DEFINE allows you to define the data reduction parameters for a new assay, or to modify a previously defined assay stored in memory.




Press **Options** to scroll through the assay list.

Press **ENTER** to select an assay.

See *Editing the Assay Name* on page 3-7 for instructions on modifying the current name.

Press **ENTER** to continue.

***Figure 3-3:*** *Options available under* DEFINE

- **METHOD:** Define the method of reading, such as Endpoint, Kinetic, or Scan, and Single or Dual Wavelength. Specify a delay before read, or incubation settings.
- **MAP:** Specify the plate layout, using blanks, controls, standards, and/or samples. Choose to map the plate manually, or let the software map it automatically.
- **FORMULA**: Define cutoff, transformation, validation, and/or general formulas. Create variables to be used within formulas.
- **CURVE**: Specify a curve fit type and x/y axis types (lin/log). Specify the method by which standard outliers can be edited. Enable or disable the extrapolation feature.

From the Main Menu, press the soft key beneath the DEFINE menu option to access the Select Assay Number screen.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | A | S | S | A | Y | | N | U | M | B | E | R | : | 0 | 1 |
| N | A | M | E | : | H | B | S | -A | G | 1 | | | | | | | | | | | |

*Assay Selection screen showing the current assay name and number*

- Use the **NUMERIC** keys to enter the number of any predefined assay stored in the reader's memory, or the **OPTIONS** key to advance one assay at a time. The cursor is positioned at the first editable field, and advances automatically. The numeric range depends on the number of assays programmed in the reader's memory. (The reader typically has 55 "open" assays available.)
- Press **ENTER** to advance to the Edit Assay Name screen. You may change the default assay number to a more descriptive one (see *Editing the Assay Name* on the following page):
  - **CLEAR:** Clears the reader's display.
  - **MAIN MENU:** Returns the display to the Main Menu screen.
  - **PREVIOUS SCREEN:** Returns the display to the previous screen.
  - **ENTER:** Saves the current settings and advances to the next screen.

| | |
|---|---|
| iii | **Note:** Within every screen, these keys will continue to function as described above. |

Use the Edit Name screen to edit the name currently assigned to the assay. The assay name can contain up to 16 alphanumeric characters.

| N | A | M | E | : | | H | B | S | - | A | G | 1 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | | | | | | | / | | | | | | : | | | | | S | P | A | C | E |

*Edit Assay Name screen*

- The cursor is positioned at the first editable field (e.g., under "H").
- Use the **ALPHA** and **NUMERIC** keys to change the Assay name.
- Use the **OPTIONS** key to sequentially advance the character positioned above the cursor. The characters will cycle through the alphabet (A-Z), with a space following Z.
- Use the **LEFT◄** and **RIGHT►** arrow keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Use **SOFT KEYS 1, 2, 3** and **4** to select a dash, forward slash, colon, or space for inclusion in the assay name.

## Define METHOD

To define read method parameters, start at the Main Menu and select DEFINE, select the assay, press **ENTER**, then select METHOD.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | E | F | I | N | E | | | | | | | | | | | | | | | | |
| M | E | T | H | O | D | | M | A | P | | F | O | R | M | U | L | A | | C | U | R | V | E |

*Define options screen*

The definable read method parameters include:

- Endpoint, Kinetic or Well Scanning Read Modes
- Delay first read
- Incubation parameters
- Wavelengths applied
- Shake parameters
- Kinetic analysis
- Number of points for well scanning
- Spectral scan (Assay 00 only)

| | |
|---|---|
| iii | **Note:** Some screens described in this section may not appear on certain reader models. |

### READ TYPE

Use the READ TYPE screen to specify the reading method, Endpoint, Kinetic, or Scan.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | A | D | | T | Y | P | E | : | K | I | N | E | T | I | C | | | | | | | | |
| E | N | D | P | O | I | N | T | | | | K | I | N | E | T | I | C | | S | C | A | N | |

*Read Type screen*

- During an ENDPOINT read, the plate is read once with one (single) or two (dual) wavelengths. One optical density (OD) value is reported for each well.

- During a KINETIC read, the plate is read a defined number of times with one (single) or two (dual) wavelengths. Three different types of data are available (see *Kinetic Analysis* on page 3-15):
    - For a rate determination, the data calculated is the rate of OD change. After determining the maximum rate of change per well using linear regression, the units are milliOD/min.
    - The R-squared value is an indication of how well the points fit the linear curve.
    - The Onset time indicates how many seconds it took to reach a user's predetermined OD value.
- During WELL SCANNING, the Optical Density values are measured at different points across the well diameter. Choose from 1 to 31 points total (15 left of center, 15 right of center). If you choose 1, then the plate is read as an endpoint assay. The data out is equal to the sum of all of the OD values recorded for the well. To find the average OD value for the region scanned, divide the scanned OD result by the total number of scans. For more information, see *Well Scanning* on page 3-16.

## Spectral Scanning

Well Scanning and **Spectral Scanning** are two different read methods.

Certain readers contain the pre-programmed assay SPECTRAL SCAN (number 00). This assay allows for the definition of start, stop, and step (increment) wavelength values. For example, if the Start/Stop/Step values are 200/600/10 nm, respectively, each well will be read at 200, 210, 220 … 600 nm.

The maximum number of wavelengths that can be specified for reading within an assay is 100. The printed report shows up to 100 points and includes the Peak OD and wavelength at Peak OD.

## DELAY FIRST READ

The Delay First Read option can be used to allow for sufficient time for microwell contents to settle, or to incubate a plate before reading.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | E | L | A | Y | | F | I | R | S | T | | R | E | A | D | | | | | | |
| T | I | M | E | : | | | | | | | | | | | | | M | M | : | S | S |

*Delay in first read entry*

- Use the **NUMERIC** keys to enter the time in minutes and seconds. The maximum delay time is 59 minutes, 59 seconds (59:59).

## INCUBATION TEMPERATURE (READER-DEPENDENT)

Certain reader models are equipped with heaters to perform plate reads at elevated temperatures (see *Chapter 1, Introduction* for information on these models). The Incubation Temperature screen is used to enable incubation for this assay, and to set the temperature.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | N | C | U | B | A | T | I | O | N | | T | E | M | P | : | | 3 | 7 | ° | C | |
| A | M | B | I | E | N | T | | | | | | T | E | M | P | E | R | A | T | U | R | E |

*Incubation Temperature entry*

- Select `AMBIENT` to read the plate at room temperature.

  **Note:** See *Cooling Fan* in Chapter 2 for information on lowering the ambient temperature by turning on the internal fan.

- Select `TEMPERATURE` to read the plate at an elevated temperature.
- If `TEMPERATURE` is selected, use the **NUMERIC** keys to enter the incubation temperature. The valid range is from 25 to 50° C. Incubation performance is specified from 4° over ambient to 50° C.

## WAVELENGTH(S)

Use the Wavelength selection screen to specify whether the plate will be read at one (SINGLE) or two (DUAL) wavelengths.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W | A | V | E | L | E | N | G | T | H | : | | D | U | A | L | | | | | | |
| S | I | N | G | L | E | | D | U | A | L | | | | | | | | | | | |

*Specifying the Wavelength mode, Single or Dual*

- If `SINGLE` is chosen, the reader measures the optical density of each well with a single wavelength.
- If `DUAL` is chosen, each well is read twice, at two different wavelengths. The microplate is not removed from the reading chamber between the two measurements. The final reported optical density is the difference between the two readings.

| | |
|---|---|
| **iii** | **Note:** Dual wavelength readings can significantly reduce optical interference caused by scratches or fingerprints that absorb light equally at both wavelengths. Choose a REF wavelength that is far from the MEAS wavelength in the spectrum for best results. |

## MEASUREMENT WAVELENGTH(S)

After specifying Single or Dual Wavelength, the MEAS or MEAS/REF selection screen appears, for wavelength selection.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | E | A | S | : | 4 | 0 | 5 | | | | | | | | | | | | | | |
| 4 | 0 | 5 | | | | | 4 | 5 | 0 | | | | 4 | 9 | 0 | | | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | E | A | S | : | 4 | 5 | 0 | | R | E | F | : | 6 | 3 | 0 | | | | | | |
| 4 | 0 | 5 | | | | | 4 | 5 | 0 | | | | 4 | 9 | 0 | | | | * | M | O | R | E |

*Specifying the wavelength(s) to be used during plate reading. Single wavelength selection is shown on top, Dual wavelength selection on bottom.*

- If reading at a single wavelength, a MEASurement wavelength must be selected. If reading at dual wavelengths, a REFerence wavelength must also be selected.
- Choose a wavelength value from those presented. Select `*MORE` to see additional wavelengths.
- **Note:** If a desired wavelength is not listed, the reader's internal wavelength table must be changed. Press the **Main Menu** button to save the current assay settings and return to the Main Menu. See *Adjusting the Reader's Wavelength Table Settings* in *Chapter 2* for instructions.

## KINETIC READ PARAMETERS

If the Kinetic read type was specified, a few accompanying parameters must be defined, including kinetic method, reading interval, and number of reads.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | : | | T | O | T | A | L | | R | E | A | D | S | | |
| T | O | T | A | L | | R | E | A | D | S | | | D | U | R | A | T | I | O | N | |

*Kinetic reading method selection screen*

- Select `TOTAL READS` to read the plate a specified number of times.
- Select `DURATION` to read the plate for a specified length of time.

### Kinetic Interval

Regardless of the kinetic reading method selected (Total Reads or Duration), a Kinetic Interval is required. This is the length of time (in hours, minutes and seconds), between each kinetic read.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | | | | | | | | | | | | | | | |
| I | N | T | E | R | V | A | L | : | | | H | H | : | M | M | : | S | S | | | |

*Kinetic Interval entry screen*

- Use the **NUMERIC** keys to enter the interval.
- The valid entry ranges are: 0-1 hours, 0-59 minutes, and 0-59 seconds.
- **Note:** If the kinetic reading method is `DURATION`, the Interval/Duration combination must result in 2 to 40 total kinetic reads.

### Total Number of Kinetic Reads

If the TOTAL READS kinetic reading method was selected, specify the total number of kinetic reads.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | | T | O | T | A | L | | | | | | | | | |
| N | U | M | B | E | R | | O | F | | R | E | A | D | S | : | | 1 | 0 | | | |

*Kinetic Reads entry screen*

- Use the **NUMERIC** keys to enter the total number of reads to perform.
- The valid range is 2 to 40 reads.

### Kinetic Duration

If the DURATION kinetic reading method was selected, specify the total length of time during which readings will be taken.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | | | | | | | | | | | | | | | |
| D | U | R | A | T | I | O | N | : | | H | H | : | M | M | : | S | S | | | | |

*Kinetic Duration entry screen*

- Use the **NUMERIC** keys to enter the time duration in hours, minutes and seconds.
- The maximum duration time is 80 hours. **Note:** The Kinetic Interval/ Duration combination must result in 2 to 40 total kinetic reads.

## PLATE SHAKING

A variety of plate shaking options are available on the *PowerWaveX* reader.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | H | A | K | E | : | B | E | F | O | R | E | | E | V | E | R | Y | | R | E | A | D | |
| F | I | R | S | T | | E | V | E | R | Y | | | N | O | N | E | | | | | | | |

*Shake Mode Selection screen*

- For **non-kinetic** reads, choose FIRST to enable shaking, or NONE to indicate no shaking.
- For **kinetic** reads, choose FIRST to shake before the first read only, EVERY to shake before every read, or NONE to indicate no shaking.

### Shake Time

If shaking is enabled, a Shake Time must be specified.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | H | A | K | E | | T | I | M | E | : | | H | H | : | M | M | : | S | S | | |
| C | O | N | T | I | N | U | O | U | S | | | | | | | | | | | | |

*Shake Time entry screen*

- Use the **NUMERIC** keys to enter the shake duration. Valid ranges are: 0-1 hours, 0-59 minutes, and 0-59 seconds.
- For a kinetic read with Shake Before Every Read enabled, the CONTINUOUS option is available. If selected, the shake duration is set automatically, according to the kinetic interval.

### Shake Speed

For the *PowerWaveX*, the shake movement is a repeated 0.021-inch movement from the shake position and back. Choose from several different speeds, including a Variable option.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | H | A | K | E | | S | P | E | E | D | : | | M | E | D | I | U | M | | | |
| L | O | W | | | M | E | D | I | U | M | | H | I | G | H | | | | V | A | R | I | |

*Shake Speed selection screen*

- Select `LOW` for low-speed (17 Hz) shaking.
- Select `MEDIUM` for medium-speed (18 Hz) shaking.
- Select `HIGH` for high-speed (19 Hz) shaking.
- Select `VARI` variable-speed shaking (1 second of each speed repeated).

For kinetic reads there are three calculation options to choose from.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | | A | N | A | L | Y | S | I | S | : | | R | - | S | Q | R | |
| R | A | T | E | | | R | - | S | Q | R | | O | N | S | E | T | | | | | | | |

*Kinetic Data Analysis selection screen*

- Select RATE to apply a linear fit to calculate the maximum slope in mOD/min. based on the number of kinetic points specified.
- Select R-SQR to calculate the R-squared value at the maximum slope, based on the linear curve fit and the number of kinetic points specified.
- Select ONSET to calculate the time it takes for each well to reach the specified onset optical density.

## Kinetic Points

For the kinetic analysis selections RATE and R-SQR, specify the number of sequential kinetic points used to calculate the steepest Rate or the R-squared value at the steepest Rate.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | I | N | E | T | I | C | | P | O | I | N | T | S | : | | | | | | 0 | 3 | |
| A | L | L | | P | O | I | N | T | S | | | | | | | | | | | | | |

*Defining the number of Kinetic Points for use in calculations*

- Select ALL to indicate all of the kinetic reads.
- To specify a subset of kinetic reads, select POINTS, then use the **NUMERIC** keys to input the number of points. The valid entry range is 2 to the total number of kinetic reads.

| | |
|---|---|
| iii | **Note:** If the number of kinetic reads is changed during assay definition, you may need to adjust the Kinetic Points setting. The number of Kinetic Points must be less than or equal to the total number of kinetic reads. |

### Onset OD

For the Kinetic Analysis selection `ONSET`, an Onset OD is required.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | |
| O | N | S | E | T | | O | D | : | | | | | | | | | 2 | . | 5 | 0 | 0 |

*Entering the Onset OD for kinetics*

- Use **NUMERIC** keys to enter the onset OD. The valid entry range is 0.000 to 3.000 OD.

## WELL SCANNING

For the Scanning Read Type, multiple readings are taken across each microwell. The number of readings, or Scan Points, is configurable.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | N | U | M | B | E | R | | | | | | | | | |
| O | F | | S | C | A | N | | P | O | I | N | T | S | ? | | 1 | 1 | | | |

*Entering the number of scan points per well*

- The maximum number of Scan Points is 31. Only odd integers are accepted, to ensure that the center of the well is always read.
- The 31 scan positions are fixed in the software. You must determine the optimal number of scans per well. If, for example, 7 scans across the well is chosen, the reader will read the centermost seven points in the well. The more scan points chosen, the closer to the well sides reads will be taken.
- The reader reports the sum of OD values for all points scanned.

| | |
|---|---|
| iii | **Note:** Exercise caution when specifying the Scan Points value. If too many points are specified, the sides of the wells may be getting read, possibly resulting in an artificially high reading. |

## Define MAP

To configure the plate layout, start at the Main Menu and select DEFINE, select an assay, press **ENTER**, then press MAP.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | E | F | I | N | E | | | | | | | | | | | | | | | | |
| M | E | T | H | O | D | | M | A | P | | F | O | R | M | U | L | A | | C | U | R | V | E |

*Selecting the Map option on the DEFINE screen*

The following MAP parameters can be defined/modified:

- Automatic or Manual Map Generation
- Mapping Direction
- Replication Direction
- Blank Map Selection
- Blanking Constant
- Number of Blanks
- Location of Blanks
- Number of Standards
- Number of Standard Replicates
- Averaging of Standards
- Concentration and Location of Standards
- Number of Controls
- Control Type Definition
- Number of Control Replicates
- Control Location
- Number of Samples
- Number of Sample Replicates
- Sample Location

"Map Generation" represents the method by which blanks, controls, standards, and/or samples are assigned to specific locations on the plate.

| MAP GENERATION: MANUAL | |
|---|---|
| AUTO MANUAL | |

*Selecting Manual or Automatic map generation*

- Select AUTO to instruct the software to automatically generate a Plate Map after the blanks, controls, standards and/or samples have been defined.
- Select MANUAL to indicate that the well assignments will be performed manually (by the user) at Define and/or Run time.

| | |
|---|---|
| iii | **Note:** Press **Shift + Clear** at the MAP GENERATION screen to "clear" a previously defined map. |

## Mapping Direction

The "Mapping Direction" is the direction (Down or Across) in which blank, control, standard, or sample *groups* will be mapped on the plate.

| MAPPING DIRECTION: DOWN | |
|---|---|
| DOWN ACROSS | |

*Selecting the direction for group mapping*

- Select DOWN to map groups down the plate columns.
- Select ACROSS to map groups across the plate rows.
- For example, if the plate contains one blank (BLK), one negative control (NC), one positive control (PC), and samples with one replicate:
  - DOWN maps the wells as A1:BLK, B1:NC, C1:PC, D1:SMP1, E1:SMP2, and so on.
  - ACROSS maps the wells as A1:BLK, A2:NC: A3:PC, A4:SMP1, A5:SMP2, and so on.
- Additional examples of mapping directions are shown in *Figure 3-4*.

## Replication Direction

The "Replicate Direction" is the direction (Down or Across) in which blank, control, standard, or sample *replicates* will be mapped on the plate.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | P | | D | I | R | E | C | T | I | O | N | : | A | C | R | O | S | S | | | |
| D | O | W | N | | | A | C | R | O | S | S | | | | | | | | | | | |

*Selecting the direction for replicate mapping*

- Select DOWN to map replicates down the plate columns.
- Select ACROSS to map replicates across the plate rows.
- For example, if the plate contains two blanks (BLK), two negative controls (NC), two positive controls (PC), and samples in duplicate:
  - DOWN maps the wells as A1/B1:BLK, C1/D1:NC, E1/F1:PC, G1/H1:SMP1.
  - ACROSS maps the wells as A1/A2:BLK, A3/A4:NC, A5/A6:PC, A7/A8:SMP1, and so on.
- Additional examples of mapping directions are shown on page 3-21.

Map Direction DOWN, Rep Direction DOWN:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | STD1 | STD5 | SMP2 | | | | | | | | | |
| B | STD1 | STD5 | SMP2 | | | | | | | | | |
| C | STD2 | PC | SMP3 | | | | | | | | | |
| D | STD2 | PC | SMP3 | | | | | | | | | |
| E | STD3 | NC | SMP4 | | | | | | | | | |
| F | STD3 | NC | SMP4 | | | | | | | | | |
| G | STD4 | SMP1 | SMP5 | | | | | | | | | |
| H | STD4 | SMP1 | SMP5 | | | | | | | | | |

Map Direction ACROSS, Rep Direction ACROSS:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | STD1 | STD1 | STD2 | STD2 | STD3 | STD3 | STD4 | STD4 | STD5 | STD5 | PC | PC |
| B | NC | NC | SMP1 | SMP1 | SMP2 | SMP2 | SMP3 | SMP3 | SMP4 | SMP4 | SMP5 | SMP5 |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

Map Direction DOWN, Rep Direction ACROSS:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | STD1 | STD1 | SMP2 | SMP2 | | | | | | | | |
| B | STD2 | STD2 | SMP3 | SMP3 | | | | | | | | |
| C | STD3 | STD3 | SMP4 | SMP4 | | | | | | | | |
| D | STD4 | STD4 | SMP5 | SMP5 | | | | | | | | |
| E | STD5 | STD5 | | | | | | | | | | |
| F | PC | PC | | | | | | | | | | |
| G | NC | NC | | | | | | | | | | |
| H | SMP1 | SMP1 | | | | | | | | | | |

Map Direction ACROSS, Rep Direction DOWN:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | STD1 | STD2 | STD3 | STD4 | STD5 | PC | NC | SMP1 | SMP2 | SMP3 | SMP4 | SMP5 |
| B | STD1 | STD2 | STD3 | STD4 | STD5 | PC | NC | SMP1 | SMP2 | SMP3 | SMP4 | SMP5 |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

***Figure 3-4:*** *Examples of Mapping Directions*

### Start Mapping at Well Location

Enter the location of the well that will be the starting point for automatic mapping.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | T | A | R | T | | M | A | P | P | I | N | G | | | | | | | | | |
| A | T | | W | E | L | L | | L | O | C | A | T | I | O | N | : | | A | 0 | 1 | | |

*Entering the starting well for automatic plate mapping*

- Use the **NUMERIC** and **ALPHA** keys to enter a letter or number at the cursor location. The valid entry range is from A01 to H12, depending on the number of blanks, standards, controls, and/or samples defined in the assay.

### Blank Map

There are several blanking methods to choose from.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | L | A | N | K | | M | A | P | : | | F | U | L | L | | | | | | | |
| A | I | R | | | F | U | L | L | | | C | O | N | S | T | | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | L | A | N | K | | M | A | P | : | | F | U | L | L | | | | | | | |
| R | O | W | | | C | O | L | U | M | N | | | | | | | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | L | A | N | K | | M | A | P | : | | F | U | L | L | | | | | | | |
| P | - | A | C | R | O | S | S | | | | P | - | D | O | W | N | | | * | M | O | R | E |

*Selecting a blanking method*

- AIR performs an initial reading "on air" just prior to the plate read, and uses that reading as the blank value. This value is subtracted from each well on the plate.
- FULL enables a single blank well or an average of blank wells (up to 48) to be subtracted from each well on the plate.
- CONST (Constant) allows entry of a user-specified absorbance value. This value will be subtracted from each well on the plate.

- ROW enables a single blank well or an average of blank wells to be selected for each row. The maximum number of blanks is 48. Use manual mapping to position blanks, standards, controls, and samples.
- COLUMN enables a single blank well or an average of blank wells to be selected for each column. The blank OD or average OD will be subtracted from other wells in the column. Use manual mapping to position blanks, standards, controls, and samples.
- P-ACROSS enables a blank in every even-numbered column to be subtracted from the well to the left of it in every odd column. Use manual mapping to set up the appropriate map by placing the standards, controls, and samples in only the odd columns.
- P-DOWN enables a blank in the B, D, F and H rows to be subtracted from the well above in the A, C, E and G rows. Use manual mapping to set up the appropriate map by placing the standards, controls, and samples in rows A, C, E, and G.

### Blanking Constant

If the CONST blanking method is selected, a Blanking Constant must be defined. This value will be subtracted from each well on the plate.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| B | L | A | N | K | I | N | G | | C | O | N | S | T | A | N | T | : | | 0 | . | 5 | 0 | 0 |

*Entering the Blanking Constant value*

- Use the **NUMERIC** keys to enter the value. The valid entry range is 0.000 to 3.000 OD.

### Number of Blanks

If the FULL, COLUMN, or ROW blanking method is selected, enter the number of blank wells on the plate.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | N | U | M | B | E | R | | O | F | | | | | | | |
| B | L | A | N | K | S | : | | | | | | | | | | | | | | 0 | 2 | |

*Entering the number of blank wells on the plate*

- Use the **NUMERIC** keys to enter the number of blank wells. The valid entry range is 0 to 48 wells.

## Blank Location

The Blank Location screen is presented if blank wells are defined and **manual** map generation is selected.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | T | H | E | | L | O | C | A | T | I | O | N | | O | F | | | |
| B | L | A | N | K | # | 1 | : | | | | | | | | | | | | | | A | 0 | 1 |

*Defining the Location of the blank wells*

- Use the **NUMERIC** and **ALPHA** keys to enter the location of the first Blank well.
- Press **ENTER** to define subsequent Blank well location(s).

## Number of Standards

For assays requiring standards, begin by entering the number of standard *groups*.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | | |
| N | U | M | B | E | R | | O | F | | S | T | A | N | D | A | R | D | S | : | | 0 | 5 | |

*Entering the number of standard groups*

- Use the **NUMERIC** keys to enter the number of standard groups.
  - Enter 00 to indicate no standards.
- The valid entry range depends on the selected curve fit method. The maximum number of standards for all curve fit methods is 12. The minimum numbers of standards are:
  - 4 for 2-P, 4-P, cubic, cubic spline
  - 3 for quadratic
  - 2 for linear, point-to-point
- **Note:** If the NUMBER OF STANDARDS setting is modified, the number of standard *replicates* (see next page) automatically defaults to 01.

### Number of Standard Replicates

The Standard Replicates entry screen is presented if the number of standard groups is greater than 0.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | N | U | M | B | E | R | | O | F | | | | | | |
| S | T | A | N | D | A | R | D | | R | E | P | L | I | C | A | T | E | S | : | | 0 | 2 | |

*Entering the number of standard replicates*

- Use the **NUMERIC** keys to enter the number of standard *replicates*. The valid entry range is from 1 to 8 replicates.
    - The software will automatically verify that the number of replicates, multiplied by the number of standards, does not exceed the number of wells on the plate.

### Average Standards

If the Number of Standard Replicates setting is greater than 01, the Average Standards? screen is presented.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | V | E | R | A | G | E | | S | T | A | N | D | A | R | D | S | ? | | Y | E | S | | |
| | Y | E | S | | | | | N | O | | | | | | | | | | | | | | |

*Choosing whether or not to Average Standards*

- Select `YES` to average the replicates for each standard group, and then use the group averages when calculating the standard curve.
- Select `NO` to use the individual standard replicates when calculating the standard curve.

## Standard Concentrations

Use the Standard Concentration screen to enter the predicted concentration value for each standard group. If manual map generation is selected, the replicate locations must also be defined.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | N | C | . | | O | F | | | | | | | L | O | C | A | T | I | O | N |
| S | T | D | # | 1 | : | 1 | . | 5 | 0 | | | | | R | E | P | # | 1 | : | A | 0 | 1 |

*Entering the predicted standard concentrations, and well locations (if manual map)*

- Use the **NUMERIC, ALPHA,** and **DECIMAL POINT** keys to enter the standard concentration values. The valid entry range is .00001 to 999999. The entry cannot exceed 6 characters, including the decimal point.
- If automatic mapping is selected, each replicate's location is available for viewing only. Pressing **ENTER** advances to the concentration value entry for the next standard.
- If manual mapping is selected, the location must be defined. Pressing **ENTER** from the standard concentration entry moves the cursor to the Location field. Pressing **ENTER** from the Location field advances to the concentration value entry for the next standard.

## Number of Controls

For assays requiring controls, begin by entering the number of control *groups*.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| N | U | M | B | E | R | | O | F | | C | O | N | T | R | O | L | S | : | | 0 | 2 |

*Entering the number of control groups*

- Use the **NUMERIC** keys to enter the Number of Control groups in the assay. For example, if the assay requires one or more positive control wells and one or more negative control wells, enter 02.
- The valid entry range depends on the number of locations on the plate that are undefined. The maximum number of control groups is 8.

## Control Type

After defining the number of controls for this assay, select the types of controls to use.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | N | T | R | O | L | # | 1 | : | P | C | | | | | | | | | |
| | | P | C | | | | N | C | | | | H | P | C | | | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | N | T | R | O | L | # | 1 | : | P | C | | | | | | | | | |
| | L | P | C | | | | C | T | L | 1 | | | | C | T | L | 2 | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | N | T | R | O | L | # | 1 | : | P | C | | | | | | | | | |
| | C | T | L | 3 | | | | C | T | L | 4 | | | | | | | * | M | O | R | E |

*Selecting the types of controls to use with this assay*

- Choose one control identifier for each type of control in your assay. The available options are: **P**ositive **C**ontrol**, N**egative **C**ontrol, **H**igh **P**ositive **C**ontrol, **L**ow **P**ositive **C**ontrol, **CTL1**, **CTL2**, **CTL3**, **CTL4**.
- After choosing an identifier for Control #1, press **ENTER** to choose the identifier for the next control.

## Number of Control Replicates

The Number of [Control] Replicates entry screen is presented if the number of control groups is greater than 0.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | U | M | B | E | R | | O | F | | P | C | | | | | | | | | |
| R | E | P | L | I | C | A | T | E | S | : | | | | | | | | | 0 | 2 |

*Entering the number of PC (Positive Control) replicates*

- The well ID associated with Control #1 appears first. Press **ENTER** to advance to the next control.
- Use the **NUMERIC** keys to enter a value for Number of [Control] Replicates.
- The valid entry range is from 1 to 12 replicates. The software automatically performs a check to ensure the number of replicates, multiplied by the number of controls, does not exceed the number of undefined wells remaining on the plate.

## Location of Controls

If mapping is **manual** and controls are defined, the locations for each control replicate must be specified.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | O | N | T | R | O | L | # | 1 | | | | | | | L | O | C | A | T | I | O | N | |
| T | Y | P | E | : | P | C | | | | | | | | | R | E | P | # | 1 | : | B | 0 | 1 |

*Entering the control locations (if manual map)*

- Use the **NUMERIC** and **ALPHA** keys to enter the well location for Rep #1 of Control #1. Press **ENTER** to advance to the next replicate or control group.

## Number of Samples

The number of sample groups on the plate can be defined here, and/or it can be defined at run-time if `UTIL ➔ READ ➔ PROMPT FOR SAMPLE COUNT?` is set to YES. See *Selecting Read Options* in Chapter 2 for more information.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| N | U | M | B | E | R | | O | F | | S | A | M | P | L | E | S | : | 2 | 4 | | |

*Entering the number of sample groups on the plate*

- Use the **NUMERIC** keys to enter the number of sample *groups* on the plate.
- The valid entry range is from 01 to the number of undefined well locations remaining on the plate.
- If the Number of Samples setting is altered, the Number of Sample Replicates setting resets to 01.

## Number of Sample Replicates

After the number of sample groups is specified, the Number of Sample Replicates entry screen is presented.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | N | U | M | B | E | R | | | | | | | | | | |
| O | F | | S | A | M | P | L | E | | R | E | P | L | I | C | A | T | E | S | : | | 0 | 2 |

*Entering the Number of Sample Replicates*

- Use the **NUMERIC** keys to enter the number of sample replicates.
- The valid entry range is from 1 to 12 replicates. The software automatically performs a check to ensure that the number of replicates multiplied by the number of samples does not exceed the number of undefined wells remaining on the plate.

## Sample Location

If mapping is **manual** and samples are defined, the locations for each sample replicate must be specified.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | A | M | P | # | 1 | | | | | | | | | L | O | C | A | T | I | O | N | |
| | | | | | | | | | | | | | | R | E | P | # | 1 | : | A | 0 | 1 |

*Defining the locations of the samples*

- Use the **NUMERIC** and **ALPHA** keys to enter the well location for Rep #1 of Sample Group #1. Press **ENTER** to advance to the next replicate or sample group.

## Define FORMULA

The *PowerWaveX* supports four types of formulas (Cutoff, Transformation, Validation, and General), as well as the ability to program variables for use within formulas. Up to three types of Validation formulas may be defined (Blank, Control, and Assay Validation).

To define formulas, start at the Main Menu and select DEFINE, select the assay, press **ENTER**, then select FORMULA.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | E | F | I | N | E | | | | | | | | | | | | | | | | | |
| M | E | T | H | O | D | | M | A | P | | F | O | R | M | U | L | A | | C | U | R | V | E |

*Selecting the Formula option on the DEFINE screen*

| | |
|---|---|
| iii | **Note:** Formulas created using Bio-Tek's Extensions™ (Define Reader Protocol) software cannot be edited via the reader front panel. |

### CALCULATION STRUCTURE

During data reduction, formulas are processed in the order shown below. The number of permitted formulas of each type are shown as well.

- Blank Validation 0-1
- Control Validation 0-4
- Assay Validation 0-4
- Transformations 0-1
- Cutoff Formulas 0-1
- Curve Fit Analysis (if a curve fit method is defined)
- General Formulas 0-4

## FORMULA TYPE

The *PowerWaveX* supports four types of formulas, as well as the ability to program variables for use within Transformation formulas.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | F | O | R | M | U | L | A | | T | Y | P | E | : | | |
| C | U | T | O | F | F | | T | R | A | N | S | | V | A | L | | | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | F | O | R | M | U | L | A | | T | Y | P | E | : | | |
| G | E | N | E | R | A | L | | | T | R | A | N | S | - | V | A | R | | | | |

*Selecting the type of formula to create or edit*

- CUTOFF formulas are used to classify results. During data reduction, results are evaluated against the cutoff formulas and each well is assigned a user-specified label (POS, NEG, or EQUIV).
- TRANSformation formulas are applied to the absorbance data in preparation for further data reduction and/or curve fit calculation.
- VALidation formulas can be used to determine whether or not blanks and/or controls are valid. In addition, Assay Validation formulas can be used to determine whether or not the entire assay should be considered valid.
- GENERAL formulas are calculated after all other calculations are complete, and the results are not used in further data reduction.

  **Note:** General formulas are not currently supported in the *PowerWaveX* on-board software.
- The TRANS-VAR option allows you to define a variable to be used in transformation formulas.

After the formula type is selected, the Formula Entry screen appears. Each formula can contain a maximum of 24 characters. Spaces are not necessary.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | O | R | M | U | L | A | # | 1 | : | | | | | | | | | | | | | |
| | M | A | T | H | | O | T | H | E | R | | | M | A | P | | | F | U | N | C | T | N |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | C | ; | x | > | = | 2 | . | 5 | 0 | 0 | | | | | | | | | | | | |
| | M | A | T | H | | O | T | H | E | R | | | M | A | P | | | F | U | N | C | T | N |

*Formula entry screen - the "Formula#1" prompt disappears to provide more spaces*

- After a moment, the FORMULA#1: prompt disappears and the formula can be entered. Use the options found under MATH, OTHER, MAP, and FUNCTN to "build" the formula.
  - To cycle through the available Math, Other, Map, or Function options, continue to press the appropriate **SoftKey**. For example, press the MATH Softkey several times to see +, -, *, /, %, =, etc. When the desired option appears, press the **RIGHT ARROW** key to select it and advance to the next editable field.
  - Press the **LEFT ARROW** key to move the cursor to the left.
  - Press **CLEAR** to delete the item above the cursor.
  - When a formula is complete, press **ENTER** to continue.
- Select MATH to insert a mathematical symbol such as +, %, or <=.
- Select OTHER to insert an opening " ( " or closing " ) " parenthesis, or logical operators AND or OR.
- Select MAP to insert a well ID such as BLK;x or NC;1.
- Select FUNCTN to insert a mathematical function such as LOG or SQRT.

| | |
|---|---|
| iii | **Note:** The reader software checks the formulas for errors during data reduction. A syntax error in a formula will result in a "Token Error" on results reports. |

### MATH

The following mathematical symbols can be used in formulas:

| | | | |
|---|---|---|---|
| + | Addition | == | Equal to |
| - | Subtraction | > | Greater than |
| * | Multiplication | >= | Greater than or equal to |
| / | Division | < | Less than |
| % | Percent | <= | Less than or equal to |

### OTHER

The following additional symbols can be used in formulas:

| | |
|---|---|
| ( | Left parenthesis |
| ) | Right parenthesis |
| AND | Logical AND |
| OR | Logical OR |

### MAP

The available MAP options depend on the formula type and the current plate map.

MAP options resemble BLK;X (mean of the blank wells), NC;1 (the first NC well), or OD (every well).

### FUNCTION

The following functions can be used in formulas:

| | | | |
|---|---|---|---|
| LOG10 | Log Base 10 | ALOG | Anti Log |
| ALOG10 | Anti Log Base 10 | LOG | Log |
| AB | Absolute Value | SQRT | Square Root |
| PWR | Power | | |

Validation formulas can be used to determine whether or not blanks and/or controls are valid. In addition, Assay Validation formulas can be used to determine whether or not the entire assay should be considered valid.

See *Formula Type* on page 3-30 for instructions on selecting an assay and accessing the Select Validation Type screen.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | V | A | L | I | D | A | T | I | O | N | | T | Y | P | E | : | |
| C | O | N | T | R | O | L | | | | | | A | S | S | A | Y | | B | L | A | N | K | |

*Selecting a Validation formula type*

## Control and Blank Validation Formulas

Blank Validation is used to ensure that the OD values for the blank replicates, or for the blank mean, meet certain criteria. Control Validation serves the same purpose as Blank Validation, but apply to the control replicates or control mean. If the criteria are not met, results are considered suspect, and the message "RESULTS INVALID! Blank (or Control) validation failed" appears on results reports.

- One blank validation formula can be defined.
- Up to 4 control validation formulas can be defined.
- Define the plate map (via `DEFINE` ➔ `MAP`) before creating blank or control validation formulas.
- Blank/Control validation can be performed on individual replicates (BLK, PC), or on the group mean (BLK;x, NC;x).

### *Examples*

If an assay protocol states that:

- Each Blank well should have an OD less than 0.050. The formula is: `BLK<0.050`
- Each Positive Control replicate must fall within the OD range of 1.000 to 2.500. This can be accomplished with one formula: `PC>1.000ANDPC<2.500,` or with two separate formulas: `PC>1.000` *and* `PC<2.500`
- The Negative Control mean must have an OD less than 0.100. The formula is: `NC;x<0.100`

## Number of Required Blanks / Controls

When a blank or control validation formula is defined, enter the number of blanks or controls that must meet the criteria established by that formula.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | C | : | | | | N | U | M | B | E | R | | O | F | | V | A | L | I | D | | | |
| R | E | P | L | I | C | A | T | E | S | | R | E | Q | U | I | R | E | D | : | | | 0 | 2 |

*Entering the number of blanks/controls that must be found valid*

- Use the **NUMERIC** keys to enter the number of blanks or controls that are required to be found valid in order for the results to be valid. For example, if an assay states that two out of three PC wells must be valid, enter 02.
- The range is 1 through the number of defined replicates of the blank or control.

## Assay Validation Formulas

Assay Validation formula(s) establish a set of criteria used to determine whether or not an assay can be considered valid. If the criteria are not met, results are considered suspect, and the message "RESULTS INVALID! Assay validation failed" appears on results reports.

- Up to four assay validation formulas can be defined.
- Define the plate map (via `DEFINE` ➔ `MAP`) before creating assay validation formulas.

### *Examples*

If an assay protocol states that in order for the assay to be valid:

- The mean of the negative controls must be less than 0.100. The formula is:

  ```
  NC;x<0.100
  ```

- The mean of the positive controls must be greater than the mean of the negative controls. The formula is:

  ```
  PC;x>NC;x
  ```

Transformation formulas can be used to transform raw or blanked absorbance data in preparation for further data reduction, including curve fit analysis.

See *Formula Type* on page 3-30 for instructions on selecting an assay and accessing the Transformation Formula definition screen.

- If a blanking method is selected in the assay, transformation formulas are applied to the blanked absorbance values, otherwise they are applied to the raw data. Turn to page
  3-29 to review the results calculation structure.
- One transformation formula may be defined per assay.
- A transformation formula can be simple (ex. `OD*100` to multiply all wells on the plate by 100), or more complex with the inclusion of a pre-defined Transformation Variable (see *TVAR*, below).

## Simple Transformation Formulas

"Simple" transformation formulas are typically applied to all wells on the plate. For example:

- To divide the OD in each well on the plate by 2 and then multiply by 100, the formula is: `(OD/2)*100`
- To raise the OD in each well to the power of 10, the formula is: `ODPWR10`

## Transformation Variable (TVAR)

For more complex transformations, a Transformation Variable (TVAR) can be defined for use within a transformation formula. This variable defines the scope of the transformation: whether to apply the transformation to all of the wells on the plate (OD), or to just the sample wells (SMP).

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S** | **C** | **O** | **P** | **E** | | **V** | **A** | **R** | **I** | **A** | **B** | **L** | **E** | **:** | | | | | **O** | **D** | | | |
| | **S** | **M** | **P** | | | | **O** | **D** | | | | | | | | | | | | | | | |

*Choosing the scope of the transformation*

- If **SMP** is chosen:
  - The transformation formula will be applied to the *sample* wells only.
  - SMP and any other well identifiers (BLK, PC, NC, STD, etc.) defined will become available as MAP options when building the transformation formula.
  - Example:

    The assay plate map contains 2 NC wells and 2 PC wells. The remainder of the map is filled with samples.

    The assay data reduction requires that the mean of the NC be subtracted from all the samples on the plate.

    The transformation formula is: `SMP-NC;x`
- If **OD** is chosen, the formula definition screen will appear so that you can define a formula for use *within* the transformation formula.
  - Use the formula keys (Math, Other, Map and Function) to define the Transformation Variable (TVAR). Once the variable has been defined, it can be used in a transformation formula. The TVAR will be available as a MAP option when building the transformation formula.

  Example:

  The assay plate map has 2 blanks, 1 control well in duplicate (CTL1), 1 negative control well in triplicate (NC), and 5 standards in duplicate (STD1-STD5) in varying concentrations.

  The assay data reduction states:

  - Subtract the mean of CTL1 from the mean of the NC. Subtract the difference from all OD's on the plate.
  - Divide the result of the above by the mean of the NC less the mean of CTL1, then multiply by 100.

  On paper, the formula reads:

  ```
  (OD - (NC;x-CTL1;x)) / (NC;x-CTL1;x) * 100
  ```

  On the reader, the formula `(NC;x-CTL1;x)` will be programmed as the TVAR, since the transformation will apply to all standards, controls and samples on the plate. To do this:

  - At the Scope Variable selection screen, choose `OD` and press **ENTER**.
  - Enter the formula `(NC;x-CTL1;x)` using the **Math, Other, Map** and **Function** keys. Press **ENTER**.

- The formula selection screen is displayed. Choose TRANS.
- Enter the formula (OD-(TVAR))/(TVAR)*100 using the Math, Other, Map and Function keys. "TVAR" is available as a MAP option.

Example:

In the case of competitive reactions, converting absorbance data to percent $B/B_0$ can be: (OD/STD1)*100. This divides all the wells by STD1, presumably the 0 standard, and multiplies the results by 100. To do this:

- At the Scope Variable selection screen, choose OD and press **ENTER**.
- Enter simply STD1 as the TVAR formula. Press **ENTER**.
- The formula selection screen is displayed. Choose TRANS.
- Enter the formula (OD/TVAR)*100 using the Math, Other, Map and Function keys. "TVAR" is available as a MAP option.

A cutoff formula calculates a **cutoff value** that is used for classifying samples. See *Formula Type* on page 3-30 for instructions on selecting an assay and accessing the Cutoff formula definition screen.

During data reduction, results are evaluated against the cutoff value (with an optional greyzone), and each well is assigned a call POS (positive), NEG (negative), or EQUIV (equivocal).

- One cutoff formula may be defined per assay.
- If Transformation Formulas are defined, cutoffs are based on the transformed results. Turn to page 3-29 to review the results calculation structure.
- A cutoff formula can consist of a simple numeric value (1.500), a well identifier (PC, or PC;x to represent the mean), or a formula combining the two (NC;x+0.050).
- A "greyzone" around the cutoff value can be defined, to indicate questionable results.
- Do not use the < or > mathematical symbols in a cutoff formula.
- **Tip:** Choose to print a Column Report to see the greyzone and cutoff values, as well as the equations used to assign calls to samples. See *Specifying Data Output and Reporting Options* in Chapter 2 for information on Column Reports.

## Greyzone

The **greyzone** is a definable area around the cutoff value. Samples falling within an area defined by the greyzone (ex. ± 5% of the cutoff value) could be considered questionable, or equivocal (EQUIV).

| E | N | T | E | R |  | G | R | E | Y | Z | O | N | E | : |  |  |  |  |  | 0 | 5 | % |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

*Defining a greyzone around the cutoff value*

- Use the **NUMERIC** keys to enter the greyzone percentage.
- The valid entry range is from 00 to 99%. An entry of 00% indicates no greyzone, although a sample equal to the cutoff value will still receive the EQUIV call.
- See *POS / NEG Calls* on the next page for information on how calls are assigned.

## POS / NEG Calls

After the greyzone is defined, calls for the sample wells (POSitive, NEGative, EQUIVocal), must be defined.

| S | A | M | P | > | C | U | T | O | F | F | + | 1 | 5 | % | : | | | | | | P | O | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | O | S | | | | N | E | G | | | | | | | | | | | | | | |

*Defining the POS/NEG/EQUIV calls for samples*

- Select POS or NEG to select the call that will be assigned to samples greater than the cutoff value plus the greyzone.
- If for example POS is selected in the screen shown above, calls will be assigned according to the following equations (SMP represents the sample wells):

  **EQUIV:** `SMP <= (CUTOFF+(CUTOFF*GREYZONE)) AND`
  `SMP >= (CUTOFF-(CUTOFF*GREYZONE))`

  **POS:** `SMP > (CUTOFF+(CUTOFF*GREYZONE))`

  **NEG:** `SMP < (CUTOFF-(CUTOFF*GREYZONE))`

## Examples

1. The cutoff between negative and positive calls should be calculated as the average of the negative controls plus the OD value 0.050. Samples greater than the cutoff should be labeled as positive. No greyzone is required.
   - For this example, NC;x (the mean of the NC wells) equals 0.100 OD
   - The cutoff formula is `NC;x+0.050`
   - The greyzone is `00%`
   - `POS` is selected for `SAMP>CUTOFF+00%`
   - Calls are assigned to sample wells as follows:
     - `EQUIV` if the sample equals 0.150
     - `POS` if the sample is greater than 0.150
     - `NEG` if the sample is less than 0.150

2. For a quantitative assay, samples with ODs greater than the STD2 mean plus a 10% greyzone should be labeled as positive, samples with ODs less than the STD2 mean minus the 10% greyzone should be labeled as negative. All other samples should be considered equivocal.

   - For this example, STD2;x (the mean of the STD2 wells) equals 1.500 OD
   - The cutoff formula is simply `STD2;x`
   - The greyzone is `10%`
   - POS is selected for `SAMP>CUTOFF+10%`
   - Calls are assigned to sample wells as follows:
     - EQUIV if the sample is greater than or equal to 1.350 *and* less than or equal to 1.650
     - POS if the sample is greater than 1.650
     - NEG if the sample is less than 1.350

## GENERAL FORMULAS

General Formulas are calculated after all other calculations are complete, and the results are not used in further data reduction.

**Note:** General Formulas are not supported in the current version of the reader.

## Define CURVE

To define curve-fitting parameters for an assay, start at the Main Menu and select DEFINE, select the assay, press **ENTER**, then select CURVE.

| D | E | F | I | N | E | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | E | T | H | O | D | | M | A | P | | F | O | R | M | U | L | A | | C | U | R | V | E |

*Selecting the Curve option on the DEFINE screen*

Configurable curve-fitting parameters include:

- Curve-Fit Type
- Editing of Outliers
- Axis Identification
- Extrapolation of Unknowns

### CURVE-FIT TYPE

The *PowerWaveX* supports 7 different curve-fitting methods, Linear, Quadratic, Cubic, 4-P, 2-P, cubic-spline, and point-to-point.

| C | U | R | V | E | - | F | I | T | | T | Y | P | E | : | | L | I | N | E | A | R | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | O | N | E | | L | I | N | E | A | R | | Q | U | A | D | | | * | M | O | R | E |

| C | U | R | V | E | - | F | I | T | | T | Y | P | E | : | | L | I | N | E | A | R | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | U | B | I | C | | | 4 | - | P | | | | 2 | - | P | | | * | M | O | R | E |

| C | U | R | V | E | - | F | I | T | | T | Y | P | E | : | | L | I | N | E | A | R | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | - | S | P | L | I | N | E | | | | P | T | - | P | T | | * | M | O | R | E |

*Selecting a curve-fit type*

- **NONE:** Choose None if no standard curve will be generated (this is the default).
- **LINEAR:** A simple best fit straight line is plotted using the values of the standards.

- **QUADratic:** Uses the Quadratic equation "$ax^2 + bx + c = y$" to plot the standards values. Utilizing this curve, any data point for a standard that deviates from the ideal value will not affect the entire curve.
- **CUBIC:** Uses the equation "$ax^3 + bx^2 + cx + d = y$" to plot the standards values. This type of curve fit is affected even less than the quadratic fit when any particular standard has a poor value.
- **2-P (Logit/Log):** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The logistic equation is algebraically transformed to a simpler form in which experimentally determined values are used for the responses at concentrations of zero and infinity.
- **C-SPLINE (Cubic-Spline):** A piecewise polynomial approximation consisting of joining a set of data points by a series of straight lines, which is then smoothed by using a cubic fit.
- **4-P (4-Parameter Logistic):** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The 4 parameters are: Left asymptote, Right asymptote, Slope and Value at the Inflection point. This fit is most recommended for immunoassay data, and is more exact than Logit/Log.
- **PT-PT (Point-to-Point):** A plot that connects each standard point with a line, with no averaging of the values to "smooth" the curve at each standard.

## EDIT STANDARD OUTLIERS

After the standard curve has been generated, one or more standards can be excluded from the recalculation of the curve.

| E | D | I | T |  | S | T | D |  | O | U | T | L | I | E | R | S | : | M | A | N | U | A | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | N | O | N | E |  | M | A | N | U | A | L |  |  |  |  |  |  |  |  |  |  |  |  |

*Choosing whether or not to enable standard outlier editing for this assay*

- Select NONE to suppress the Edit Standard Outliers capability for this assay.
- Choose MANUAL to enable the capability.
  - If AVERAGE STANDARDS is set to NO, the individual standard replicates are available for editing. If set to YES, the standard *groups* are available for editing.

- After the assay is run and reports are generated, press `REPORT` from the Main Menu. Press `RESULT`, select the assay, then press **`ENTER`**. The `EDIT STD OUTLIERS? YES/NO` prompt will appear. See *Editing Standard Outliers* on page 3-53 for further instructions.

## X/Y Axis Type

After the curve-fit type is selected, select the X/Y Axis Type.

| X | / | Y |  | A | X | I | S |  | T | Y | P | E | : |  | L | I | N |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L | I | N |  |  | L | I | N | / | L | O | G |  | L | O | G |  | L | O | G | / | L | I | N |

*Selecting the X/Y Axis Type*

- Choose the method by which the X and Y axes will be scaled.
- This option is not available for the 2-P and 4-P curve-fit types. The X/Y scaling for these curves is always LIN/LIN.

## Extrapolation of Unknowns

The *PowerWaveX* provides the option to "extrapolate" the curve to evaluate samples outside of the absorbance range defined by the standards.

| E | X | T | R | A | P | O | L | A | T | E |  | U | N | K | N | O | W | N | S | ? | Y | E | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Y | E | S |  |  |  |  | N | O |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

*Choosing whether or not to extrapolate unknowns*

- Select `YES` to enable Extrapolation, otherwise select `NO`.
- On printed reports, extrapolated concentrations (RSLT values) are surrounded by < >, for example `<44.425>`.

| iii | **Note:** If extrapolation is chosen for the Point-to-Point curve fit, unknown concentrations will be extrapolated linearly from the nearest segment of the curve. If the plot includes both increasing and decreasing segments, the curve printout will be labeled "Ambiguous." The resulting values, which actually are extrapolated, may not be indicated as such. All calculated results for an "Ambiguous" curve should be considered unreliable. |
|---|---|

## Panel Assays

A Panel assay is a collection of up to eight assays to be run on one plate.

- The most common reason to use a Panel assay is for confirmatory tests based on a screening test in clinical applications.
- Only one panel assay can be defined on the reader at any time.
- The assays specified within the Panel must be pre-defined in any of the assay positions 1-55**.**
- The assays specified within the panel must all use the Endpoint read method.
- The assays specified within the panel must all read at the same wavelength(s).
- Any curve-fit type, formulas or standard concentrations previously defined for each assay will be used when the assay is selected for a Panel.
- The type and number of controls, blanks, standards and replicates in the assays chosen for the Panel will be "copied" into the Panel definition. To change any of the map or assay parameters in the Panel, they must be changed in the pre-defined assay first.
- Consider printing a Map Report for each assay that will be included in the panel, for use with mapping the Panel.

To create a panel assay, start at the Main Menu, press `DEFINE`, then choose assay number **99**. Enter the panel assay name.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | **A** | **M** | **E** | **:** | | | | **P** | **A** | **N** | **E** | **L** | | | | | | | | | |
| | **-** | | | | | | | **/** | | | | | | **:** | | | | **S** | **P** | **A** | **C** | **E** |

*Editing the name of the Panel assay*

- The default name is "PANEL".
- Use the **ALPHA** and **NUMERIC** keys to update the Assay name, if desired.
- Press **ENTER** to continue. The Number of Assays entry screen will appear.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | U | M | B | E | R | | O | F | | A | S | S | A | Y | S | : | | | | | | 2 |
| | | | | | | | | | | | | | | | | | | | | | | |

*Entering the number of assays to include in the Panel*

- Specify the number of assays to include in the panel (1 to 8).
- Press **ENTER** to continue. The Mapping Direction selection screen will appear.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | A | P | P | I | N | G | | D | I | R | E | C | T | I | O | N | : | D | O | W | N | |
| D | O | W | N | | | A | C | R | O | S | S | | | | | | | | | | | |

*Choosing the Panel's mapping direction*

- This option ensures that all assays will be mapped in the same direction. Select DOWN or ACROSS.

| | |
|---|---|
| iii | **Note:** The original map directions for the pre-defined assays are overridden by the Panel's direction. If the assays include replicates, they will follow the Panel map direction. |

After selecting the mapping direction of the assays, choose which assays to include in the panel.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | A | S | S | A | Y | | N | U | M | B | E | R | : | | | 2 | 2 |
| N | A | M | E | : | | H | B | S | - | A | G | 1 | | | | | | | | | | | |

*Selecting the assays to include in the panel*

- Press **OPTION** to cycle through the assay numbers and names, or use the **NUMERIC** keys to enter an assay number. Press **ENTER** to make a selection.
- After selecting an assay, its starting location must be defined.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | T | A | R | T | | M | A | P | P | I | N | G | | | | | | | | | |
| A | T | | W | E | L | L | | L | O | C | A | T | I | O | N | : | | | | A | 0 | 1 |

*Defining the starting location for the selected assay*

- Use the **ALPHA** and **NUMERIC** keys to choose the well location to begin the assay. Wells A01 through H01 are valid for ACROSS mapping; A01 through A12 are valid for DOWN mapping.
- This process will be repeated for each assay within the panel. Remain aware of the total number of controls, standards and blanks that were originally mapped in each individual assay while mapping for the Panel assay.

  For example, to include Assays 1, 8 and 22 in the Panel assay (DOWN mapping is selected for the Panel):

  - Assay 1 has a total of 12 wells defined for controls, blanks and standards. In the Panel, the mapping for Assay 1 begins in well A01. The user wants to run 6 samples in Assay 1. Assay 1 now fills wells A01 through B03.
  - The mapping for Assay 8 can begin in well B04, or any well other than A01 to B03. The reader will "beep" if you try to map into a well that is already assigned for use with the Panel.
  - The mapping for Assay 22 may begin at the next available well location after Assay 8 mapping is complete.
  - After all the assays have been entered into the Panel, consider printing the Panel's Map Report to verify the map before reading the plate. Choose REPORT (from the **Main Menu**), MAP, ASSAY 99. The reader will print the map of each assay configured in the Panel.
  - The Panel Assay results are sorted by Sample (unless a custom assay has been programmed by Bio-Tek).

| | |
|---|---|
| iii | **Note:** The Interpretation of Results reports for each assay in the panel will print first, and then the Sample results will print. See *Appendix D* for a sample Panel Report. |

## Reading a Microplate

Use the Main Menu option READ, or press the **READ** key on the front panel, to initiate a plate read.

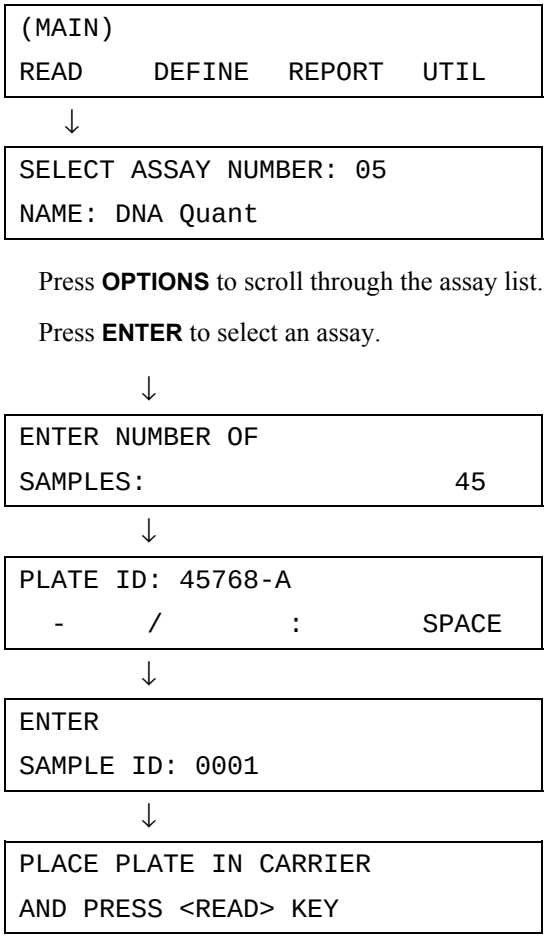



***Figure 3-5:*** *Options available under READ*

| | |
|---|---|
| iii | **Notes:** The options to present the Enter Number of Samples, Plate ID, and/or Sample ID screens are configurable via UTIL ➔ READ. Custom assays may present additional screens for the entry of special parameters; refer to the Assay Reference Guide (PN 7271006) for more information. |

| | |
|---|---|
| iii | **Notes:** Before reading a plate, ensure that the reporting options are set correctly under UTIL ➔ OUTPUT. See *Selecting Read Options* in Chapter 2 for more information. |

## SELECTING AN ASSAY

From the Main Menu, press READ and then select the desired assay.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | A | S | S | A | Y | | N | U | M | B | E | R | : | 6 | 5 |
| N | A | M | E | : | H | B | S | - | A | G | 1 | | | | | | | | | | |

*Selecting an assay to run*

- Use the **NUMERIC** keys to enter the number of any predefined assay stored in the reader's memory, or the **OPTION** key to advance one assay at a time.
- Press **ENTER** to continue.

## RUN-TIME PROMPTS

After the assay is selected, one or more informational prompts may be presented, depending on preferences selected via UTIL ➔ READ, whether or not the assay specifies manual mapping, or if this is a custom pre-programmed assay.

- Prompts enabled via UTIL ➔ READ can include Enter Number of Samples, Plate ID, and Enter Sample ID.
- If the assay specifies manual mapping, prompts for information will include the locations for the sample wells.
- If running a custom assay, typical prompts might include (refer to the *Assay Reference Guide* for more information):
  - The number of samples
  - Standard concentrations
  - Assay ID
  - Fill pattern
  - Blank method
  - First well location
  - Replicate count for each well type
  - Wavelength mode
  - Report preferences, etc.

## Enter Number of Samples

If the Enter Number of Samples prompt is presented, indicate the number of sample *groups* on the plate. The number of sample replicates is typically pre-defined in the assay, but if this is a custom assay, you may also be prompted to enter the replicate count.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| N | U | M | B | E | R | | O | F | | S | A | M | P | L | E | S | : | | 2 | 0 | | | |

*Entering the number of sample groups on the plate*

- Use the **NUMERIC** keys to enter the number of sample groups.
- The valid entry range is from 01 to the maximum number of wells remaining on the plate after any blank, control, or standard wells are mapped.
- If you enter a value greater than the number of empty wells remaining on the plate, the reader will "beep" and automatically change the value to the maximum permissible number of samples.

## Enter Plate ID

If the Plate ID prompt is presented, enter a unique plate identifier to be stored in memory with the assay name and absorbance data.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | L | A | T | E | | I | D | : | | 0 | 0 | 1 | - | A | | | | | | | |
| | - | | | | | / | | | | : | | | | S | P | A | C | E | | | | | |

*Entering a Plate ID*

- Use up to 10 alphanumeric characters. See *Figure 3-1* for instructions on using the keypad.
- Although the reader does not require it, consider entering a unique ID for each plate, especially if reading multiple plates for the same assay.
- If the internal bar code scanner option is installed, the reader will automatically scan the plate/bar code label and use that for the Plate ID.

| | |
|---|---|
| iii | **Note:** Use caution when creating multiple Plate IDs. The reader does not provide a warning when the maximum of 8 Plate IDs stored in memory is about to be exceeded. If a 9th Plate ID is added, it will overwrite the first Plate ID stored in memory. |

## Enter Sample ID

If the Enter Sample ID prompt is presented, enter a *starting* sample identification number.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | N | T | E | R | | | | | | | | | | | | | | | | | |
| S | A | M | P | L | E | | I | D | : | | 0 | 0 | 0 | 1 | | | | | | | |

*Entering the starting sample identification number*

- The valid entry range is from 0001 to 9999.
- The software will automatically increment each subsequent sample identification by 1.
- The sample IDs will be assigned in accordance with the plate map defined in the assay.

## Enter Well Location

If the assay specifies **manual** plate mapping and if Prompt for Sample Count is set to Yes under UTIL ➔ READ, sample well locations can be defined at run-time.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | A | M | P | L | E | # | | 1 | | | | | L | O | C | A | T | I | O | N | | | |
| | | | | | | | | | | | | | R | E | P | # | | 1 | : | A | 0 | 2 | |

*Entering the sample well locations at run-time*

- The sample well locations originally defined in the assay will be presented. If desired, use the keypad to enter new well location for each sample replicate.
- Press **ENTER** to advance to the next replicate.

When all required prompts have been responded to, the Place Plate in Carrier prompt appears.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | L | A | C | E | | P | L | A | T | E | | I | N | | C | A | R | R | I | E | R | | |
| A | N | D | | P | R | E | S | S | | < | R | E | A | D | > | | K | E | Y | | | | |

*Final prompt before the plate read begins*

- Before reading the plate, make sure that the printer is connected, turned on, and full of paper.
- Place the plate on the carrier, then press the **READ** key to initiate the plate read.
- After the read is complete, data reduction will perform ("Calculating Results…"), and then the reports will be generated ("Generating Reports…").
- To halt in read in progress, press the **STOP** key.

| | |
|---|---|
| **iii** | **Note:** If using the incubation option, the reader will wait for the incubator to reach temperature before reading the plate. |

## Printing Reports

Reports are automatically generated after a plate has been read (see *Specifying Data Output and Reporting Options* in Chapter 2 for information on selecting reports). Results reports also can be regenerated manually by using the REPORT option from the Main Menu. In addition, Map, Assay, and Assay List reports can be printed.

**Note:** See *Appendix D* for sample reports.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | A | D | Y | | | | 9 | : | 4 | 5 | A | M | | | 0 | 7 | / | 2 | 0 | / | 9 | 9 |
| R | E | A | D | | | D | E | F | I | N | E | | R | E | P | O | R | T | | U | T | I | L |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | R | I | N | T | | R | E | P | O | R | T | ? | | | | | | | | | |
| R | E | S | U | L | T | | M | A | P | | A | S | S | A | Y | | L | I | S | T | |

*Generating reports via the REPORT option from the Main Menu*

- Select RESULT to obtain an exact copy of results from the plate reading (the 8 most recent sets of plate data are stored in memory).
  - The form in which the results are presented is determined by the report settings (Matrix, Column, Curve Fit) specified under UTIL ➔ OUTPUT.
- Select MAP to print a matrix showing the locations of the Blanks, Standards, Controls and Samples for a particular assay.
- Select ASSAY to print a plate map and a listing of all of an assay's settings, such as wavelengths, numbers of well types, formulas, and curve fit parameters.
- Select LIST to print a list of all assays (name and number), currently programmed in the reader.

## Results Report

The reader stores the absorbance data for the 8 most recent plate reads. Results reports can be generated for these plates if, for example, the data that automatically printed after the read needs to be printed in a different format, or if the standard curve contains outliers that require editing.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | E | P | O | R | T | : | H | B | S | - | A | G | | | | | | | | | |
| I | D | : | | 0 | 0 | 0 | 1 | | | | | | | 0 | 7 | / | 2 | 2 | / | 9 | 9 |

*Choosing a plate for the Results Report*

- The most recently read plate is presented first, showing the assay name, the plate ID (if one was entered), and the date the plate was read.
- Press **OPTIONS** to see the next plate in memory.
- Press **ENTER** to select a plate and continue.
- If a standard curve was generated and `EDIT STANDARD OUTLIERS` was set to `MANUAL` in the assay definition, the `EDIT STD OUTLIERS?` prompt is presented; otherwise, the Print Results? prompt is presented.

### *Editing Standard Outliers*

If a standard curve was generated and if `EDIT STANDARD OUTLIERS` was set to `MANUAL` in the assay definition, the option to edit outliers is presented.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | T | D | | O | U | T | L | I | E | R | S | ? | | | | |
| | Y | E | S | | | N | O | | | | | | | | | | | | | | |

*Choosing whether or not to edit standard outliers*

- Select `NO` to include all standards in the curve fit calculations.
- Select `YES` to indicate that one or more standard replicates or groups should be temporarily excluded from curve fit calculations.
  - If `AVERAGE STANDARDS` was set to `NO` in the assay definition, one or more standard *replicates* can be chosen for exclusion.

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | T | D | 1 | | R | E | P | 1 | ? | | Y | E | S | | | | | |
| | Y | E | S | | | N | O | | | | | | | | | | | | | | | | |

*Choosing to remove replicate 1 of Standard 1 from curve fit calculations*

- Select YES to exclude the replicate from curve fit calculations.
- Select NO to retain the replicate.
- Press **ENTER** to advance to the next replicate.

➢ If AVERAGE STANDARDS was set to YES in the assay definition, one or more standard *groups* can be chosen for exclusion.

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | T | D | 1 | ; | x | ? | | Y | E | S | | | | | | | | |
| | Y | E | S | | | N | O | | | | | | | | | | | | | | | | |

*Choosing to remove all replicates of Standard 1 from curve fit calculations*

- Select YES to exclude the group from curve fit calculations.
- Select NO to retain the group.
- Press **ENTER** to advance to the next group.

| | |
|---|---|
| iii | **Note:** Each curve-fit type requires a minimum number of standards for curve generation: 4 for 2-P, 4-P, cubic, and cubic-spline, 3 for quadratic, and 2 for linear and point-to-point. Exercise caution when editing outliers. If the assay is left with insufficient standards, the curve fit will fail. |

### *Printing Results*

After the assay is selected and standard outliers are edited (if necessary), the results report can be printed.

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | R | I | N | T | | R | E | S | U | L | T | S | ? | | | | | | | | | | |
| | Y | E | S | | | N | O | | | | | | | | | | | | | | | | |

*Print Results screen*

- Ensure that the printer is connected, turned on, and full of paper.
- Press YES to print reports, or NO to return to the Main Menu.

## Map Report

The Map Report contains a matrix in Row x Column format, showing the location of every well identifier defined in the plate map.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | A | S | S | A | Y | | N | U | M | B | E | R | : | 0 | 7 |
| N | A | M | E | : | | | | R | N | A | | Q | u | a | n | t | | | | | |

*Choosing an assay for the Map Report*

- Press **OPTIONS** to cycle through the list of available assays, or enter the number of the desired assay.
- Press **ENTER** to print the report.

## Assay Report

The Assay Report lists the assay definition parameters and their current settings.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | E | L | E | C | T | | A | S | S | A | Y | | N | U | M | B | E | R | : | 0 | 1 |
| N | A | M | E | : | | H | B | S | - | A | G | | | | | | | | | | |

*Selecting an assay for the Assay Report*

- Press **OPTIONS** to cycle through the list of available assays, or enter the number of the desired assay.
- Press **ENTER** to print the report.

## List Report

The List Report lists the all of the assays (name and number), currently programmed on the reader.

- Select `REPORT` from the Main Menu, then select `LIST` to print the report.

# 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 *PowerWaveX* supports most 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. 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 for one shift seven days a week.

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

**Table 4-1**
**Recommended Test Schedule**

| | Installation Qualification | Performance Qualification / *Monthly* | Performance Qualification / *Quarterly* | Operation Qualification / *Annually* |
|---|---|---|---|---|
| System Self-Test, p. 4-4 | ✓ | ✓ | | ✓ |
| Universal Plate Test, p. 4-7 | ✓ | ✓ | | ✓ |
| Liquid Test 1, p. 4-17 | ✓ | | ✓ | ✓ |
| Liquid Test 2, p. 4-19 | ✓* | | ✓* | ✓* |
| Liquid Test 3, p. 4-22 Optional for 340 nm | ✓ | | | ✓ |
| Robotic Lube, p. 4-25 | **Every six months, or after 10,000 cycles.** | | | |

* Run Liquid Test 2 only if you do not have a Universal Test Plate. If you run Liquid Test 2 or 3, you do not have to also run Liquid Test 1.

## Installation and Performance Qualification Procedures

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

Your *PowerWaveX* 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. After the initial confirmation of operation, the Universal Plate Test and Liquid Testing should be performed at least monthly. The System Self Test and Universal Plate Test can be run from KCjunior via **Utilities**, **Diagnostics**, and the Universal Plate Test can be run from KC4 via **System**, **Diagnostics**.

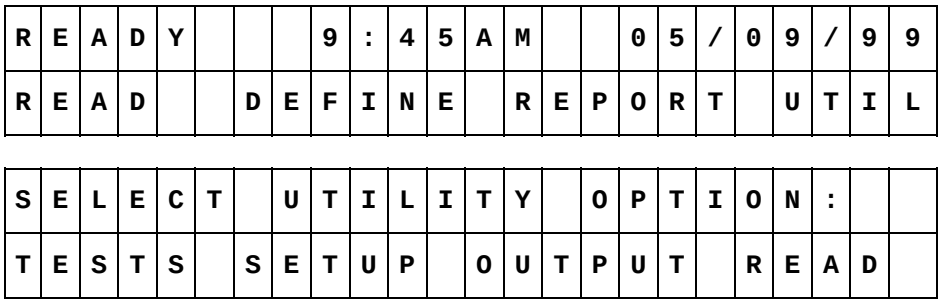
- **System Self Test:** Verifies proper gains, bulb operation, low electronic noise, and optional incubator functionality. Test results are sent to an attached printer.
- **Universal Plate Test:** Confirms the optical accuracy, linearity, alignment, channel-to-channel variation, and wavelength accuracy of the instrument
- **Liquid Testing:** Quantifies the channel-to-channel variation of the instrument using liquids, which verifies operation in a way that glass test filters cannot.

## Routine Procedure

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

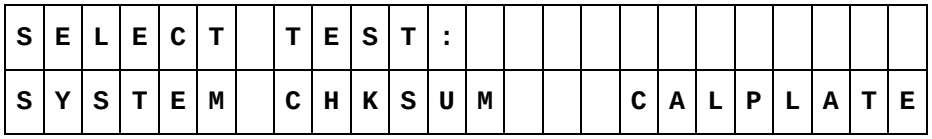
- Select Reader System Test to verify that the light levels and electronic noise at all set wavelengths fall within factory acceptance criteria.
- Select Universal Test Plate to run the calibration plate test to confirm the alignment, repeatability, and accuracy/linearity of the reader. The report will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

From the Main Menu, press the soft key that corresponds to `UTIL` to access the Utility Options Menu. The Select Utility Option screen will appear.




*Selecting UTIL from the Main Menu*

- Select `TEST` to advance to the Select Test screen.




*Choosing a performance test to run*

- Select `SYSTEM` to verify that the light levels and electronic noise at all wavelengths fall within factory acceptance criteria.
- Select `CHKSUM` to instruct the reader to compare the software to the internally recorded checksum values to ensure that the programming has not been corrupted. In addition, use this option to view the part numbers and versions for the software currently installed on the reader. This information is needed when contacting Bio-Tek for technical assistance.
- Select `CALPLATE` to run the calibration plate test, to confirm the alignment, repeatability, and accuracy/linearity of the reader.

The System Test confirms that the light levels and electronic noise at all wavelengths fall within factory acceptance criteria, and accomplishes this by measuring the air and dark readings and evaluating them to ensure they fall within specified ranges.

The reader automatically runs an internal System Test each time it is powered on. An error will be displayed if the power on System Test fails. No report will be produced at the power on System Test.

To obtain a report of the System Test values for either periodic testing documentation or troubleshooting (*Figure 4-1*), start at the Main Menu and press `UTIL` → `TESTS` → `SYSTEM`. The *PowerWaveX* will conduct the instrument's System Test and report results in a pass/fail format.

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

### Photodiodes

The Optics portion of the System Self Test confirms that the eight reading and one reference channels have adequate signal range without saturating the electronics.

### Xenon Flash Lamp

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

### Incubation

If the *PowerWaveX* has the 4-Zone™ incubation option installed, the System Self-Test will verify the four thermistors and compare these readings to internal voltage references to confirm proper operation.

Operator ID: 9219

Notes: 182427

02:51PM 08/30/02 SYSTEM SELF TEST

| Lambda: 200 | Gain: 1.64 | Resets: 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 31445 | 37883 | 34074 | 39602 | 36231 | 38224 | 36345 | 36493 | 39331 |
| Dark: | 9695 | 9743 | 9767 | 9718 | 9763 | 9810 | 9819 | 9808 | 9718 |
| Delta: | 21750 | 28140 | 24307 | 29884 | 26468 | 28414 | 26526 | 26685 | 29613 |

| Lambda: 352 | Gain: 1.84 | Resets: 4 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 29680 | 37687 | 35488 | 38364 | 37300 | 38312 | 39652 | 36991 | 37723 |
| Dark: | 9706 | 9730 | 9750 | 9716 | 9747 | 9781 | 9786 | 9778 | 9717 |
| Delta: | 19974 | 27957 | 25738 | 28648 | 27553 | 28531 | 29866 | 27213 | 28006 |

| Lambda: 639 | Gain: 2.42 | Resets: 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 30309 | 39090 | 35718 | 39052 | 37655 | 39196 | 39901 | 38448 | 38423 |
| Dark: | 9679 | 9749 | 9785 | 9712 | 9779 | 9848 | 9861 | 9845 | 9712 |
| Delta: | 20630 | 29341 | 25933 | 29340 | 27876 | 29348 | 30040 | 28603 | 28711 |

| Lambda: 790 | Gain: 1.54 | Resets: 1 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 30435 | 39040 | 35751 | 39081 | 37564 | 39318 | 39895 | 38492 | 38449 |
| Dark: | 9673 | 9766 | 9802 | 9721 | 9796 | 9871 | 9889 | 9870 | 9718 |
| Delta: | 20762 | 29274 | 25949 | 29360 | 27768 | 29447 | 30006 | 28622 | 28731 |

| Lambda: 860 | Gain: 1.95 | Resets: 1 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 30306 | 38759 | 35633 | 38765 | 37221 | 39047 | 39715 | 38223 | 38136 |
| Dark: | 9657 | 9775 | 9822 | 9719 | 9813 | 9908 | 9932 | 9907 | 9714 |
| Delta: | 20649 | 28984 | 25811 | 29046 | 27408 | 29139 | 29783 | 28316 | 28422 |

| Lambda: 962 | Gain: 2.23 | Resets: 1 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Air: | 30088 | 38770 | 35545 | 38758 | 37241 | 39089 | 39940 | 38235 | 38095 |
| Dark: | 9648 | 9780 | 9836 | 9716 | 9825 | 9934 | 9961 | 9931 | 9712 |
| Delta: | 20440 | 28990 | 25709 | 29042 | 27416 | 29155 | 29979 | 28304 | 28383 |

| Channel: | Ref | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Noise Max: | 9634 | 9686 | 9775 | 9648 | 9766 | 9902 | 9915 | 9888 | 9658 |
| Noise Min: | 9632 | 9684 | 9773 | 9647 | 9764 | 9899 | 9910 | 9884 | 9656 |
| Delta: | 2 | 2 | 2 | 1 | 2 | 3 | 5 | 4 | 2 |

02:51PM 08/30/02 INCUBATOR SELF TEST

Temperature Setpoint: 37.0 Current Average: 36.9 A/D Test: PASS

| | | | | |
|---|---|---|---|---|
| Zone 1: 36.9 | Min: 36.9 | Max: 37.0 | Range: PASS | Thermistor: PASS |
| Zone 2: 37.0 | Min: 36.9 | Max: 37.1 | Range: PASS | Thermistor: PASS |
| Zone 3: 36.9 | Min: 36.9 | Max: 37.1 | Range: PASS | Thermistor: PASS |
| Zone 4: 37.0 | Min: 36.9 | Max: 37.1 | Range: PASS | Thermistor: PASS |

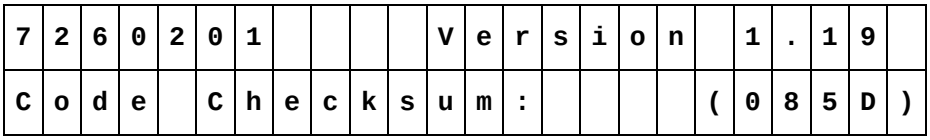
SYSTEM TEST PASS

***Figure 4-1:*** *Sample Output for the System Test*

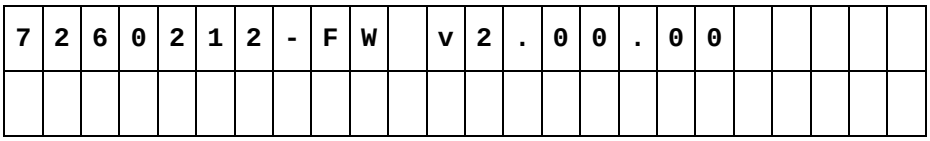
## CHECKSUM TEST (CHKSUM)

The Checksum test runs automatically when the reader is powered on. The test compares the software to the internally recorded checksum values to ensure that the programming has not been corrupted. If there are any errors during the power-on checksum test, they will be displayed.

To verify the checksum manually, and to view the part numbers and versions of software currently loaded onto your reader, start at the Main Menu and press `UTIL` ➔ `TESTS` ➔ `CHKSUM`. The information displayed will *resemble* the following; the actual checksum will be dependent upon the software version that is loaded:




*The initial checksum test display, showing the **basecode** software part number, version number, and checksum. After a few moments, a second screen will display.*




*The second checksum test display, showing the **assay configuration** software part number and version number.*

## Calibration Verification

It is considered good laboratory practice to periodically verify the calibration of the *PowerWaveX*. Verification should be performed monthly using the tests in this section:

- Universal Plate Test (see below)
- Liquid Tests (see page 4-14)

### UNIVERSAL PLATE TEST

The Universal Plate Test (also referred to at the Calibration Plate Test), confirms the alignment, repeatability, and accuracy/linearity of the *PowerWaveX*. An alternative method used to determine accuracy, repeatability and linearity is the Liquid Test 2 described later in this chapter.

The Universal Test Plate (PN 7260522) allows comparison of the reader's optical density measurements and mechanical alignment to NIST traceable values. Accuracy/ linearity, repeatability, and alignment are tested. Specific standard calibration values must be entered for each wavelength to be tested (see *Entering the Universal Test Plata Data* on the following page).

The Universal Plate Test confirms the following:

- **Accuracy** of the Optical Density readings -- the comparison of the optical density readings with those given with the Universal Test Plate insert will confirm the accuracy of the optical density readings at specific wavelengths.
- **Linearity** of the Optical Density readings are confirmed by default if the optical density readings are accurate. This can be proven by analyzing the data in a program such as Microsoft® Excel.
- **Alignment** of the plate carrier and standard microplates are confirmed by the four corner positional accuracy check.
- **Channel-to-channel variation** can be tested by completing the turnaround test. This tests the reader's ability to read samples accurately in different plate positions.
- **Wavelength setting accuracy.** To check the accuracy of wavelength settings, use the Universal Test Plate. The Test Plate provides a multiband test filter (Didymium glass V10) in location C6.

## Requirements

To run the Universal Plate Test from the reader front panel, you need Bio-Tek's Universal 7-Filter Test Plate (PN 7260522), with its accompanying Data Sheet, shown in *Figure 4-2* below.

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

**WAVELENGTH (nm)**

| Well | 405nm | 450nm | 490nm | 550nm | 620nm | 630nm | 690nm | 750nm |
|---|---|---|---|---|---|---|---|---|
| C1 | 0.139 | 0.134 | 0.130 | 0.127 | 0.133 | 0.134 | 0.125 | 0.130 |
| E2 | 0.640 | 0.596 | 0.593 | 0.576 | 0.592 | 0.589 | 0.502 | 0.466 |
| G3 | 1.188 | 1.105 | 1.099 | 1.069 | 1.095 | 1.090 | 0.924 | 0.852 |
| H6 | 1.742 | 1.628 | 1.608 | 1.561 | 1.614 | 1.605 | 1.367 | 1.256 |
| F5 | 2.154 | 1.925 | 1.881 | 1.822 | 1.795 | 1.772 | 1.443 | 1.218 |
| D4 | 2.837 | 2.519 | 2.445 | 2.415 | 2.357 | 2.324 | 1.932 | 1.595 |

Set # 4796 Serial # 174819

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

## Entering the Universal Test Plate Data

| | |
|---|---|
| iii | **Note:** Before defining the standard calibration values, set the reader's internal wavelength settings to correspond with up to six of the wavelengths shown on the Universal Test Plate Data Sheet (see *Figure 4-2* above). See *Adjusting the Reader's Wavelength Table Settings* in *Chapter 2* for instructions. Additional wavelengths, **above 405 nm**, are available at extra cost. Contact your Bio-Tek dealer. |

Refer to the Data Sheet provided with the Universal Test Plate (*Figure 4-2*), when entering the standard values. To enter the values into the reader, start at the Main Menu and press `UTIL` ➔ `SETUP`. The Edit Setup Information menu will appear.

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | : |
| | D | A | T | E | | | T | I | M | E | | L | A | M | B | D | A | | * | M | O | R | E |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | D | I | T | | S | E | T | U | P | | I | N | F | O | R | M | A | T | I | O | N | : |
| R | S | 2 | 3 | 2 | | | C | A | L | | P | L | A | T | E | | | | * | M | O | R | E |

*Edit Setup Information menu. Press *MORE to see the Cal Plate option.*

- From the Edit Setup Information menu, press `*MORE`, then select `CAL PLATE`.

The Calibration Lambda entry screen will appear.

| C | A | L | I | B | R | A | T | I | O | N | | L | A | M | B | D | A | : | | 4 | 0 | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 0 | 5 | | | | 4 | 5 | 0 | | | | 4 | 9 | 0 | | | | 6 | 3 | 0 | | |

*Selecting a wavelength for which to enter standard calibration values*

- Choose a wavelength (e.g., 405), then press **ENTER**.

| W | A | V | E | L | E | N | G | T | H | : | 4 | 0 | 5 | | | W | E | L | L | : | C | 0 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A | L | I | B | R | A | T | I | O | N | | V | A | L | U | E | S | | 0 | . | 1 | 4 | 7 |

*Entering standard calibration values, referencing the Universal Test Plate Data Sheet*

- Enter the absorbance value from the Data Sheet for the selected wavelength and current well location (C01 in the above example).
- After each entry, press **ENTER** to advance to the next consecutive well location.
- When the sixth value has been entered, press **ENTER** to return to the Calibration Lambda selection screen to select another wavelength, or **Main Menu**.

## Running the Universal Plate Test

Before running the Universal Plate Test, ensure that the standard calibration values for the test plate have been defined on the reader (see *Entering the Universal Test Plate Data* on the previous page).

To run the Universal Plate Test:

- Ensure that the printer is attached, turned on, and full of paper.
- From the reader's Main Menu, press `UTIL` ➔ `TESTS` ➔ `CAL PLATE`.

  The Calibration Lambda selection screen will appear.

| C | A | L | I | B | R | A | T | I | O | N | | L | A | M | B | D | A | : | | 4 | 0 | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 0 | 5 | | | | 4 | 5 | 0 | | | | 4 | 9 | 0 | | | | 6 | 3 | 0 | | |

*Selecting a wavelength for the calibration plate test*

- Select a wavelength for the test, then press **ENTER**.

The Place Plate in Carrier prompt will appear.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | L | A | C | E | | P | L | A | T | E | | I | N | | C | A | R | R | I | E | R | | |
| A | N | D | | P | R | E | S | S | | < | R | E | A | D | > | | K | E | Y | | | | |

*Place the Universal Test Plate in the reader, then press READ*

- Place the Universal Test Plate in the carrier so that well A1 is in the upper left corner of the carrier, then press **READ** to begin the test.

  The plate will be read twice, then the Rotate Plate prompt will appear.

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | o | t | a | t | e | | P | l | a | t | e | | 1 | 8 | 0 | | d | e | g | r | e | e | s |
| P | r | e | s | s | | < | R | E | A | D | > | | t | o | | C | o | n | t | i | n | u | e |

*Rotate the plate, then press READ*

- Rotate the plate so that well A1 is in the lower right corner of the carrier, then press **READ** to complete the test.
- When the test is complete, results will print. See the sample Calibration Plate Analysis report in Figure 4-3.

CALIBRATION PLATE ANALYSIS

05:50PM 08/30/02 Read Lambda: 405

Operator ID: 7696

Notes: 182427

A1 Top Left
Alignment Results

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A01=0.000 | PASS | A12=0.001 | PASS | H01=0.000 | PASS | H12=0.000 | PASS |

A1 Top Left
Accuracy Results

| | C01 | D04 | E02 | F05 | G03 | H06 |
|---|---|---|---|---|---|---|
| STANDARD | 0.160 | 0.617 | 1.221 | 1.804 | 2.210 | 2.919 |
| DATA | 0.155 | 0.623 | 1.230 | 1.818 | 2.228 | 2.921 |
| RESULT | PASS | PASS | PASS | PASS | PASS | PASS |

Repeatability Results

| | C01 | D04 | E02 | F05 | G03 | H06 |
|---|---|---|---|---|---|---|
| READ 1 | 0.155 | 0.623 | 1.230 | 1.818 | 2.228 | 2.921 |
| READ 2 | 0.155 | 0.623 | 1.230 | 1.819 | 2.228 | 2.923 |
| RESULT | PASS | PASS | PASS | PASS | PASS | PASS |

H12 Top Left
Accuracy Results

| | C01 | D04 | E02 | F05 | G03 | H06 |
|---|---|---|---|---|---|---|
| STANDARD | 0.160 | 0.617 | 1.221 | 1.804 | 2.210 | 2.919 |
| DATA | 0.163 | 0.627 | 1.227 | 1.807 | 2.223 | 2.931 |
| RESULT | PASS | PASS | PASS | PASS | PASS | PASS |

Repeatability Results

| | C01 | D04 | E02 | F05 | G03 | H06 |
|---|---|---|---|---|---|---|
| READ 1 | 0.163 | 0.627 | 1.227 | 1.807 | 2.223 | 2.931 |
| READ 2 | 0.162 | 0.627 | 1.227 | 1.808 | 2.224 | 2.923 |
| 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 PN 7260522 Calibration Test Plate.
- **Accuracy:** Accuracy is a measure of the absorbance (optical density) of Calibration Plate wells C01, D04, E02, F05, G03 and H06 with known standard values contained in the Specification Sheet that accompanies each Calibration Test Plate.
- **Linearity:** If the accuracy specifications are met, then the reader also proves to be linear. Linearity can also be proven by performing a regression analysis on the OD values in a program such as Microsoft® Excel. See the instruction on the following page.

In Microsoft® Excel, open a spreadsheet and label one column “Assigned” and the next column “Observed.”

1. Enter the Assigned OD data for each glass filter in the first column from the data sheet provided with the Test Plate.
2. (Analyze one wavelength at a time.) Next, enter the Observed OD values for the same glass filters in the adjacent column.
3. Under Tools, select Data Analysis, and then Regression.
4. Use the Regression “Input” box to enter the Assigned values as the “Input Y Range” and the Observed OD as the “Input X Range.”
5. Click the OK box and the Summary Output sheet will be displayed. An R Square value of at least 0.99 is expected and the results will often be 0.999 or better.

- **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.
- **Turnaround:** (Second Repeatability Result) This test confirms the reader's ability to read samples accurately in different plate positions. Each channel is compared to at least one other channel in this test: A to H, B to G, C to F, and D to E. This ensures that all channels have comparable performance.

## Wavelength Accuracy

The C6 filter should be scanned between 580 and 590 nm in 1-nm increments using KC4 or KC*junior* software. If neither KC4 nor KC*junior* is available, select six wavelengths at 1-nm increments near the expected peak. 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* below). The accuracy of the wavelength should be ± 3 nm (± 2 nm instrument, ± 1 nm filter allowance).

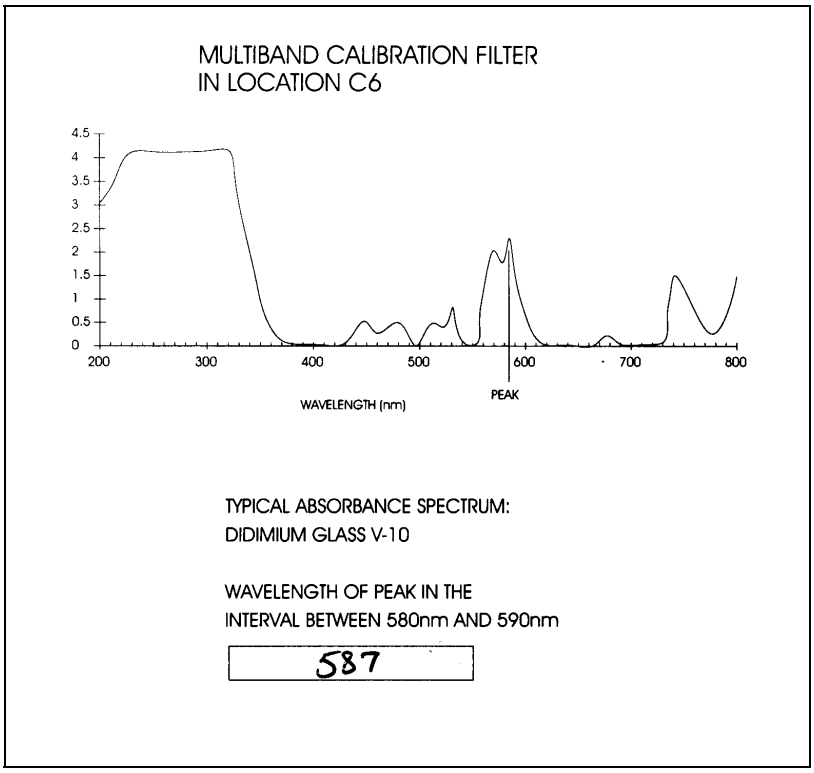



***Figure 4-4:*** *Sample data sheet showing wavelength of peak in the interval between 580 and 590 nm*

For example:

If the data sheet value is 587 nm and the allowable range is ± 3 nm, and if the reader reports a peak value of 590 nm, then the reader meets its specifications. If the reader reports 591 nm, then the reader does not meet specifications.

## 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 or contamination that can contribute to errant readings.

- If you have the Universal Test Plate, you will need to run the simple Liquid Test 1 for routine testing.
- If you do not have a Universal Test Plate, test the linearity and repeatability 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 described dye solution (see *Table 4-3*). The purpose of the recipe is to create a solution that absorbs light in a well-defined manner and yields ~ 2.000 OD at full strength when dispensed at 200 microliters in a 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 are shown below.
- If you need to test a Universal Test Plate 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 |

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 (Polyoxyethylene sorbitan 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 2.000 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 2.000 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 on page 4-15 or Procedure B on page 4-16), 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 2.000 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. Next, turn the microplate around so that well A1 is now in the H12 position and read the plate five more times.
6. The plate data can be exported to an Excel® spreadsheet using KC4 or KC*junior*. The mathematical computations described below may then be performed and the template kept for future data reduction.

**Channel-to-Channel Variation:**

7. Calculate the means of the wells in columns 1 and 2 in the normal plate position, and in the turnaround position.

   This test results in four comparisons of each channel to its corresponding channel, two in column 1, and two in column 2.

   Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the other wells to their corresponding mean values with the well in the turnaround position. (Compare B1 to G12, C1 to F12, D1 to E12, E1 to D12, F1 to C12, G1 to B12, H1 to A12, A2 to H11, B2 to G11, etc.). 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.)

If any set of well values is out of the expected range, review the other three sets for the same channel pair. Thus, if A1 and H12 are not within range of each other, review the compliance of H1 to A12, A2 to H11, and H2 to A11. This will confirm that there is a problem in one of the eight read channels or indicate that the result of one set of wells was in error. If two or more sets of well values for a channel pair are out of the allowed accuracy range, there may be contamination on, or a problem with, one of the lenses.

### Accuracy Specification:

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

± 1% ± 0.010 Abs from 0.000 to 2.000 Abs

± 3% ± 0.010 Abs from 2.000 Abs to 3.000 Abs

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 on page 4-15, or Procedure B on page 4-16.
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 or KC*junior*. 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 2.000 Abs, calculate the allowed deviation using the repeatability specification for a 96-well format of ±1% ± 0.005 Abs. If above 2.000 Abs, apply the ±3% 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 0.000 to 2.000 Abs

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

## Channel-to-Channel Variation and 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 four comparisons of each channel to its corresponding channel, two in column 1, and two in column 2.
2. Calculate the means of the wells in columns 1 and 2 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 other wells to their corresponding mean values with the well in the turnaround position. (Compare B1 to G12, C1 to F12, D1 to E12, E1 to D12, F1 to C12, G1 to B12, H1 to A12, A2 to H11, and B2 to G11, etc.). 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.)

   If any set of well values is out of the expected range, review the other three sets for the same channel pair. Thus, if A1 and H12 are not within range of each other, review the compliance of H1 to A12, A2 to H11, and H2 to A11. This will confirm that there is a problem in one of the eight read channels, or indicate that the result of one set of wells was in error. If any two sets of well values for a channel pair are out of the allowed accuracy range, there may be contamination on, or a problem with, one of the lenses.
3. If the four corner wells are within the repeatability range, the reader is also in alignment.

## LIQUID TEST 3

To verify operation of the *PowerWaveX* at 340 nm, perform the Liquid Test 3 Procedure described on page 4-23. This test is optional as the front end of the instrument shows good correspondence at various wavelengths. Thus, if the device performs properly using the test plate at 405 nm, it will also perform adequately at 340 nm.

***Required Materials:***

- Deionized Water
- Pipettes
- Costar® #3590 Flat-Bottom Microplate
- Beakers and Graduated Cylinder
- Analytical balance
- Tween® 20 (Polyoxyethylene sorbitan 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 0.700 to 1.000. 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 a Microsoft Excel spreadsheet using KC4™ or KCjunior™. 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 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.

## Maintenance of Robotic Units

Use the following procedure for periodic lubrication of *PowerWaveX* robotic units.

1. Remove the shroud.
2. Lubricate the components shown in *Figures 4-5* and *4-6* every six months, or after 10,000 cycles, according to the following instructions. Use Bio-Tek lubricant PN 66039.

| Component Location | Procedure |
|---|---|
| A | Lubricate the underside of the bracket. |
| B | Lightly lubricate the shaft indicated. |
| C | Apply a heavy coating of lubricant to both sides of the motor shaft. Run the motor shaft back and forth through the motor to ensure that the internal drive nut is heavily lubricated. Use the robotic motor adjustment procedure provided below to work the lubricant into the internal drive nut. |
| D | Ensure that the mylar sheet (BTI P/N 7262113) is in place on top of the main PCB and below the motor assembly before lubricating the motor. Tape to the chassis with black electrical tape. (This may already be in place.) |
| E | Lightly lubricate the surface of the roller. |
| F | Lightly lubricate the underside of the hook-in bracket. |

3. Reassemble the shroud.
4. After lubrication/reassembly, the robotic door needs to be set to the open and closed positions.
5. Press the following keys to adjust the door. At the **Main Menu**:
   - Press the **UTIL** soft key.
   - Press the **Setup** soft key.
   - Press the **hidden key** between the **Main Menu** and **Previous Screen** keys.
6. You should see the **Door Adjustment** screen.
7. Press the **CLOSE** soft key.

8. Press the **UP** soft key until you just see the door move up. You may have to press the key many times before the door moves. When the door does move, press the **DOWN** soft key 4 times. This positions the door lift ram just behind the door roller in the closed position.
9. Press the **OPEN** soft key.
10. Press the **UP** soft key to raise the door to the desired height. Press the DOWN soft key if you want to adjust the door downward.
11. Press the **CLOSE** soft key. This will close the door. The door should rest closed on the top shroud.
12. Press the **Main Menu** key.

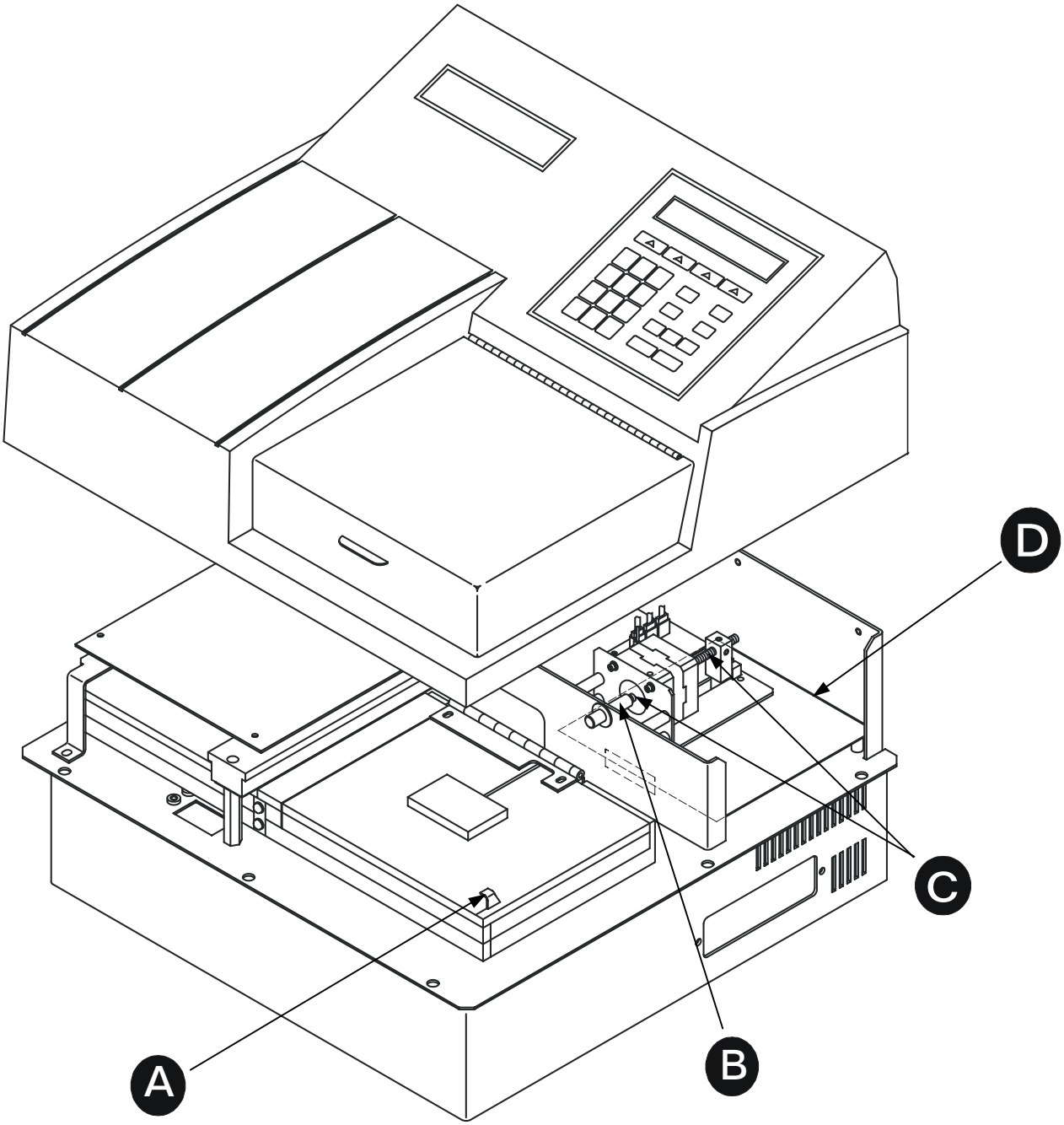



***Figure 4-5:*** *Location of bracket, motor shaft, mylar sheet, and main PCB/motor assembly*

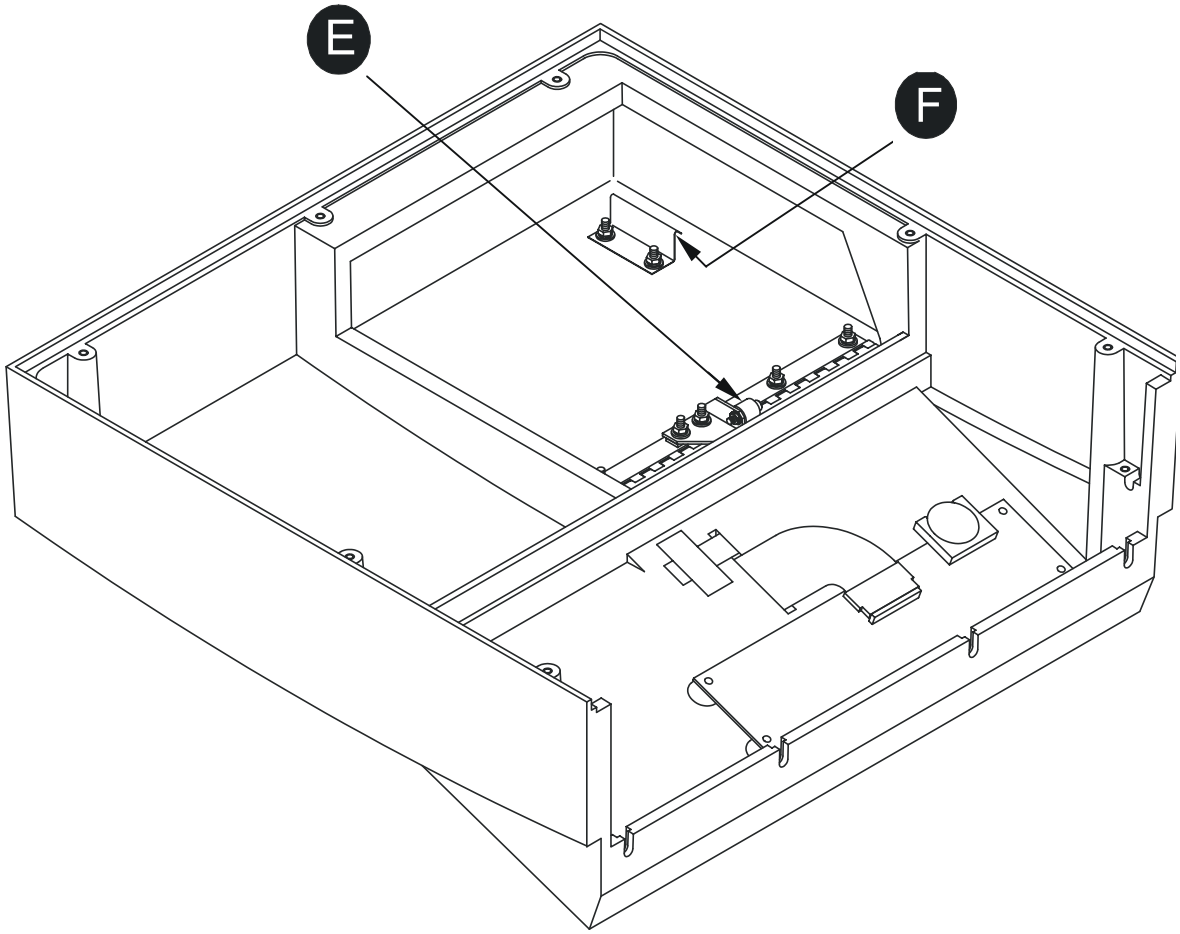



***Figure 4-6:*** *Roller surface and hook-in bracket*

# Appendix A
# *Decontamination and Cleaning*

*This appendix contains the procedures for decontaminating and cleaning the PowerWaveX.*

## Decontamination Procedure

If the *PowerWaveX* 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 be 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**.**

| | |
|---|---|
| iii | **Important!** Disconnect the unit from the power supply for all decontamination or cleaning operations. |

**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.

## Cleaning Procedure

Exterior surfaces may be cleaned (not decontaminated ) with a cloth moistened (not soaked) with water or water and mild detergent. Do not immerse instrument or spray with liquids.

# Appendix B
# *Computer Control*

*The PowerWaveX can be controlled either from the reader's front panel or from a computer connected to the reader via the computer's serial port. This chapter describes the features of computer control, and explains how to configure the computer to control the reader.*

## Overview

With the computer control feature, the user is provided even more power and flexibility. For example, the *PowerWaveX* can run computer-controlled kinetic assays using up to six wavelengths on the same microplate. Spectral scan assays may be performed that use the entire wavelength range from 200 nm to 999 nm, at 1 nm increments.

When using computer control, the blanking and data reduction capabilities on the *PowerWaveX* are suppressed, and all data is returned to the user for evaluation. Readings higher than 3.000 OD may be transmitted.

This appendix details the protocols necessary for communicating with the *PowerWaveX* reader. In addition, instructions are provided for controlling the reader with Bio-Tek's KCjunior or KC4 software packages.

## Controlling the Reader with KC4™

The *PowerWaveX* can be operated using a computer running Bio-Tek's KC4 software. Follow the steps below:

1. Power off the computer and the reader. Connect the appropriate serial cable (PN 75053) between the two machines. See *Table B-1* on the following page for a pin-out description of the cable.
2. Power up both machines.
3. Install KC4 on the computer's hard drive.
4. Once installed, start KC4.
5. Select **System**, **Readers**.
6. Scroll through the list of **Available Readers** and select the appropriate *PowerWaveX* reader model. Click the **Port** button (and subsequent **Setup** button), to define the following communications parameters:
    - Port: **COM1**, **2**, **3**, or **4** (the serial port used for the RS-232 cable connection).
    - Baud Rate: **9600**
    - Data Bits: **8**
    - Parity: **None**
    - Stop Bits: **2**
7. Click the **Current Reader** button to attempt to establish communications with the reader, using the currently defined communication parameters.
8. If the test passes, click **OK** to save the settings and close the dialog box. If the test fails, KC4 will provide appropriate instructions for resolving any problems. See also the *Problems* section below.

To learn more about KC4 and how to define assays and read plates, refer to the KC4 User's Manual.

### *Problems*

If KC4 fails to communicate with the reader and it displays a serial communications error, check the cable plug-in location to ensure that it matches the setup choices and is not a Null cable. If this is suspected, add another Null and try again.

If an **Incorrect Reader Model Connected** dialog box is displayed, click **OK** to clear the screen and select **System**, **Readers**, **Available Readers**. Verify that the reader selected is correct.

**Table B-1**

**Serial Cable Pin-Out Description**

| Serial Cable Pin-Out* | |
|---|---|
| **PC (9-pin female)** | **Reader (25-pin female)** |
| 1+6 | 4 |
| 2 | 2 |
| 3 | 3 |
| 5 | 7 |
| 7 | 5+6+8 |
| 8 | 20 |
| Shell | Shell (shielding) |
| * For a 25-pin PC connection using Bio-Tek serial cable (PN 75053) plus 9pM-25pF adapter (PN 49755). | |

### *Getting Started with KC4*

The following instructions *briefly* describe how to read a plate using KC4. Refer to KC4's Help system and User's Guide early and often to learn how to create protocols, assign well identifiers, read plates, print reports, and more.

To read a plate using KC4:

1. Select Data|New Plate.
2. If prompted to select a protocol, select "Empty Protocol" and click OK. If not prompted, select Protocol|New, or use KC4's Protocol Wizard to step through protocol creation.
3. Select Protocol|Reading. The Reading parameters dialog will appear.
4. Select a Reading Type of Endpoint, Kinetic, or Spectrum.
5. Define the wavelength(s) at which the plate will be read.
6. Select a Plate Geometry of 8x12 (96-well plate) or 16x24 (384-well plate).
7. Define other reading parameters as necessary. Click the Help button for assistance.
8. When complete, click OK.
9. Select Data|Read Plate. The Plate Reading dialog will appear.
10. Enter any comments, place the plate on the carrier, then click Start Reading to begin the plate read.
    - The plate will be read and then the raw data results will display in KC4.
    - To analyze, manipulate, or print results, **protocol** parameters should be defined. Refer to KC4's Help system or User's Guide for instructions.

## Controlling the Reader with KCjunior™

The *PowerWaveX* can be operated using a computer running Bio-Tek's KCjunior software. Follow the steps below:

1. Power off the computer and the reader. Connect the serial cable (PN 75053) between the two machines. See *Table B-1*.
2. Power up both machines.
3. Install KCjunior on the computer's hard drive.
4. Once installed, start KCjunior.
5. Select **Setup**, then **Reader1**. To select the *PowerWaveX* reader and define the communications parameters, choose the following setup parameters:
    - Reader: **PowerWaveX**
    - Com Port: **COM1** or **COM2** (the serial port used for the RS-232 cable connection)
    - Baud Rate: **9600**
    - Data Bits: **8**
    - Parity: **None**
    - Stop Bits: **2**
    - EOT Character: Keep the default number.
6. Click the **Test Communications** button to attempt to establish communications with the reader, using the currently defined communication parameters. If a **Serial Write Error** dialog box is displayed, an incorrect Com Port may have been selected. Select a different port and then repeat this step.
7. If the test passes, click **OK** to save the settings and close the dialog box. If the test fails, follow the directions provided by KCjunior, then click **Test Communications** again.

To learn more about KCjunior and how to define assays and read plates, refer to the KCjunior User's Manual.

### *Problems*

If KCjunior fails to communicate with the reader, 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.

### *Getting Started with KCjunior*

The following instructions *briefly* describe how to read a plate using KCjunior. Refer to KCjunior's Help system and User's Guide early and often to learn how to create protocols, assign well identifiers, read plates, print reports, and more.

To read a plate using KCjunior:

1. Click Read Plate from KCjunior's main screen. The Read Plate Dialog will appear.
2. If desired, enter a Results ID and a Plate Description, and then click Read Plate. The Protocol Definition dialog will appear.
3. Select a Read Method Type of Endpoint, Kinetic, Multi-Wavelength, or Spectrum.
4. Define the wavelength(s) at which the plate will be read.
5. Select a Plate Geometry of 8x12 (96-well plate) or 16x24 (384-well plate).
6. Define other reading parameters as necessary. Click the Help button for assistance.
7. When complete, click OK to return to the Read Plate dialog. If desired, enter a Plate ID.
8. Place the plate on the carrier, then click OK to start the plate read.
    - The plate will be read and then the raw data results will display in KCjunior. Print the raw data by selecting Plate|Print Results.
    - To analyze or manipulate results, a **protocol** should be defined. Refer to KCjunior's Help system or User's Guide for instructions.

## Controlling the Reader Using Serial Protocol

At baud rates of 1200, 2400, and 9600, the *PowerWaveX* 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 RS-232C serial port on the *PowerWaveX* is configured as a DTE (see the section *Setting Up the Serial Port for Communications* in Chapter 2); that is, the unit is wired to look like a modem. Data is received on Pin 3 (the RX Pin), and transmitted on Pin 2 (the TX pin).

## Computer Control Command Set

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

While awaiting a command, the *PowerWaveX* 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-2* below for the ASCII Control Characters used in the computer control protocol.

**Table B-2**

**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 | <--- |

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

Lower wavelength limits shown are for the *PowerWaveX* 200 only. The *PowerWaveX* 340 is limited to wavelengths above 340 nm, and will not accept lower values.

The following section describes the supported computer control command set.

### STORE PLATE CARRIER ('A')

This command causes the plate carrier to move inside the instrument.

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

### 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> |
| | | status string (5) |

### CONFIGURE WAVELENGTHS ('M')

This command downloads the wavelength setup table for use in future read cycles.

| **host:** | **valid limits:** | **response:** |
|---|---|---|
| 'M' | | <ACK/NAK> |
| data bytes: | | |
| 1 - 3 | 1st wavelength ("200" - "999", ASCII) | |
| 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 wavelength setup table is stored in non-volatile memory, and only needs updating when a change in current wavelength configuration is desired.

After wavelength configuration, a self-test ('*****' command) sequence must be run 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.

## READ PLATE ('S')

This command causes a microplate to be read according to the currently loaded assay definition table.

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

Reader response protocol (ASCII format):

status string (5)

for each wavelength specified (up to six):

| | | |
|---|---|---|
| wavelength start code (1) | | <CR> |
| for each row: | | |
| for each column (well) within specified strip range: | | |
| comma separator (1) | | ',' |
| sign (1) | | '+' or '-' |
| data (4) | | "1234" |
| row terminator (2) | | <CR>, <LF> |
| data terminator (1) | | <^Z> |
| checksum (1) | | 0 - 255 (not ASCII) |

status string (5) (only if read error)

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.

Checksum calculation starts with the first byte AFTER the data 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).

### SET ASSAY DEFINITION ('V')

This command downloads to the reader a 170-byte assay definition table to be used in subsequent plate reads.

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

| **host:** | | **valid limits:** | **response:** |
|---|---|---|---|
| 'V' | | | <ACK/NAK> |
| data bytes: | | | |
| 1 | | don't care | |
| 2 - 7 | | assay name (ASCII characters) | |
| 8 - 30 | | don't care | |
| 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 | | number of scans per well for well scanning (must be an odd integer from 1 to 31) | |
| 33 - 49 | | don't care | |
| 50 - 55 | | "000000" (ASCII) | |
| 56 | | read type (0: endpoint, 2: kinetic, 3: scan)<br>Note: 3 indicates well scanning. To perform spectral scanning, use the '&' SCAN PLATE command. | |
| 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 ("200" - "999", ASCII) | |

| host: | valid limits: | response: |
|---|---|---|
| 70 - 84 | remaining wavelengths (“000” - “999”, ASCII) | |
| 85 - 162 | don’t care | |
| 163 - 164 | shake time (seconds, 0 - 999) | |
| 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) |

Up to six wavelengths may be specified for a single plate read definition.

The assay definition table must be downloaded before other plate-specific commands are sent, such as “Set Plate Geometry” and “Set Strip Range”. If a wavelength is not to be used, it must be replaced by “000”.

### GET 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 (ASCII format):

for each wavelength configured (six):

| | |
|---|---|
| wavelength | (“000” - “999”) |
| comma terminator | ‘,’ |

status string (5)

### HALT ('X')

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

| host: | valid limits: | response: |
|---|---|---|
| 'X' | no arguments | none (see below) |
| | | no status string |

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 be 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, nor is a status string.

### 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 | geometry (2 or 3) | |
| | 2: 96 wells (8 x 12) | |
| | 3: 384 wells (16 x 24) | |

If no Set Plate Geometry command is sent after the assay definition table, the default 96 well plate is assumed (it is recommended, however, that you issue this command even if using the 96 well plate).

If only a range of strips is desired, the Set Strip Range command must be sent AFTER the Set Plate Geometry command.

### SET STRIP RANGE ('%')

This command selects a range of adjacent strips (numbered plate columns) to be read.

| **host:** | **valid limits:** | **response:** |
|---|---|---|
| '%' | | <ACK/NAK> |
| data bytes: | | |
| 1 | first strip (1 - 24) | |
| 1 | last strip (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, either from the front panel or by computer control. The strip range is also reset when the Set Geometry command is sent.

### GET MINIMUM 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, strip range, and even baud rate selected.

| **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).

### SCAN PLATE ('&')

This command causes the reader to perform a spectral scan on the indicated microwell.

| **host:** | **valid limits:** | **response:** |
|---|---|---|
| '&' | | <ACK/NAK> |
| data bytes: | | |
| 1 - 2 | row ("00" - "16", ASCII) | |
| | "00" indicates scan entire strip (column)<br>Note: Do not use 00 if reading 384-well plate. | |
| 3 - 4 | column ("01" - "24", ASCII) | |
| 5 - 7 | start wave ("200" - "999", ASCII) | |
| 8 - 10 | stop wave ("201" - "999", ASCII) | |
| 11 - 13 | wave step ("001" - "799", ASCII) | |
| 14 | series option ('0' - '3', ASCII) | |
| | '0': single scan | |
| | '1': first scan in series | |
| | '2': next scan | |
| | '3': last scan | |
| 15 | calibrate option ('0' - '1', ASCII) | |
| | '0': calibrate only if necessary | |
| | '1': calibrate before scanning | |
| 16 - 17 | shake time in seconds ("00" - "99", ASCII) | |
| 18 | shake speed | |
| | '0': slow | |
| | '1': medium | |
| | '2': fast | |
| | '3': cycle through speeds listed above | |
| <ETX> | | <ACK/NAK> |

| **host:** | **valid limits:** | **response:** |
|---|---|---|
| Reader response protocol (ASCII format): | | |
| status string (5) | | |
| for each wavelength undergoing calibration (none to all waves selected): | | |
| wavelength (3) | | “200” - “999” |
| wave terminator (1) | | <CR> |
| calibration terminator (1) | <^Z> | |
| for each wavelength specified for first pass scan (none, or all waves selected): | | |
| wavelength (3) | | “200” - “999” |
| wave terminator (1) | | <CR> |
| first pass terminator (1) | | <^Z> |
| data start code (1) | | <CR> |
| for each wavelength specified for scan: | | |
| wavelength (3) | | “200” - “999” |
| for each well selected in strip (1 or 8 wells): | | |
| comma separator (1) | | ',' |
| sign (1) | | '+' or '-' |
| data (4) | | “1234” |
| well/strip terminator (2) | <CR>, <LF> | |
| data terminator (1) | | <^Z> |
| checksum (1) | | 0 - 255 (not ASCII) |
| status string (5) (only if error) | | |

The reader sequentially scans the well or strip 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 or equal to the difference.

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.

Due to the fact that even-row detectors are offset left by one column, the reader uses two passes to collect data when an entire strip is selected to be scanned. During the first pass, only the wavelength being scanned is returned. Absorbance data is returned for each wavelength for the entire strip during the second pass. The first pass terminator is always returned even if no first pass is necessary.

If more than one well or strip is to be scanned, the series option can be used to make the process more efficient. If a sequence of strips is to be scanned using the series option, they must be contiguous and selected in sequence from left to right, since first pass data for the next strip is collected during the second pass for the currently-selected strip. The last strip selected (last scan) then requires no first pass. Calibration is only performed if the "single" or "first" scan option is selected, and remains valid through the remaining wells or strips in the series.

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, a <NAK> response is returned by the reader.

The Set Geometry command must be sent before any Scan Plate commands if a microplate other than the standard 96-well plate is to be used.

### SELF-TEST ('*')

This command causes the reader to perform a system self-test and calibration. This should be performed any time the wavelength configuration is changed ('M' command).

| **host:** | **valid limits:** | **response:** |
|---|---|---|
| '*' | no arguments | <ACK/NAK> |
| | | 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.

| **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.

### GET TEMPERATURE (‘]’)

This command returns the current temperature in the incubation chamber.

| 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. 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 PLATE BARCODE (‘s’)

This command returns the barcode for the microplate currently in the instrument carrier.

| host: | valid limits | response: |
|---|---|---|
| 's' | no arguments | <ACK/NAK> |
| | | barcode (33) |
| | | status string (5) |

The barcode is returned as a 33-character string, with a null terminator (0x00) as the last character. If the barcode is less than 32 characters it is null-terminated, then padded with blank characters (0x20) up to the final null. If the barcode is more than 32 characters it is truncated.

If no barcode is detected after two passes of the microplate carrier in front of the scanner, the null-terminated string "NR" is returned, followed by the remainder of the 33-character string of blanks and the final null. A barcode error is flagged and returned with the standard status response string.

### GET INSTRUMENT CONFIGURATION ('}')

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

| **host:** | **valid limits** | **response:** |
|---|---|---|
| '}' | no arguments | <ACK/NAK> |
| | | configuration (2) |
| | | no status string |

Instrument configuration data is returned low byte first.
Encoding is defined as follows:

| **code:** | **description:** |
|---|---|
| 0x0030 | PowerWave 200 |
| 0x0031 | PowerWave 340 |
| 0x0032 | PowerWave$_X$ |
| 0x0038 | PowerWave 200 w/ Incubator |
| 0x0039 | PowerWave 340 w/ Incubator |
| 0x003A | PowerWave$_X$ w/ Incubator |
| 0x003B | PowerWave$_X$ 340 w/ Incubator |
| 0x0072 | PowerWave$_X$ Select |
| 0x007A | PowerWave$_X$ Select w/ Incubator |
| 0x00B0 | PowerWave 200 w/ Robot Access Door |

| code: | description: |
|---|---|
| 0x00B1 | PowerWave 340 w/ Robot Access Door |
| 0x00B2 | PowerWave$_X$ w/ Robot Access Door |
| 0x00B8 | PowerWave 200 w/ Incubator and Robotic Access Door |
| 0x00B9 | PowerWave 340 w/ Incubator and Robotic Access Door |
| 0x00BA | PowerWave$_X$ w/ Incubator and Robotic Access Door |
| 0x00F2 | PowerWave$_X$ Select w/ Robot Access Door |
| 0x00FA | PowerWave$_X$ Select w/ Incubator and Robotic Access Door |

### OPEN PLATE ACCESS DOOR ('(')

This command opens the robotic plate access door

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

### CLOSE PLATE ACCESS DOOR (')')

This command closes the robotic plate access door

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

## Using the Stop Key to Halt Plate Scans and Reads

Pressing the **STOP** key on the reader while a computer-control-initiated plate read or spectral scan is in progress causes the following to occur.

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, nor is a status string.

## Status String Format

Following execution of each command, the *PowerWave* sends a status string back to the computer. This 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 strip 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.

Instrument failures are usually accompanied by an error code on the reader display.

**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.

Items described in curly braces ({}) are indicated by an ASCII digit replacing the next-to-last '0' character in the status code.

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

| | |
|---|---|
| iii | **Note**: Errors listed on the following pages 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 powerup |
| DR ALLOC ERR | “A500” | // DR steps alloc/free error <assay num) |
| 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 |
| 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 |
| 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 |

***Non-Fatal Errors (continued)***

| | | |
|---|---|---|
| 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> |
| SENSOR POS ERR | "2400" | // test sensor position incorrect <motor> |
| FLASH MISS ERR | "2500" | // motor went by flash location too soon |
| XY LIMIT ERR | "2600" | // physical limit exceeded for area scan request |
| PANEL METHOD ERR | "2700" | // <assay> method doesn't match first panel assay |
| MOTOR TIMER ERR | "2800" | // <motor> timer not available |
| VREF ERR | "2900" | // voltage reference failed <test type> |
| PLATE JAM ERR | "2A00" | // <motor> didn't find middle sensor |

***Test Type Codes (lowest digit in returned error code)***

| | | |
|---|---|---|
| FAIL POWER 5V | '1' | // 5V power failed |
| FAIL POWER 24V | '2' | // 24V power failed |

***Motor Codes (lowest digit in returned error code)***

| | | |
|---|---|---|
| Carrier X Axis | '0' | |
| Filter Wheel | '1' | |
| Robotic Door | '2' | ('R' instruments only) |
| Monochromator | '3' | |
| Carrier Y Axis | '4' | (Select only, '2' on HT) |

***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.*

***Data Flash Codes (2nd lowest digit in returned error code)***

| | | |
|---|---|---|
| Readback Error | '0' | // data read back 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' |

# Appendix C
# *Error Codes*

*This chapter describes Error Codes that may appear on the reader. If an error is displayed and you cannot solve the problem, call Bio-Tek's Technical Assistance Center. Refer to Chapter 1, Technical Support for contact information.*

## Error Codes

An error code is displayed on the microplate reader as a four-digit identifier. The first digit is usually 0 or A. A 0 denotes a non-critical error, which means the instrument will still respond to keypad input. An A denotes a serious error, which requires that the reader be powered down, and then powered back up before any diagnostics can be performed.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0200** | Plate carrier did not find the home sensor |
| **ERROR 0201** | Order sorting filter wheel did not find the home sensor |
| **ERROR 0202** | Robot lid lift did not find the home sensor |
| **ERROR 0204** | Y axis could not find the optosensor |

Errors 0200, 0201, 0202, and 0204 indicate that an axis was not able to correctly travel to its "home" position. Both axes have optical sensors which, when interrupted, indicate that the specific axis has been successfully homed.

**PROBABLE CAUSE:**

- **Carrier Axis Case - defective sensor.** The X-axis movement is limited so that the optical sensor cannot be interrupted.
- **Order Sorting Wheel Case - defective sensor.** The order sorting filter wheel movement is limited so that the sensor cannot be interrupted.

| | |
|---|---|
| **iii** | **Note:** In cases where a sensor is not functioning, the motor will 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** | Order sorting 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.
- **Order Sorting Filter Wheel Motor -** The filter wheel drive gear is loose or motor drive failure is impeding filter wheel movement.
- **Flash Lamp** – The flash lamp is not flashing due to a defective lamp, poor alignment, or blocked optics.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0400** | Track carrier failed position verify |
| **ERROR 0401** | Order sorting filter wheel failed position verify |
| **ERROR 0402** | Robot lid lift failed position verify |
| **ERROR 0403** | Wavelength select (monochromator) failed position verify |
| **ERROR 0404** | Y axis failed position verify (*PowerWaveX Select* models only) |

Errors 0400, 0401, 0402, 0403, and 0404 indicate that an axis failed its position verify test. The position verify test verifies that the axis returns to the opto or the zero order (mono case) in the same number of steps as it is expected to be away from home. If the axis does not return home in the required number of steps, the test fails. Each axis is allowed to gain or lose three steps.

**PROBABLE CAUSE:**

- Belt slipping caused by incorrect tension, loose motor pulley, or loose belt clamp.
- Defective motor drive circuit or Main PCB.

- In the wavelength select case, failure to verify home correctly usually indicates an alignment problem.
- Anti-backlash spring on the monochromator has become free of the mounting post.
- A defective flash lamp.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0500** | Saturation error |

This error indicates that the optic system detected saturation conditions at the wavelength selected during the read.

**PROBABLE CAUSE:**

- Optic system is defective.

| 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 flash lamp, misaligned optics, or defective analog board.
- A defective order sorting filter.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0700** | Reader failed noise test |

The last digit indicates the channel, 0-8, where 0 is the reference channel.

The reader noise test checks the DARK current signal level for stability. Dark current is measured with the light blocked at maximum measurement channel gain. Four groups of 96 readings are taken at 100 ms intervals. This data is reduced to four averages that cannot vary by 20 counts or a 0700 error will result. The outgoing production specification for this test is 12 counts of variation. The unit will not flag an error until a 20-count variation is seen.

**PROBABLE CAUSE:**

- External signals getting into the measurement circuit. The bottom and top shrouds should be correctly installed.
- This problem in a correctly assembled unit could indicate a bad main or analog board. Failure indicates excessive variation in the dark current (background) noise levels of the measurement circuit.
- Defective ground on the track zone 3 ground wire.
- Malfunctioning incubation board.
- Defective photodetector on the analog PCB.

| 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. The noise signal level must be between 144 and 2019 counts.

**PROBABLE CAUSE:**

- Ambient light leak (track access door open during self check).
- The analog board could be dirty or defective.
- The digital board power supplies could be outside their intended values.
- The photodiode could be defective.

| 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 from the same reading taken during the power-up self-check. The last digit indicates the wavelength and the second to last digit indicates the channel.

**PROBABLE CAUSE:**

- The measurement electronics background noise has changed since the last power-up self-check. This could be caused by a large increase in external ambient light since power-up. See Probable Cause for Error 0800.
- Running the System Test will reset the value to the new baseline.

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

Error A00 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. The flash lamp could be near failure.
- Running the System Test will restore to new baseline.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0B00** | Invalid assay |

This error indicates that an assay number that is not programmed was selected.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0C00** | Printer timeout |

Error 0C00 indicates that the printer in use is not responding.

**PROBABLE CAUSE:**

- Printer not connected or powered up.
- Printer's parallel port may not be correctly selected in the on-board software. See *Printing and Data Communications* in Chapter 2.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0D00** | Calibration checksum error |

This error indicates that the stored checksum value for the calibration data does not match the actual checksum. This indicates that the data in the quick flash memory has been corrupted, probably due to an electronic defect.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0E00** | Wavelength not detected in reader's Lambda table |

Error 0E00 indicates that the specified assay wavelength is not in the Lambda table and must be programmed in.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 0F00** | Wavelength signal is out of specified range |

This error indicates that the measurement frequency selected has a signal out of range. The causes could be many. Essentially, the signal produced at a specific frequency is either too high or too low to allow performance within the specification. The lamp could have gone bad or a part of the optics system could be out of alignment. This can also be a flag for filters starting to degrade.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1000** | Configuration data is missing |

This error indicates that necessary configuration data is missing from memory, which probably means it was never downloaded or it was downloaded incorrectly.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1100** | Failed configuration checksum test |

This error indicates that the stored checksum value from the configuration data does not match the actual checksum of the current configuration data. This means that the configuration data has changed and the checksum stored is no longer valid. The error is produced when outdated versions (old) of Extensions or Define Assay are used to create an assay configuration file. This file is incompatible with the operation code within the instrument's memory. The fix for this problem is to recreate the assay definition on the correct version of assay definition software and re-download it. This can also be caused by a bad Flash memory. Reloading the basecode and assay configuration may fix this.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1200** | Calibration data missing |

This error means that AUTOCAL has not been performed after a memory erase or in the case of a new unprogrammed board immediately after the basecode or assay definition download. The system must have the AUTOCAL sequence performed. This can also indicate a bad Flash memory.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1300** | Motor not correctly homed |

This error will occur if the error 0200 or error 0300 is ignored. The situation needs to be fixed before the instrument is used.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1400** | Assay incubation error |

Assay requires incubation but incubation is not available.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1500** | Assay incubation error |

This error indicates that incubator failed to hold temperature within tolerances during the assay.

### Incubator Failure Error Data

| Bits | Zone | Error |
|---|---|---|
| 7 | 3 | Temperature out of range |
| 6 | 2 | Temperature out of range |
| 5 | 1 | Temperature out of range |
| 4 | 0 | Temperature out of range |
| 3 | 3 | Thermistor out of range or shorted |
| 2 | 2 | Thermistor out of range or shorted |
| 1 | 1 | Thermistor out of range or shorted |
| 0 | 0 | Thermistor out of range or shorted |

**Examples:**

```
                                  Bit 6  Bit 0
                                  ↓      ↓
Error code (1540) → 40 hex = 0100 0000
```

Zone 2 temp out of range, unknown reason (A/D failure?)

```
Error code (1544) → 44 hex = 0100 0100
```

Zone 2 temp out of range, due to zone 2 thermistor bad.

```
Error code (15FF) → FF hex = 1111 1111
```

All zones temp out of range, due to all thermistors bad.

**Note:** Any failure indicates a need to run a System Test and review of the printout (or KC4 screen).

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1600** | Computer control assay definition error |

Assay programming via computer control has tried to define an invalid assay sequence or parameter.

| Displayed Error | Potential Cause |
|---|---|
| **ERROR 1700** | Kinetic interval too short for selected options |
| **ERROR 1800** | Too many kinetic intervals programmed |
| **ERROR 1900** | Memory allocation failure |
| **ERROR 1A00** | Store curve error |
| **ERROR 1B00** | Get curve error |
| **ERROR A100** | Task control block error |
| **ERROR A200** | Reader function already in use |
| **ERROR A300** | Device not available |
| **ERROR A400** | Failed code checksum on power-up |
| **ERROR A500** | Power good error, power dropped below safe level |
| **ERROR A600** | Quick flash configuration time-out |

# Appendix D
# *Sample Reports*

*This appendix contains samples of the different types of reports available on the PowerWaveX.*

Reports are automatically generated after a plate has been read (see *Specifying Data Output and Reporting Options* in Chapter 2 for information on selecting report formats).

In addition, Map, Assay, and Assay List reports can be printed via the REPORT option from the Main Menu.

This Appendix contains samples of the different types of reports available on the *PowerWaveX*.

**Bio-Tek Instruments**

Assay:ASSAY1 Date:07/01/99 Lot: _____
Time:10:36:10AM Operator:
Wavelength:405 Temp: 30 C Plate ID:123

**COMMENTS**
Some concentrations may lie outside curve asymptotes(*.***)
< > around RSLT indicate extrapolated concentration.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| CALL | | | POS | EQUIV | NEG | NEG | POS | NEG | NEG | POS | NEG | EQUIV |
| CalcOD | 0.031 | 1.089 | 1.332 | 1.057 | 0.779 | 0.509 | 1.320 | 0.788 | 0.786 | 1.389 | 0.802 | 1.015 |
| Well | BLK | STD4 | SMP1 | SMP5 | SMP9 | SMP13 | SMP17 | SMP21 | SMP25 | SMP29 | SMP33 | SMP37 |
| RSLT | <-24.629> | 39.786 | 47.526 | 38.776 | 29.703 | 19.394 | 47.118 | 29.987 | 29.947 | 49.405 | 30.476 | 37.439 |
| B | | | | | | | | | | | | |
| CALL | | | POS | EQUIV | NEG | NEG | POS | NEG | NEG | POS | NEG | EQUIV |
| CalcOD | -.031 | 1.092 | 1.314 | 1.144 | 0.807 | 0.672 | 1.347 | 0.816 | 0.820 | 1.392 | 0.754 | 1.050 |
| Well | BLK | STD4 | SMP1 | SMP5 | SMP9 | SMP13 | SMP17 | SMP21 | SMP25 | SMP29 | SMP33 | SMP37 |
| RSLT | *.*** | 39.862 | 46.914 | 41.505 | 30.651 | 25.899 | 48.011 | 30.946 | 31.103 | 49.495 | 28.813 | 38.562 |
| C | | | | | | | | | | | | |
| CALL | | | NEG | NEG | EQUIV | EQUIV | NEG | EQUIV | POS | NEG | POS | NEG |
| CalcOD | 0.290 | 1.324 | 0.751 | 0.825 | 1.047 | 1.148 | 0.804 | 1.073 | 1.307 | 0.975 | 1.334 | 0.775 |
| Well | STD1 | STD5 | SMP2 | SMP6 | SMP10 | SMP14 | SMP18 | SMP22 | SMP26 | SMP30 | SMP34 | SMP38 |
| RSLT | <8.163> | 47.244 | 28.703 | 31.254 | 38.467 | 41.629 | 30.560 | 39.272 | 46.697 | 36.152 | 47.584 | 29.557 |
| D | | | | | | | | | | | | |
| CALL | | | NEG | NEG | POS | POS | NEG | EQUIV | POS | NEG | POS | NEG |
| CalcOD | 0.324 | 1.339 | 0.808 | 0.819 | 1.320 | 1.236 | 0.777 | 1.036 | 1.486 | 0.966 | 1.320 | 0.792 |
| Well | STD1 | STD5 | SMP2 | SMP6 | SMP10 | SMP14 | SMP18 | SMP22 | SMP26 | SMP30 | SMP34 | SMP38 |
| RSLT | 10.227 | 47.752 | 30.670 | 31.070 | 47.118 | 44.432 | 29.640 | 38.108 | 52.758 | 35.864 | 47.131 | 30.125 |
| E | | | | | | | | | | | | |
| CALL | | | POS | EQUIV | POS | POS | POS | NEG | POS | EQUIV | NEG | EQUIV |
| CalcOD | 0.526 | 1.619 | 1.334 | 1.095 | 1.644 | 2.479 | 1.323 | 0.815 | 1.288 | 1.001 | 0.779 | 1.031 |
| Well | STD2 | STD6 | SMP3 | SMP7 | SMP11 | SMP15 | SMP19 | SMP23 | SMP27 | SMP31 | SMP35 | SMP39 |
| RSLT | 20.136 | 57.917 | 47.582 | 39.966 | 59.017 | *.*** | 47.224 | 30.915 | 46.086 | 36.993 | 29.675 | 37.945 |
| F | | | | | | | | | | | | |
| CALL | | | POS | EQUIV | POS | POS | POS | NEG | POS | NEG | NEG | EQUIV |
| CalcOD | 0.557 | 1.610 | 1.321 | 1.080 | 1.816 | 2.475 | 1.316 | 0.768 | 1.274 | 0.954 | 0.737 | 1.049 |
| Well | STD2 | STD6 | SMP3 | SMP7 | SMP11 | SMP15 | SMP19 | SMP23 | SMP27 | SMP31 | SMP35 | SMP39 |
| RSLT | 21.416 | 57.558 | 47.165 | 39.488 | 69.631 | *.*** | 46.984 | 29.315 | 45.629 | 35.488 | 28.232 | 38.524 |
| G | | | | | | | | | | | | |
| CALL | | | NEG | POS | POS | POS | NEG | NEG | POS | NEG | NEG | POS |
| CalcOD | 0.818 | 1.873 | 0.777 | 1.275 | 1.519 | 2.347 | 0.822 | 0.774 | 1.241 | 0.755 | 0.790 | 1.286 |
| Well | STD3 | STD7 | SMP4 | SMP8 | SMP12 | SMP16 | SMP20 | SMP24 | SMP28 | SMP32 | SMP36 | SMP40 |
| RSLT | 31.012 | *.*** | 29.619 | 45.671 | 53.985 | *.*** | 31.151 | 29.509 | 44.582 | 28.869 | 30.082 | 46.007 |
| H | | | | | | | | | | | | |
| CALL | | | NEG | POS | POS | POS | NEG | NEG | EQUIV | NEG | NEG | POS |
| CalcOD | 0.853 | 1.914 | 0.758 | 1.248 | 1.495 | 2.394 | 0.767 | 0.810 | 1.183 | 0.802 | 0.773 | 1.303 |
| Well | STD3 | STD7 | SMP4 | SMP8 | SMP12 | SMP16 | SMP20 | SMP24 | SMP28 | SMP32 | SMP36 | SMP40 |
| RSLT | 32.187 | *.*** | 28.969 | 44.808 | 53.100 | *.*** | 29.288 | 30.745 | 42.727 | 30.469 | 29.498 | 46.578 |

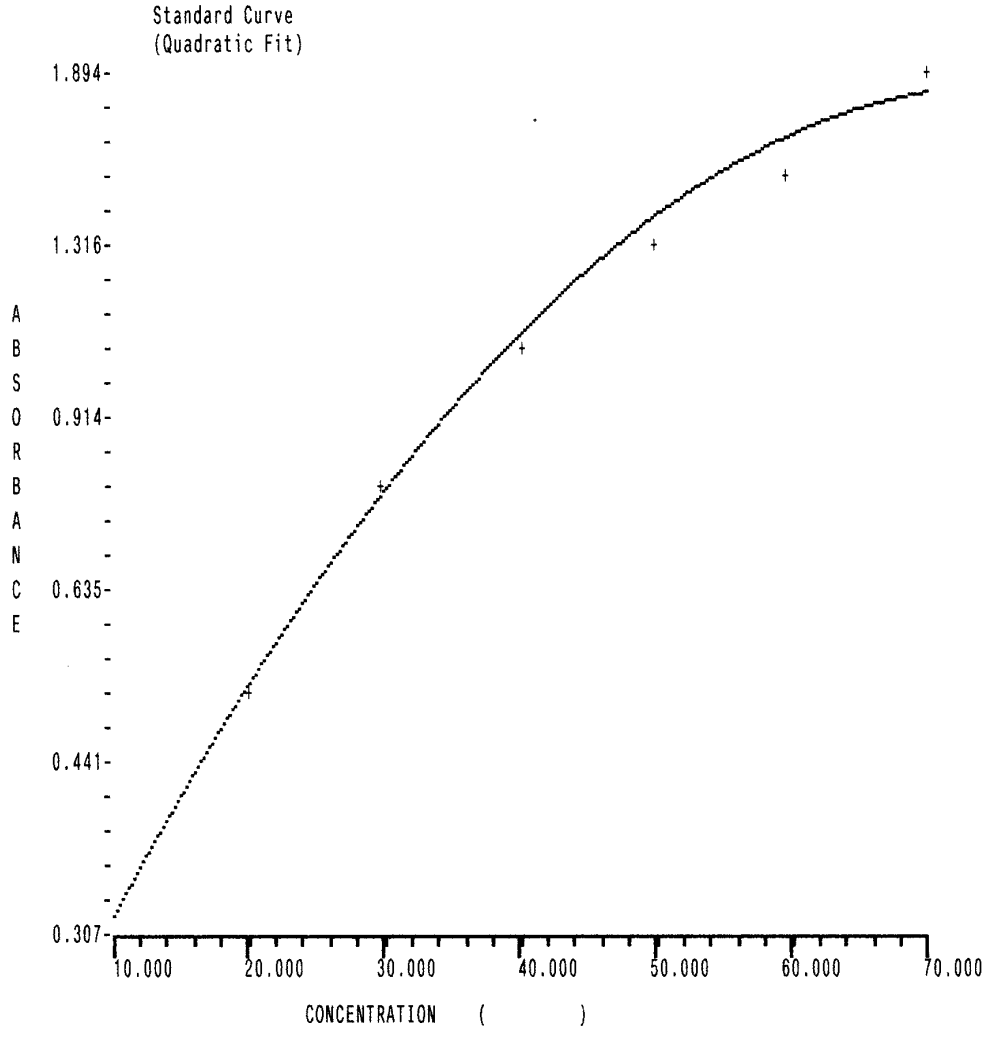
***Figure D-1:*** *Matrix Report showing well IDs, calculated OD values and concentrations, calls on samples, and comments*

**Bio-Tek Instruments**

| | | |
|---|---|---|
| Assay:ASSAY1 | Date:07/01/99 | Lot: _____ |
| | Time:10:36:10AM | Operator: |
| Wavelength:405 | Temp: 30 C | Plate ID:123 |

**COMMENTS**

Some concentrations may lie outside curve asymptotes(*.***)
< > around RSLT indicate extrapolated concentration.




Curve: log y = Ax^2 + Bx + C
Coefficients: A=-.000 B=0.027 C=-.743
R-Sqr: 0.9952

***Figure D-2:*** *Curve Fit Report showing the curve method, equation, coefficients, and r-squared value*

**Bio-Tek Instruments**

Assay:ASSAY1 Date:07/01/99 Lot: _____
Time:10:36:10AM Operator:
Wavelength:405 Temp: 30 C Plate ID:123

**COMMENTS**

Some concentrations may lie outside curve asymptotes(*.***)
< > around RSLT indicate extrapolated concentration.

**Interpretation of Results**

```
blk_ave=BLK;x          =0.175
GREYZONE=0.10          =0.100
CUTOFF=1.1             =1.100

SMP : SMP<=(CUTOFF+(CUTOFF*GREYZONE))AND(SMP>=(CUTOFF-(CUTOFF*GREYZONE))) : EQUIV
SMP : SMP>(CUTOFF+(CUTOFF*GREYZONE)) : POS
SMP : SMP<(CUTOFF-(CUTOFF*GREYZONE)) : NEG
```

| Well | ID | BlkdOD | CalcOD | Call | Pred Conc | RSLT | Std Dev | CV% | Notes: |
|---|---|---|---|---|---|---|---|---|---|
| A01 | BLK | 0.031 | 0.031 | | | <-24.629> | | | |
| B01 | BLK | -.031 | -.031 | | | *.*** | | | |
| AVE | | 0.000 | 0.000 | | | <-80.227> | 0.044 | error | |
| C01 | STD1 | 0.290 | 0.290 | | | <8.163> | | | |
| D01 | STD1 | 0.324 | 0.324 | | | 10.227 | | | |
| AVE | | 0.307 | 0.307 | | 10.000 | <9.215> | 0.024 | 7.841 | |
| E01 | STD2 | 0.526 | 0.526 | | | 20.136 | | | |
| F01 | STD2 | 0.557 | 0.557 | | | 21.416 | | | |
| AVE | | 0.541 | 0.541 | | 20.000 | 20.781 | 0.022 | 4.023 | |
| G01 | STD3 | 0.818 | 0.818 | | | 31.012 | | | |
| H01 | STD3 | 0.853 | 0.853 | | | 32.187 | | | |
| AVE | | 0.835 | 0.835 | | 30.000 | 31.602 | 0.025 | 2.969 | |
| A02 | STD4 | 1.089 | 1.089 | | | 39.786 | | | |
| B02 | STD4 | 1.092 | 1.092 | | | 39.862 | | | |
| AVE | | 1.090 | 1.090 | | 40.000 | 39.824 | 0.002 | 0.158 | |
| C02 | STD5 | 1.324 | 1.324 | | | 47.244 | | | |
| D02 | STD5 | 1.339 | 1.339 | | | 47.752 | | | |
| AVE | | 1.331 | 1.331 | | 50.000 | 47.498 | 0.011 | 0.824 | |
| E02 | STD6 | 1.619 | 1.619 | | | 57.917 | | | |
| F02 | STD6 | 1.610 | 1.610 | | | 57.558 | | | |
| AVE | | 1.614 | 1.614 | | 60.000 | 57.737 | 0.006 | 0.373 | |
| G02 | STD7 | 1.873 | 1.873 | | | *.*** | | | |
| H02 | STD7 | 1.914 | 1.914 | | | *.*** | | | |
| AVE | | 1.894 | 1.894 | | 70.000 | *.*** | 0.029 | 1.540 | |

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***Figure D-3:*** *First page of Column Report, showing plate data for blanks and Standards, the Interpretation of Results, and comments*

| Well | ID | BlkdOD | CalcOD | Call | Pred Conc | RSLT | Std Dev | CV% | Notes: |
|---|---|---|---|---|---|---|---|---|---|
| A03 | SMP0001 | 1.332 | 1.332 | POS | | 47.526 | | | |
| B03 | SMP0001 | 1.314 | 1.314 | POS | | 46.914 | | | |
| AVE | | 1.323 | 1.323 | POS | | 47.219 | 0.013 | 1.001 | |
| C03 | SMP0002 | 0.751 | 0.751 | NEG | | 28.703 | | | |
| D03 | SMP0002 | 0.808 | 0.808 | NEG | | 30.670 | | | |
| AVE | | 0.779 | 0.779 | NEG | | 29.694 | 0.040 | 5.183 | |
| E03 | SMP0003 | 1.334 | 1.334 | POS | | 47.582 | | | |
| F03 | SMP0003 | 1.321 | 1.321 | POS | | 47.165 | | | |
| AVE | | 1.328 | 1.328 | POS | | 47.373 | 0.009 | 0.681 | |
| G03 | SMP0004 | 0.777 | 0.777 | NEG | | 29.619 | | | |
| H03 | SMP0004 | 0.758 | 0.758 | NEG | | 28.969 | | | |
| AVE | | 0.768 | 0.768 | NEG | | 29.295 | 0.013 | 1.727 | |
| A04 | SMP0005 | 1.057 | 1.057 | EQUIV | | 38.776 | | | |
| B04 | SMP0005 | 1.144 | 1.144 | EQUIV | | 41.505 | | | |
| AVE | | 1.101 | 1.101 | EQUIV | | 40.142 | 0.061 | 5.566 | |
| C04 | SMP0006 | 0.825 | 0.825 | NEG | | 31.254 | | | |
| D04 | SMP0006 | 0.819 | 0.819 | NEG | | 31.070 | | | |
| AVE | | 0.822 | 0.822 | NEG | | 31.162 | 0.004 | 0.471 | |
| E04 | SMP0007 | 1.095 | 1.095 | EQUIV | | 39.966 | | | |
| F04 | SMP0007 | 1.080 | 1.080 | EQUIV | | 39.488 | | | |
| AVE | | 1.087 | 1.087 | EQUIV | | 39.727 | 0.011 | 0.988 | |
| G04 | SMP0008 | 1.275 | 1.275 | POS | | 45.671 | | | |
| H04 | SMP0008 | 1.248 | 1.248 | POS | | 44.808 | | | |
| AVE | | 1.262 | 1.262 | POS | | 45.239 | 0.019 | 1.511 | |
| A05 | SMP0009 | 0.779 | 0.779 | NEG | | 29.703 | | | |
| B05 | SMP0009 | 0.807 | 0.807 | NEG | | 30.651 | | | |
| AVE | | 0.793 | 0.793 | NEG | | 30.179 | 0.020 | 2.473 | |
| C05 | SMP0010 | 1.047 | 1.047 | EQUIV | | 38.467 | | | |
| D05 | SMP0010 | 1.320 | 1.320 | POS | | 47.118 | | | |
| AVE | | 1.184 | 1.184 | EQUIV | | 42.760 | 0.193 | 16.275 | |
| E05 | SMP0011 | 1.644 | 1.644 | POS | | 59.017 | | | |
| F05 | SMP0011 | 1.816 | 1.816 | POS | | 69.631 | | | |
| AVE | | 1.730 | 1.730 | POS | | 63.294 | 0.122 | 7.041 | |
| G05 | SMP0012 | 1.519 | 1.519 | POS | | 53.985 | | | |
| H05 | SMP0012 | 1.495 | 1.495 | POS | | 53.100 | | | |
| AVE | | 1.507 | 1.507 | POS | | 53.540 | 0.017 | 1.132 | |
| A06 | SMP0013 | 0.509 | 0.509 | NEG | | 19.394 | | | |
| B06 | SMP0013 | 0.672 | 0.672 | NEG | | 25.899 | | | |
| AVE | | 0.590 | 0.590 | NEG | | 22.774 | 0.116 | 19.626 | |
| C06 | SMP0014 | 1.148 | 1.148 | EQUIV | | 41.629 | | | |

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***Figure D-4:*** *Second page of Column Report, showing plate data for samples*

# Bio-Tek Instruments

Assay:ASSAY3 | Date:07/01/99 | Lot: _____
Time:10:55:07AM | Operator:
Wavelength:405 | Temp: | Plate ID:456

**COMMENTS**
RESULTS INVALID! Control validation failed.

**Interpretation of Results**

```
blk_ave=BLK;x              =0.175
GREYZONE=0.10              =0.100
CUTOFF=1                   =1.000


SMP : SMP<=(CUTOFF+(CUTOFF*GREYZONE))AND(SMP>=(CUTOFF-(CUTOFF*GREYZONE))) : EQUIV
SMP : SMP>(CUTOFF+(CUTOFF*GREYZONE)) : POS
SMP : SMP<(CUTOFF-(CUTOFF*GREYZONE)) : NEG

CTL1 : CTL1;x > 0.500
CTL2 : CTL2;x > CTL1;x
CTL3 : CTL3;x > CTL2;x
CTL4 : CTL4;x > CTL3;x
```

| Well | ID | BlkdOD | CalcOD | Call | Pred Conc | RSLT | Std Dev | CV% | Notes: |
|---|---|---|---|---|---|---|---|---|---|
| A01 | BLK | 0.030 | 0.030 | | | | | | |
| B01 | BLK | -.030 | -.030 | | | | | | |
| AVE | | -.00 | -.00 | | | | 0.043 | error | |
| C01 | CTL1 | 0.311 | 0.311 | INV | | | | | |
| D01 | CTL1 | 0.333 | 0.333 | INV | | | | | |
| AVE | | 0.322 | 0.322 | | | | 0.016 | 4.926 | |
| E01 | CTL2 | 0.517 | 0.517 | VAL | | | | | |
| F01 | CTL2 | 0.554 | 0.554 | VAL | | | | | |
| AVE | | 0.535 | 0.535 | | | | 0.026 | 4.782 | |
| G01 | CTL3 | 0.814 | 0.814 | VAL | | | | | |
| H01 | CTL3 | 0.857 | 0.857 | VAL | | | | | |
| AVE | | 0.835 | 0.835 | | | | 0.030 | 3.636 | |
| A02 | CTL4 | 1.118 | 1.118 | VAL | | | | | |
| B02 | CTL4 | 1.092 | 1.092 | VAL | | | | | |
| AVE | | 1.105 | 1.105 | | | | 0.018 | 1.667 | |

End of Report

***Figure D-5:*** *Column Report without Samples*

**Bio-Tek Instruments**

Assay:PANEL

Date:06/29/99 Lot: _____
Time:03:31:17PM Operator:
Temp: Plate ID:123456

**COMMENTS**

| Specimen | Assay | Well | BlkdOD | CalcOD | Call | RSLT | Std Dev | CV% | Notes: |
|---|---|---|---|---|---|---|---|---|---|
| SMP0001 | ASSAY 01 | G01 | 0.729 | 0.729 | NEG | | | | |
| | | H01 | 0.709 | 0.709 | NEG | | | | |
| | | AVE | 0.719 | 0.719 | NEG | | 0.014 | 1.889 | |
| | ASSAY 02 | G05 | 1.662 | 1.662 | POS | | | | |
| | | H05 | 1.695 | 1.695 | POS | | | | |
| | | AVE | 1.679 | 1.679 | POS | | 0.023 | 1.399 | |
| | ASSAY 03 | G09 | 1.402 | 1.402 | NEG | | | | |
| | | H09 | 1.361 | 1.361 | NEG | | | | |
| | | AVE | 1.382 | 1.382 | NEG | | 0.029 | 2.132 | |
| SMP0002 | ASSAY 01 | A02 | 1.271 | 1.271 | NEG | | | | |
| | | B02 | 1.205 | 1.205 | NEG | | | | |
| | | AVE | 1.238 | 1.238 | NEG | | 0.046 | 3.742 | |
| | ASSAY 02 | A06 | 0.818 | 0.818 | NEG | | | | |
| | | B06 | 0.850 | 0.850 | NEG | | | | |
| | | AVE | 0.834 | 0.834 | NEG | | 0.022 | 2.653 | |
| | ASSAY 03 | A10 | 1.686 | 1.686 | POS | | | | |
| | | B10 | 1.653 | 1.653 | POS | | | | |
| | | AVE | 1.669 | 1.669 | POS | | 0.024 | 1.409 | |
| SMP0003 | ASSAY 01 | C02 | 0.719 | 0.719 | NEG | | | | |
| | | D02 | 0.744 | 0.744 | NEG | | | | |
| | | AVE | 0.731 | 0.731 | NEG | | 0.017 | 2.392 | |
| | ASSAY 02 | C06 | 1.296 | 1.296 | NEG | | | | |
| | | D06 | 1.357 | 1.357 | NEG | | | | |
| | | AVE | 1.326 | 1.326 | NEG | | 0.043 | 3.248 | |
| | ASSAY 03 | C10 | 1.173 | 1.173 | NEG | | | | |
| | | D10 | 1.190 | 1.190 | NEG | | | | |
| | | AVE | 1.181 | 1.181 | NEG | | 0.012 | 1.025 | |
| SMP0004 | ASSAY 01 | E02 | 1.069 | 1.069 | NEG | | | | |
| | | F02 | 1.090 | 1.090 | NEG | | | | |
| | | AVE | 1.080 | 1.080 | NEG | | 0.015 | 1.391 | |
| | ASSAY 02 | E06 | 2.606 | 2.606 | POS | | | | |
| | | F06 | 2.626 | 2.626 | POS | | | | |
| | | AVE | 2.616 | 2.616 | POS | | 0.014 | 0.542 | |
| | ASSAY 03 | E10 | 1.205 | 1.205 | NEG | | | | |
| | | F10 | 1.121 | 1.121 | NEG | | | | |
| | | AVE | 1.163 | 1.163 | NEG | | 0.060 | 5.133 | |
| SMP0005 | ASSAY 01 | G02 | 1.367 | 1.367 | NEG | | | | |
| | | H02 | 1.076 | 1.076 | NEG | | | | |
| | | AVE | 1.222 | 1.222 | NEG | | 0.206 | 16.869 | |
| | ASSAY 02 | G06 | 2.465 | 2.465 | POS | | | | |
| | | H06 | 2.538 | 2.538 | POS | | | | |
| | | AVE | 2.502 | 2.502 | POS | | 0.052 | 2.068 | |

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***Figure D-6:*** *Panel Report*

10:48AM 07/01/99

---

| | | |
|---|---|---|
| NAME: | ASSAY2 | |
| READ TYPE: | ENDPOINT | |
| WAVELENGTH: | 405/630 | |
| BLANK MAP: | FULL | |
| AVERAGE STANDARDS? | YES | |
| CONCN: | STD1 | 0.0 |
| | STD2 | 2.0 |
| | STD3 | 5.0 |
| | STD4 | 10.0 |
| | STD5 | 20.0 |
| | STD6 | 50.0 |
| CURVE-FIT TYPE: | 4-P | |
| EDIT STD OUTLIERS: | MANUAL | |
| X/Y AXIS TYPE: | LIN | |
| EXTRAPOLATE UNKNOWNS? | YES | |

---

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BLK | BLK | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| B | STD1 | STD1 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| C | STD2 | STD2 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| D | STD3 | STD3 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| E | STD4 | STD4 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| F | STD5 | STD5 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| G | STD6 | STD6 | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |
| H | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP | SMP |

***Figure D-7:*** *Assay Detail Report*

Assay List
Version: 2.0

| | |
|---|---|
| 01 | Raw_OD |
| 02 | Raw_OD w/blk |
| 03 | Bio-Cell Read |
| 04 | K-Factor Bio-Cell |
| 05 | DNA Quant |
| 06 | 260/280 Ratio |
| 07 | Protein Quant |
| 08 | Oligo Quant |
| 09 | Protein Quant |
| 10 | Phenol |
| 11 | EDTA test |
| 12 | A320 Scattering |
| 13 | Lowry Protein |
| 14 | Bradford Protein |
| 15 | BCA Protein |
| 16 | B=galactosidase |
| 17 | NADH |
| 18 | HRP 5AS |
| 19 | HRP ABTS |
| 20 | HRP OPD-S |
| 21 | HRP OPD |
| 22 | HRP TMB-S |
| 23 | HRP-TMB |
| 24 | Alk phosphatase |
| 25 | B-galact ONPG |
| 26 | Urease |
| 27 | MTT |
| 28 | XTT |
| 29 | WST-1 |
| 30 | Methylene Blue |
| 31 | Open assay 01 |
| 32 | Open assay 02 |
| 33 | Open assay 03 |
| 34 | Open assay 04 |
| 35 | Open assay 05 |
| 36 | Open assay 06 |
| 37 | Open assay 07 |
| 38 | Open assay 08 |
| 39 | Open assay 09 |
| 40 | Open assay 10 |
| 41 | Open assay 11 |
| 42 | Open assay 12 |
| 43 | Open assay 13 |
| 44 | Open assay 14 |
| 45 | Open assay 15 |
| 46 | Open assay 16 |
| 47 | Open assay 17 |
| 48 | Open assay 18 |
| 49 | Open assay 19 |
| 50 | Open assay 20 |
| 51 | Open assay 21 |
| 52 | Open assay 22 |
| 53 | Open assay 23 |
| 54 | Open assay 24 |
| 55 | Open assay 25 |

***Figure D-8:*** *Assay List*