

ELx808™

Operator's Manual



ELx808™

Absorbance Microplate Reader, All Models Operator's Manual

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Revision P
BioTek® Instruments, Inc.

Notices

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Contact Information

❖ See also *Product Support and Service* on page 7.

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Revision History



Rev	Date	Changes
A	1/96	First Release
B	5/96	Revised reader specifications. Added Scanning method to software.
C	1/97	Added Panel and Reuse of Standard Curve, and revised serial port pin-out description.
D	2/98	Changed Chapter 4, Performance Verification to reflect changes to the Universal Test Plate. Added Liquid Tests 1 and 2. Changed Appendix C: Error Codes.
E	8/98	Added printer information. Updated Appendix B: Computer Control.
F	3/99	Changed European address. Corrected printer compatibility. Corrected the sequence of steps for processing a curve-fit method.
G	7/99	Added comment about blanking, P-Down and P-Across being inactive in this version of software. Clarified the need for at least one sample to be defined on a plate. Added scanning computer control commands.
H	5/00	Updated Chapter 4- Performance Verification to include a Liquid Test 3 to verify 340 nm instruments. Corrected the voltage range for Range 2 in the Introduction. Corrected Incubation Temperature Control range to 6 deg above ambient. Changed Note on page 4-7 dealing with air readings during Self-Test. Clarified Liquid Test 1. Changed Table 4-1 to include additional maintenance items. Reworked Liquid Test 2.
I	7/02	Updated contact information (pages iii, 1-8, 1-9, 2-8, 2-20, and 4-13). Added IQ/OQ/PQ procedures and updated liquid testing information (Chapter 4). Revised lamp replacement procedure (page 2-15).
J	11/03	<p>Preface: Updated contact information in Notices (page iii). Added Document Conventions (page vi). Updated Warnings section (pages vii and viii). Updated Electromagnetic Compatibility section (pages ix and x). Added the following safety symbols and text: "Consult instructions for use" (page xii) "In vitro diagnostic medical device" (page xii) "Separate collection for (disposal of) electrical and electronic equipment" (page xiii). Expanded the Intended Use Statement (page xiv).</p> <p>Chapter 1: Updated contact information in Technical Support. Removed About This Manual section (page 1-5). Added "Absorbance Test Plate" to the Optional Accessories list (page 1-8). Clarified lamp replacement procedure (Chapter 2, pages 2-14 to 2-16). Clarified description of cutoff formulas (Chapter 3, pages 3-42 to 3-44).</p> <p>Chapter 4: Changed title to "Performance Verification and IQ, PQ, OQ Tests." Added IQ/PQ/OQ test procedure information. Clarified procedures for liquid tests.</p>
J	11/03	<p>Revised decontamination instructions and added cleaning procedure (Appendix A). Added KC4 startup information to Appendix B. Added new Appendix E with two examples of assay kit instructions and directions for programming an assay.</p> <p>General: Edited and formatted text. Modified appearance of display screens. Standardized the presentation of significant digits. Changed "Abs" to "OD" throughout.</p>

Rev	Date	Changes
K	4/05	<p>Updated the cover with a current photo of the instrument. Updated contact information and warranty. Changed "Automated Microplate Reader" to "Absorbance Microplate Reader" throughout. Changed "Universal Test Plate" to "Absorbance Test Plate" throughout. Removed references to internal barcode scanner and "R" models. Removed references to General Formulas with respect to open assays. Updated specifications to match product spec rev D. Added text from rev J1 insert (Fuse Installation/Replacement). Updated the installation instructions in Chapter 2 to better reflect actual practice. Updated the IQ/OQ/PQ/PM steps in Chapter 4 to better reflect actual practice. Created Appendix F to provide instructions for adjusting the line input voltage range and replacing fuses for instruments with an older-style power input module than the one described in Chapter 2.</p>
L	4/06	<p>Updated the manual primarily to support the introduction of Gen5™ software. In general, changed 'Bio-Tek' to 'BioTek,' and added Gen5 instructions wherever KCJunior™ and KC4™ instructions were present.</p> <p>Cover: Updated BioTek logo to new graphic.</p> <p>Preface: Added Contact Information section, updated Hazards, Precautions, and safety standards. Removed Registration Card and Warranty (these items ship separately).</p> <p>Chapter 1: Added external power supply to Hardware Features, Package Contents, and Specifications. Also added 'Reading Speeds' section to specs. Replaced Technical Support section with one-page Product Support & Service section.</p> <p>Chapter 2: Added instructions for connecting the external power supply in Connect Power section. Moved instructions in Check/Adjust the Power Input Voltage Setting section to Appendix F.</p> <p>Chapter 4: Changed chapter title from 'Performance Verification, Periodic Maintenance, and IQ, PQ, OQ Tests' to 'Instrument Verification'. Moved maintenance instructions to new Chapter 5, Preventive Maintenance (see below). For Liquid Tests 1, 2, and 3, added recommendation to shake the plate for four minutes (or wait for 20 minutes) after pipetting the diluted test solution, before reading the plate.</p> <p>Chapter 5 (new chapter): In Recommended Maintenance table, removed 3-month interval for cleaning the lamp contacts and changed 'Replace the Lamp' to 'Replace the Lamp and Clean the Contacts.' Added cleaning instructions from Appendix A, Decontamination.</p> <p>Appendix B: Added new section: 'Controlling the Reader with Gen5'.</p>
M	3/08	<p>General: Applied the BioTek template to the manual. Changed "Bio-Tek" to "BIOTEK" in startup screens to reflect this change in the reader's basecode software.</p> <p>Preface: Added Microsoft®, Windows®, Windows XP, Windows 2000, Windows Vista™, and Excel® to the "Trademarks" section. In the "Contacting BioTek Instruments" section, updated the TAC fax number and added "Authorized European Representative" to the "European Coordination Center" heading.</p> <p>Chapter 1: Added part number disclaimer to "Package Contents" and "Optional Accessories" and updated the TAC fax number in the "Product Support and Service" section.</p> <p>Chapter 2: Added a packaging disclaimer to the section "Unpack and Inspect the Instrument."</p> <p>Chapter 3: Enhanced section on the keypad with graphics that illustrate components of the ELx808™ keypad/ display. Updated "Panel Assays" section. In</p>

Rev	Date	Changes
		<p>the "Printing Reports and Assay Lists" section, added a note containing an explanation of the OUT of range indication on reports.</p> <p>Chapter 4: Clarified the description of the System Test Report to state that the incubation test is done 'separately' and that the overall system test results given at the bottom of the test report refer only to the Optics portion of the test. Capitalized the "l" in "μl" and "ml."</p> <p>Chapter 5: Merged this chapter with Appendix A, Decontamination.</p> <p>Reformatted and renamed Appendix C, Error Codes, as "Chapter 6, Error Codes". Added a "Fatal Errors" section, and added information on Error Codes 2311 to 2386.</p> <p>Renamed Appendix B, Computer Control, as Appendix A.</p> <p>Renamed Appendix D, Reports, as Appendix B, Sample Reports. In the "Overview," added a note containing an explanation of the OUT of range indication on reports.</p> <p>Renamed Appendix E, Programming a New Assay as Appendix C.</p> <p>Renamed Appendix F, Adjusting the Power Input Voltage Setting as Appendix D.</p>
N	6/11	<p>Throughout: Removed references to outdated software KC4 and KCjunior.</p> <p>Preface: Updated Trademark. Updated Warnings and Precautions. Updated Intended Use statement.</p> <p>Chapter 2: Removed Parallel and Serial Port Pinout charts. Added Install Software/Connect to Computer.</p> <p>Chapter 3: Added "Getting Started with Gen5". Gave Baud Rate information for Gen5. Removed references to outdated software Extensions.</p> <p>Chapter 4: Removed Installation Qualification. Updated Autocal Analysis. Updated sample Absorbance Test Plate Results. Updated Stock Solution Formulation for Liquid Tests 1 and 2. Removed outdated instructions for creating former Solution A: 10x Concentrate PBS from Liquid Test 3. Simplified test procedure as a result.</p> <p>Chapter 5: Updates lamp replacement instructions (added text from former revision M1 insert).</p> <p>Chapter 6: Added error code 1201.</p> <p>Appendices: Removed former Appendix A: Computer Control.</p>
O	9/12	<p><i>General:</i> Updated Gen5 instructions to reflect terminology in Gen5 v2.x.</p> <p><i>Preface:</i> Updated the Intended Use Statement and the heading for the In Vitro Diagnostics directive to refer to the instrument's IVD label (if one exists). Added 'Service' and 'Accessories' hazard warnings. Added 'Spare Parts' precaution.</p> <p><i>Chapter 1, Introduction:</i> Updated part number for the power supply.</p> <p><i>Chapter 2, Installation:</i> Added note that improper packaging that results in damage to the instrument may lead to additional charges.</p> <p><i>Chapter 4, Qualification:</i> Added a note to the Absorbance Test Plate section stating that the test tests the accuracy and repeatability of the reader to 2.500 OD. It does not indicate a PASS or FAIL above 2.500 OD.</p>
P	4/13	<p><i>Preface:</i> Added EN 61010-2-081 and EN 61010-2-010 to Directive 2006/95/EC Low Voltage (Safety) and EN 61010-2-101 to Directive 98/79/EC In Vitro Diagnostics.</p>

Document Conventions

See also *Safety Symbols* on page xix.

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

Intended Use Statement

- The ELx808 is an absorbance microplate reader. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. The user must evaluate this instrument and (if used) PC-based software in conjunction with their specific assay(s). This evaluation must include the confirmation that performance characteristics for the specific assay(s) are met.
- If the instrument has an “IVD” label it may be used for clinical and non-clinical purposes, including research and development. If there is no such label the instrument may only be used for research and development or other non-clinical purposes.

Quality Control

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

Warranty & Product Registration

Take a moment to review the warranty information that shipped with your product. Please also register your product with BioTek to ensure that you receive important information and updates about the product(s) you have purchased. You can register online through the Customer Resource Center (CRC) at www.biotek.com or by calling 888-451-5171 or 802-655-4740.

Repackaging and Shipping



If you need to ship the instrument to BioTek for service or repair, contact BioTek for a **Return Materials Authorization (RMA)** number, and be sure to use the original packing materials. Other forms of commercially available packaging are not recommended and can void the warranty. If the original packing materials have been damaged or lost, contact BioTek for replacement packing.

Warnings



Operate the instrument on a level, stable surface away from excessive humidity.

Bright light or strong incandescent light can reduce the linear performance range of the instrument.

Measurement values may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these may vary depending on the instrument model. See **Hazards** and **Precautions**.

Hazards

The following hazard warnings are provided to help avoid injury:



Warning! Internal Voltage. Always turn off the power switch and unplug the power cord or power supply before cleaning the outer surface of the instrument or removing its top case.



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

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

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

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

Warning! Liquids. Avoid spilling liquids on the reader; fluid seepage into internal components creates a potential for shock hazard or instrument damage. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

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

Warning! Software Quality Control. The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. **Failure to conduct quality control checks could result in erroneous test data.**

Warning! Reader Data Reduction Protocol. When the reader is operated in standalone mode the onboard assay software will flag properly defined controls when they are out of range. The software will present the data with the appropriate error flags for the operator to determine control and assay validity. When operated via computer control, no limits are applied to the raw measurement data. All information exported via computer control must be thoroughly analyzed by the operator.



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



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

Precautions

The following precautions are provided to help avoid damage to the instrument:



Caution: Service. The instrument should be serviced by BioTek-authorized personnel. Only qualified technical personnel should perform service procedures on internal components.

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

Caution: Environmental Conditions. Do not expose the system to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in the *Specifications* section. Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

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

Caution: Power Supply. Use only the power supply shipped with the instrument. Operate the power supply within the range of line voltages listed on it.

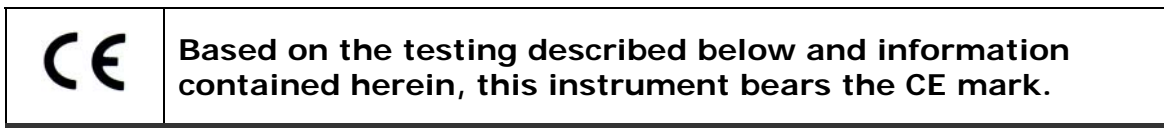
Caution: Warranty. Failure to follow preventive maintenance protocols may void the warranty.

Caution: Disposal. This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)" or local ordinances.

Caution: Electromagnetic Environment. Per IEC 61326-2-6 it is the user's responsibility to ensure that a compatible electromagnetic environment for this instrument is provided and maintained in order that the device will perform as intended.

Caution: Electromagnetic Compatibility. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), because these may interfere with the proper operation.

CE Mark



❖ See the Declaration of Conformity for more information.

Directive 2004/108/EC: Electromagnetic Compatibility

Emissions—Class A

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of **EN 61326-1: Class A** for Radiated Emissions and Line Conducted Emissions. Verification of compliance was conducted to the limits and methods of **EN 55011 - CISPR 11, Class A**. In a domestic environment it may cause radio interference, in which case you may need to mitigate the interference.

Immunity

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of **EN 61326-1** and **EN 61326-2-6** for Immunity. Verification of compliance was conducted to the limits and methods of the following:

- EN 61000-4-2 Electrostatic Discharge**
- EN 61000-4-3 Radiated EM Fields**
- EN 61000-4-4 Electrical Fast Transient/Burst**
- EN 61000-4-5 Surge Immunity**
- EN 61000-4-6 Conducted Disturbances from RFI**
- EN 61000-4-11 Voltage Dips, Short Interruptions and Variations**

Directive 2006/95/EC Low Voltage (Safety)

The system has been type-tested by an independent testing laboratory and was found to meet the requirements of this Directive. Verification of compliance was conducted to the limits and methods of the following:

- EN 61010-1, "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."
- EN 61010-2-081, "Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."
- EN 61010-2-010, "Particular requirements for laboratory equipment for the heating of materials."

Directive 2002/96/EC: Waste Electrical and Electronic Equipment

Disposal Notice: This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to **Directive 2002/96/EC**, “on waste electrical and electronic equipment (WEEE)” or local ordinances.

Directive 98/79/EC: In Vitro Diagnostics (if labeled for this use)

- Product registration with competent authorities
- Traceability to the U.S. National Institute of Standards and Technology (NIST)
- EN 61010-2-101, “Particular requirements for in vitro diagnostic (IVD) medical equipment.”

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

RADIO AND TELEVISION INTERFERENCE

NOTE: 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 their own expense.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and television reception.

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

User Safety





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

- Underwriters Laboratories UL 61010-1, “Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: General requirements.”
- Canadian Standards Association CAN/CSA C22.2 No. 61010-1, “Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: General requirements.”
- EN 61010 Standards, see **CE Mark** starting on page xvi.

Safety Symbols

Some of these symbols may appear on the instrument or accessories:

 <p>Alternating current Courant alternatif Wechselstrom Corriente alterna Corrente alternata</p>	 <p>Both direct and alternating current Courant continu et courant alternatif Gleich - und Wechselstrom Corriente continua y corriente alterna Corrente continua e corrente alternata</p>
 <p>Direct current Courant continu Gleichstrom Corriente continua Corrente continua</p>	 <p>Earth ground terminal Borne de terre Erde (Betriebserde) Borne de tierra Terra (di funzionamento)</p>
 <p>On (Supply) Marche (alimentation) Ein (Verbindung mit dem Netz) Conectado Chiuso</p>	 <p>Protective conductor terminal Borne de terre de protection Schutzleiteranschluss Borne de tierra de protección Terra di protezione</p>
 <p>Off (Supply) Arrêt (alimentation) Aus (Trennung vom Netz) Desconectado Aperto (sconnessione dalla rete di alimentazione)</p>	 <p>Caution (refer to accompanying documents) Attention (voir documents d'accompagnement) Achtung siehe Begleitpapiere Atención (vease los documentos incluidos) Attenzione, consultare la doc annessa</p>
 <p>Warning, risk of electric shock Attention, risque de choc électrique Gefährliche elektrische schlag Precaución, riesgo de sacudida eléctrica Attenzione, rischio di scossa elettrica</p>	 <p>Warning, risk of crushing or pinching Attention, risque d'écrasement et pincement Warnen, Gefahr des Zerquetschens und Klemmen</p>  <p>Precaución, riesgo del machacamiento y sejeción Attenzione, rischio di schiacciare ed intrappolarsi</p>
 <p>Warning, hot surface Attention, surface chaude Warnen, heiße Oberfläche Precaución, superficie caliente Attenzione, superficie calda</p>	 <p>Warning, potential biohazards Attention, risques biologiques potentiels Warnung! Moegliche biologische Giftstoffe Atención, riesgos biológicos Attenzione, rischio biologico</p>

	<p>In vitro diagnostic medical device Dispositif médical de diagnostic in vitro Medizinisches In-Vitro-Diagnostikum Dispositivo médico de diagnóstico in vitro Dispositivo medico diagnostico in vitro</p>		<p>Separate collection for electrical and electronic equipment Les équipements électriques et électroniques font l'objet d'une collecte sélective Getrennte Sammlung von Elektro- und Elektronikgeräten Recogida selectiva de aparatos eléctricos y electrónicos Raccolta separata delle apparecchiature elettriche ed elettroniche</p>
	<p>Consult instructions for use Consulter la notice d'emploi Gebrauchsanweisung beachten Consultar las instrucciones de uso Consultare le istruzioni per uso</p>		<p>Laser radiation: Do not stare into beam Rayonnement laser: Ne pas regarder dans le faisceau Laserstrahlung: Nicht in den strahl blicken Radiación de láser: No mire fijamente al rayo Radiazione di laser: Non stare nel fascio</p>

Chapter 1

Introduction

This chapter introduces the ELx808 Absorbance Microplate Reader and describes its hardware and software features, and technical specifications. Instructions for contacting BioTek are provided on page 7.

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ELx808 Absorbance Microplate Reader

BioTek's ELx808 is an eight-channel reader-assay system. The reader can serve as a standalone system, or can be controlled via BioTek's Gen5 software.

Designed to automatically perform endpoint and kinetic analysis, the reader can measure the optical density of solutions in 96-well microplates between 380 nm and 900 nm. The UV option measures down to 340 nm.

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

The instrument's onboard processor, 2-line x 24-character LCD screen, and membrane keys 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 ELISA-based data manipulation applications.

Reader Variations

- **ELx808:** Absorbance Microplate Reader (standard model).
- **ELx808IU:** Reader with incubation and UV capability. The ELx808IU has a four-zone incubation chamber that controls temperatures up to 50°C, and is capable of reading plates between 340 nm and 900 nm wavelengths.

Hardware Features

- Eight optics channels, with an additional reference channel
- A wavelength range of 380-900 nm (ELx808), 340-900 nm (ELx808IU)
- A user-accessible, 6-position filter wheel
- A 2-line x 24-character LCD display
- A membrane keypad with alphanumeric keys
- Adjustable plate shake frequency and times
- Reads 96-well microplates with 0.355" (9.017 mm) well centers
- Internal, four-voltage range power input module that can be adjusted for 100, 120, 230, or 240 V~ @ 50-60 Hz (readers manufactured *before* December 2005)
- 24-volt external power supply compatible with 100-240 V~ ± 10% @ 50-60 Hz (readers manufactured *after* December 2005)
- One serial COM port (25-pin male) and one parallel port (25-pin female)
- 4-Zone incubation chamber option (ELx808IU)

Software Features

- Easy-to-use, menu-driven interface
- Endpoint, Kinetic, and Linear Well 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 validation and cutoff formulas
- 55 assays are available onboard; up to 75 custom assays can be preprogrammed and exported to the reader using BioTek's *Define Protocol* software.
- Automatically stores results for the last 10 plates.

Package Contents

❖ Package contents and part numbers are subject to change. Please contact BioTek Customer Care with questions.

- ELx808 Absorbance Microplate Reader
- Power cord (PN varies according to country of use)
- Power supply for readers manufactured after December 2005 (PN 01281)
- Filter wheel with four standard filters: 405 nm, 450 nm, 490 nm, 630 nm and two blank filters. The ELx808IU also includes a 340 nm filter.
- Operator's Manual (PN 7341000)
- Serial cable (PN 75053)
- Dust cover (PN 7342066)

Optional Accessories

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

- Additional filters (please inquire for availability):
 - 340 to 630 nm, PN 3100XXX (XXX is wavelength in nanometers)
 - 640 to 900 nm, PN 3404XXX (XXX is wavelength in nanometers)

- Replacement lamp assembly (PN 3400508)
- Filter wheel plug (PN 3122037)
- Absorbance Test Plate (PN 9000547 or PN 7260522)
- ELx808 IQ/OQ/PQ Package (PN 7340543)
- Gen5 Software (visit biotek.com or contact your local dealer for details)
- Absorbance Liquid Test Solutions:
 - BioTek QC Check Solution No. 1 (PN 7120779, 25 mL; PN 7120782, 125 mL)
 - BioTek wetting agent (PN 7773002)
- Adapter to connect the reader to a USB-only printer (PN 75135)

Specifications

Microplates

- Standard 96-well, flat- or round-bottom plates

Electrical

- Power source and voltage range:
 - **Readers manufactured after December 2005:** 24-volt external power supply compatible with 100-240 V~ ± 10% @ 50-60 Hz
 - **Readers manufactured before December 2005:** Internal, adjustable power input module with four voltage ranges accommodated by the voltage selection switch:

Range 1	100 V~	90 to 110 V~, 50 to 60 Hz
Range 2	120 V~	108 to 132 V~, 50 to 60 Hz
Range 3	230 V~	207 to 253 V~, 50 to 60 Hz
Range 4	240 V~	216 to 264 V~, 50 to 60 Hz
- Power consumption: 100 VA

Physical

- **Dimensions:** 16.0" D x 15.5" W x 8.75" H (40.6 cm x 39.37 cm x 22.2 cm)
- **Weight:** 35 lb. maximum (15.87 kg maximum)

Environmental

- **Operating temperature:** 18° to 40°C (64° to 104°F)
- **Humidity:** 10% to 85% noncondensing

Hardware

- **Light source:** Tungsten halogen-filled bulb
- **Display:** 2-line x 24-character LCD

Reading Speeds

- **Single wavelength:** 8 seconds rapid mode; 12 seconds normal/regular mode
- **Dual wavelength:** 13 seconds rapid mode; 20 seconds normal/regular mode
- **Single wavelength higher than 400 nm:** 6-second minimum kinetic interval, rapid mode

Standard Model: ELx808

❖ The following specifications apply only to standard 96-well, flat- or round-bottom microplates.

- **Wavelength Range:** 380 to 900 nm
- **Filters:** 10 nm half-bandwidth interference filters. User-accessible filter wheel. Up to 6 filters may be installed on the instrument at one time. Filters supplied: 405 nm, 450 nm, 490 nm, 630 nm, and two blank filters.
- **Absorbance Measurement Range:** 0.000 to 4.000 OD

Optical specifications for single-wavelength endpoint measurements with a 12-second read (normal/regular read mode):

Accuracy: $\pm 1.0\% \pm 0.010$ OD from 0.000 to 2.500 OD @ 405 nm

Linearity: $\pm 1.0\%$ from 0.000 to 2.500 OD at 405 nm
 $\pm 2.0\%$ from 2.500 OD to 3.500 OD @ 405 nm

Repeatability: $\pm 0.5\% \pm 0.005$ OD from 0.000 to 2.500 OD @ 405 nm
 $\pm 1.5\% \pm 0.005$ OD from 2.500 to 3.500 OD @ 405 nm
 $\pm 2.5\% \pm 0.005$ OD from 3.500 to 4.000 OD @ 405 nm

Optical Specifications for single-wavelength kinetic measurements with read intervals of less than 12 seconds:

Accuracy: $\pm 2.0\% \pm 0.010$ OD from 0.000 to 2.500 OD @ 405 nm

Linearity: $\pm 2.0\%$ from 0.000 to 2.500 OD @ 405 nm

Repeatability: $\pm 1.0\% \pm 0.010$ OD from 0.000 to 2.500 OD @ 405 nm

❖ The plate can be read at **6-second** intervals (rapid mode) with wavelengths higher than 400 nm.

Incubator/Ultraviolet Model: ELx808IU

❖ The following specifications apply only to standard 96-well, flat- or round-bottom microplates.

- **Wavelength Range:** 340 to 900 nm
- **Filters:** 10 nm half-bandwidth interference filters. User-accessible filter wheel. Up to 6 filters may be installed on the instrument at one time. Filters supplied: 340, 405, 450, 490, 630 nm.
- **Absorbance Measurement Range:** 0.000 to 4.000 OD for 400 to 900 nm range, 0.000 to 3.000 OD for 340 to 400 nm range

Optical specifications for the 400 to 900 nm range (12-second read in normal/regular read mode):

Accuracy, Linearity, Repeatability: Same as Standard Model.

Optical specifications for the 340 to 400 nm range (12-second read in normal/regular mode):

Accuracy: $\pm 1.0\% \pm 0.010$ OD from 0.000 to 2.000 OD @ 340 nm

Linearity: $\pm 1.0\%$ from 0.000 to 2.000 OD @ 340 nm

Repeatability: $\pm 1.0\% \pm 0.005$ OD from 0.000 to 2.000 OD @ 340 nm

- **Incubation:** The following specifications apply to a 96-well sealed plate with 200 μ L of liquid in all wells:
 - **Temperature Control:** Temperature controlled to 50°C
 - **Temperature Variation:** $\pm 0.50^\circ\text{C}$ @ 37°C with the plate sealed

Product Support and Service

If your instrument or software fails to function properly, if you have questions about how to use or maintain your products, or if you need to send an instrument to BioTek for service or repair, please contact our Technical Assistance Center (“TAC”).

Contacting the Technical Assistance Center

TAC is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays. You can send a fax or an e-mail any time.

Phone: 800-242-4685 (in the U.S.) or 802-655-4740 (outside the U.S.)

Fax: 802-654-0638

E-Mail: tac@biotek.com

Web: www.biotek.com

Please be prepared to provide the following information:

- Your name and company information
- A daytime phone or fax number, and/or an e-mail address
- The product name, model, and serial number
- The onboard software part number and version (available via the keypad by selecting **UTIL > TESTS > CHKSUM**)
- Gen5 software version information (**Help > About Gen5**).

Returning Instruments for Service/Repair

If you need to return an instrument to BioTek for service or repair, please contact the TAC for a Return Materials Authorization (RMA) number and the shipping address. Repackage the instrument according to the instructions at the end of **Chapter 2: Installation**.

Chapter 2

Installation

This chapter includes instructions for unpacking and setting up the ELx808, and instructions for connecting printers and/or serial devices.

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Product Registration

Please register your product(s) with BioTek to ensure that you receive important information and updates about the product(s) you have purchased. Register online through BioTek's Customer Resource Center (CRC) at www.biotek.com or by contacting BioTek Customer Care.

1: Unpack and Inspect the Instrument



Important! Save all packaging materials! If you need to ship the reader to BioTek for repair or replacement, you must use the original packaging materials. Using other forms of commercially available packaging materials is not recommended and can **void the warranty**. Improper packaging that results in damage to the instrument may lead to additional charges.

If the original packaging materials have been damaged or lost, contact BioTek and ask for PN 7343000. See **Product Support & Service** in **Chapter 1** for contact information.

See **Before Repackaging the Instrument** at the end of this chapter for complete shipping instructions.

❖ The instrument's packaging is subject to change. If the instructions in this section do not apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance.

The ELx808 and its accessories are securely packaged inside custom-designed shipping materials. This packaging should protect the instrument from damage during shipping. Inspect the shipping box, packaging, instrument, and accessories for signs of damage.

If the shipping box has been damaged: Inspect the instrument for visible dents and scratches as you unpack it.

If the reader is damaged: Notify the carrier and your BioTek sales representative. Keep the shipping cartons and the packing materials for the carrier's inspection. BioTek will arrange for repair or replacement of your reader immediately.

1. (Refer to **Figure 1** on the next page.) Carefully open the top of the box, and remove any accessories. These include a power cord and power supply, a filter wheel in a padded envelope, and an operator's manual.
2. Remove the end caps from the reader.

3. Lift the reader out of the box, and place it on a level surface. Remove the reader from the plastic bag.
4. Remove the filter wheel from the shipping envelope
5. Place all packing material back into the shipping box for reuse if the instrument needs to be shipped again.

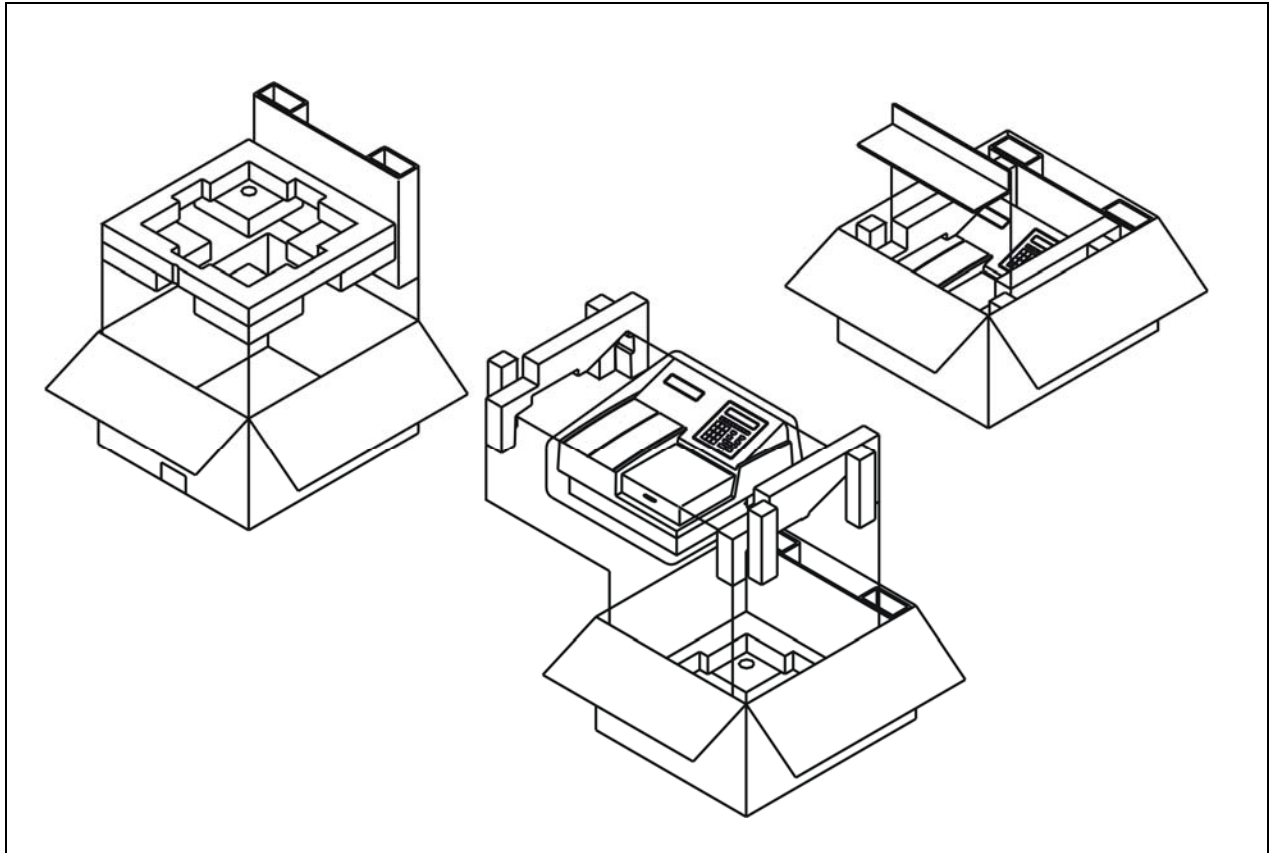


Figure 1: Unpacking the ELx808

2: Select the Operating Environment

For best operation, install the reader on a level surface in an area where ambient temperatures between 18°C (64°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. The humidity must be in the range of 10% to 85%, noncondensing.
- **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.

3: Install the Filter Wheel

The filters that ship with the ELx808 are installed in the six-position filter wheel. For example, the standard models have 405, 450, 490, 630 nm filters; the UV model's filter set is 405, 450, 490, 630 and 340 nm.

The filter wheel, which is packaged in a shipping envelope, must be installed before the reader is used. If you plan to install additional filters, or change the filter locations, use the following instructions to gain access to the filter wheel (refer to

Figure 2 on page 13):

1. If the reader is on, turn it off and disconnect the power cord or power supply.
2. Remove the seven screws around the perimeter of the shroud with a screwdriver.

❖ **Tip:** Bring the reader to the edge of the work surface to access the screws without having to turn the reader upside down.

3. Carefully lift up the shroud from the front. (Note that the shroud is hinged along its back edge.) Hold the plate access door steady, or tape it closed as the shroud is being lifted to prevent the door from moving.
4. Ensure that all locations on the filter wheel contain either a filter or a blank. Each location on the filter wheel must be occupied for the reader to operate properly. Take a moment to record the filter values in each location (e.g., 405 nm in position 1).



Important! Keep track of all filter locations. The physical location of the filters must match the filter locations mapped in the reader's software filter table. The filter wheel must have no empty locations; all locations must be filled with either a filter or a blank plug. Install all filters with the arrow denoting the light direction pointing downward.

5. The filter wheel mount is located in the left-rear corner inside the reader. The filter wheel is held in place by a magnet on the filter wheel motor hub. To install the filter wheel:
 - Line up the registration notch on the hub and the corresponding peg on the filter wheel.
 - Apply firm pressure to attach the filter wheel to the hub. The peg must be engaged in the notch for proper installation.

- Ensure that the filter wheel is positioned flat against the hub and that it rotates freely.
- Lower the top shroud back into position and remove tape if used to steady the plate access door.
- Reinstall the seven screws removed from the perimeter of the shroud.



Important! Be sure to replace the seven **perimeter screws**; they increase the reader's ability to withstand electrostatic discharges and electromagnetic interference. In addition, the screws **MUST** be installed to hold the shroud in place if you plan to ship the instrument back to BioTek for service or repair.

6. To remove the filter wheel, grasp the center hub of the filter wheel and pull it toward the lamp. The wheel should easily disconnect from the hub. Lift the wheel from the instrument.
7. To properly store interference filters for the ELx808 during extended periods of non-use, package the filters in a light-tight envelope or container, away from high humidity and direct sunlight. This will ensure the longest life for the filters. When handling the filters, keep the surfaces clean from fingerprints and debris by simply wiping with a lens tissue or other lint-free cloth.

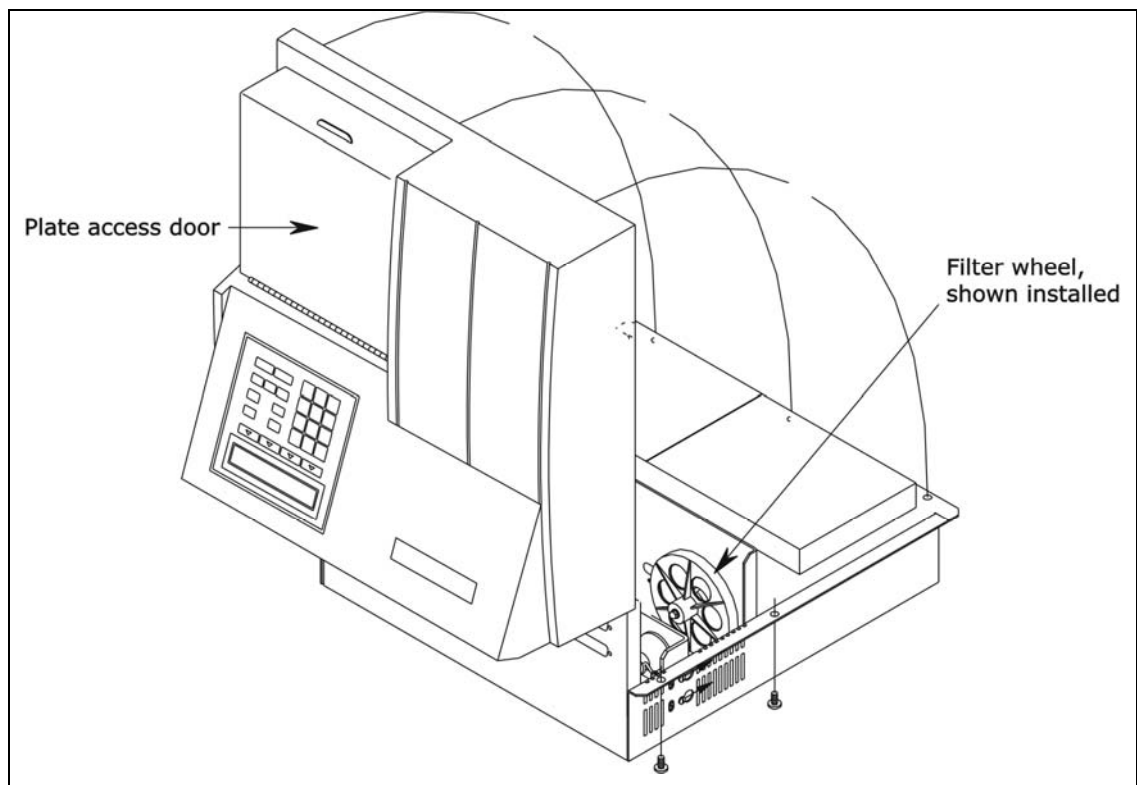


Figure 2: Installing the Filter Wheel

4: Check/Adjust the Power Input Voltage Setting

For ELx808 readers manufactured before December 2005 only: The reader is powered by an internal, adjustable four-voltage range power input module, instead of by an external power supply. For instructions for checking or adjusting the power input voltage setting, and for reconfiguring or replacing the fuses, refer to **Appendix C, *Adjusting the Power Input Voltage Setting***.



5: Connect Power

ELx808 with the external power supply:

1. Connect the power cord to the external power supply.
2. Locate the power inlet on the right side of the reader.
3. Plug the rounded end of the power supply's line cord into the power inlet.
4. Plug the other end of the power cord into an appropriate power receptacle.

ELx808 with the internal power input module:

1. Locate the power inlet on the right side of the reader.
2. Plug the rounded end of the power cord into the power inlet.
3. Plug the other end of the cord into an appropriate power receptacle.

	<p>Warning! Power Rating. The ELx808 or power supply 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.</p>
	<p>Warning! Electrical Grounding. Never use a plug adapter to connect primary power to the ELx808 power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power supply cord directly to an appropriate receptacle with a functional ground.</p>

6: (Optional) Connect Printer

If the ELx808 will operate in standalone mode (that is, without BioTek's Gen5 software running on a host PC), connect a printer directly to the reader using the supplied cable.



Important! To avoid system instability, make sure the printer and reader are turned **OFF** before connecting them.

1. If the reader and/or printer are on, turn them off. Place the printer in a location adjacent to the reader.
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.
4. Make sure the securing screws on both ends of the cable are tightened.
5. Turn on the printer.

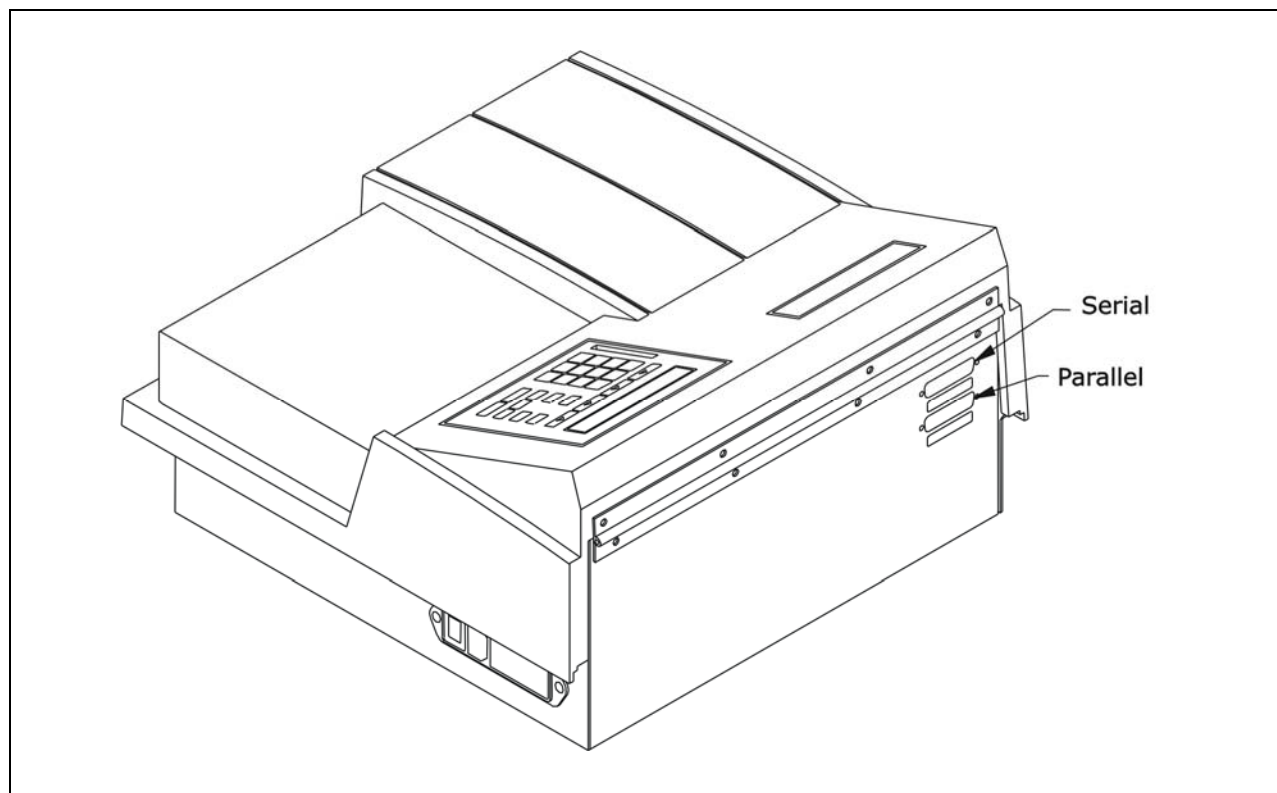


Figure 3: Serial and parallel ports

Printers

The ELx808's parallel port (LPT1) allows connection to compatible printers. The reader supports printers using either HP's PCL3 language, such as the HP DeskJet series, or Epson's LQ language. For a list of compatible printers call BioTek's Technical Assistance Center or visit our website (refer to *Chapter 1* for contact information).

7: Turn on Reader and Run System Test

If all of the required steps preceding this one have been performed successfully, locate the reader's power switch (on the right side) and turn on the reader. The ELx808 will automatically perform a System Test. The System Test conducts a series of tests at each wavelength defined in the filter table to confirm adequate light levels, low electronic noise, adequate photodiode sensitivity, overall system cleanliness, and (if equipped) proper function of the incubator. The testing verifies that the reader will give in-specification performance for each set wavelength over the specified OD range.

If a printer is connected to the reader, the test results automatically print. This test can also be run through Gen5 running on a host PC; consult the Gen5 Help system for instructions.

- If the test passes, the reader will beep once and the display will show the software's main menu, which will resemble one of the following:

Standard ELx808:

R E A D Y	0 1 : 3 0 P M	1 0 / 0 9 / 1 0
R E A D	D E F I N E	R E P O R T U T I L

ELx808IU:

R E A D Y	0 1 : 3 0 P M	3 7 . 0 ° C
R E A D	D E F I N E	R E P O R T U T I L

- **If the test fails**, the reader will beep repeatedly and the display will show an error code. If this happens, write down the error code and then press the **STOP** key on the keypad to stop the beeping. Look up the error code in *Chapter 6, Error Codes* to determine its cause.

If the problem is something you can fix (for example, if the error code is 0201 or 0301, indicating that the filter wheel is missing), turn off the reader, fix the problem, and then turn the reader back on. If the cause is not something you can fix, contact BioTek's Technical Assistance Center. See *Chapter 1, Introduction* for contact information.

8: Check/Adjust the Reader's Filter Table

After installing the filter wheel (or new filters), ensure that the ELx808's filter table accurately maps the physical location of the filters in the filter wheel.

1. Turn on the reader if it is not already on.
2. At the Main Menu screen, select **UTIL**.

```

R E A D Y           0 1 : 3 0 P M   1 0 / 0 9 / 1 0
R E A D   D E F I N E   R E P O R T   U T I L
  
```

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
  
```

3. From the Select Utility Option menu, select **SETUP**. The EDIT SETUP INFORMATION screen 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   F I L T E R   * M O R E
  
```

4. Select **FILTER**. The wavelength currently defined for Filter #1 appears:

```

E N T E R
F I L T E R # 1   W A V E L E N G T H : 4 0 5
  
```

If you need to change the filter wavelength number, use the numeric keypad to enter a number at the cursor location. To save the entry and move to the next filter on the filter table, press the **Enter** key. When the last filter has been entered, the software exits the filter routine, and displays the following screen:

```

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   F I L T E R   * M O R E
  
```

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

9: Configure Reader Settings

The ELx808 may be configured a number of ways, depending on user preference. Configuration options are accessed via the Select Utility Option menu, and include:

- **SETUP:** Set the date and time.
- **OUTPUT:** Select whether reports will be output to a Printer, the Computer, or both, choose the Column or Matrix report format, and determine if Curves will be printed.
- **READ:** Indicate whether prompts shall be displayed at run-time for Plate IDs, Sample IDs, and Sample Counts.
- **TESTS:** Access functions to test reader optics and instrument calibration. Check the version of basecode software installed. See *Chapter 4, Instrument Qualification* for more information.

To select the SETUP, OUTPUT, and READ user-configurable options:

- At the Main Menu screen, select **UTIL** to access the Select Utility Option menu.

```
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
```

SETUP Options

1. At the Select Utility Option screen, select **SETUP**.

```
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   F I L T E R   * M O R E
```

2. At the Edit Setup Information screen, select **DATE**.

```
D A T E   :       1 0 / 0 9 / 0 7                M D Y
M M D D Y Y                D D M M Y Y
```

3. Enter the new date, using the numeric keys. The cursor is positioned under the first editable field, and advances automatically. To change the date format, select **MMDDYY** or **DDMMYY**. The display updates to reflect the new format.
4. Press **Enter** to return to the Edit Setup Information screen.

```
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
```


- To edit the Time, select **TIME**. At the Time Entry Screen, use the numeric keys to enter the correct time.
- Select a 12- or 24-hour format by pressing the soft key beneath these options. The display automatically updates with the new time AM/PM format.
- Press the **Previous Screen** key to return to the Select Utility Option menu.

OUTPUT Options

- At the Select Utility Option screen, select **OUTPUT** to set report output preferences.

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

- Any previously defined selection appears on the top line of the display. Select the desired option (PRINT, COMPUTER, BOTH) to change the output device.
- Press **Enter** to advance to the Select Printer menu screen. If **COMPUTER** is selected, all results will be transferred directly to the computer screen via the RS232 serial port.

```
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
```

REPORT Type

❖ These selections only apply when the reader is run in standalone mode (without Gen5). See **Appendix A** for examples of Reports.

- At the Select Printer screen, press **Enter** to advance to the REPORT TYPE menu screen.

```
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
```

- The current selection appears on the top line of the display. Select the desired option (COLUMN, MATRIX, BOTH) to change the Report Type.

3. Press **Enter** to advance to the SAMPLES ON COL RPT screen.

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

4. Select **YES** to print samples on the column report, or **NO** to omit them.
5. Press **Enter** to advance to the PRINT CURVE-FIT screen.

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

6. The current selection is displayed on the top line of the screen. To change the report option, select **YES** or **NO**. The display updates to reflect the selection. (Select YES if there are quantitative assays defined on board and you wish to print the curve.)
7. Press **Enter** to return to the Select Utility Option screen.

READ Options

1. At the Select Utility Option screen, press the soft key beneath **READ** to set up Reader Prompt preferences. Select **YES** to prompt the operator to enter identifications and sample counts before a microplate is read. The prompts are shown below.
2. Press **Enter** after each selection to advance the display.

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

3. PROMPT FOR PLATE ID allows the user to enter an alphanumeric name of up to 10 characters. Select **YES** to present this prompt at run-time.

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

4. PROMPT FOR SAMPLE ID allows the user to identify samples with a 4-character alphanumeric name. The starting ID is entered and automatically incremented by the software. Select **YES** to present this prompt at run-time.

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

- PROMPT SAMPLE COUNT allows the user to enter the total number of samples on the plate at runtime; only those sample results will be printed. Select **YES** to present this prompt at run-time.

Read Speed

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

RAPID MODE reads a 96-well plate at 6-second intervals with wavelengths higher than 400 nm. Selecting NO means a plate with single-wavelength kinetic measurements will be read in intervals less than 12 seconds (“Regular” or “Normal” Mode). Specifications for these modes are outlined in *Chapter 1*.

10: Install Software/Connect to Computer (Optional)

The ELx808 has serial (RS232) port located on back of the reader (see *Figure 3* on page 15). This port allows the reader to communicate with a computer using the BioTek Gen5 software. It also allows for upgrades to the ELx808 basecode (on-board) software.

Attach the Cable

- Turn off the computer and the reader.
- Connect the supplied serial cable to both machines.

Install Gen5 Software on the Host Computer



If applicable, install Gen5 on the host computer. There is a certain sequence of events that **must** be followed to ensure that the software is properly installed and configured. Please follow the instructions provided in *Gen5 Getting Started Guide* to install the software.

Establish Communication

1. Start Gen5 and log in if prompted. The default System Administrator password is **admin**.
2. Go to the Gen5 main screen:
 - Gen5 version 2.x users: From the Task Manager, select **Setup > Go to System Menu**.
 - Gen5 version 1.x users: From the Welcome screen, select **System Menu**.
3. Select **System > Instrument Configuration** and click **Add**.
4. Set the Reader Type to **ELx808**.
5. Set the Com Port to the computer's COM port to which the reader is connected.
6. Click **Test Comm**. Gen5 attempts to communicate with the reader. If the communication attempt is successful, return to the Gen5 main screen.

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

- Is the reader connected to the power supply and turned on?
- Is the communication cable firmly attached to both the reader and the computer?
- Did you select the correct Reader Type in Gen5?
- Try a different COM port.
- Make sure the reader display is at its Main Menu.

If you remain unable to get Gen5 and the reader to communicate with each other, contact BioTek's Technical Assistance Center.

Changing the Baud Rate on the ELx808

❖ Gen5 requires the baud rate to be set to 9600.

If you need to change the baud rate from the default of 9600 to either 1200 or 2400:

1. Turn on the instrument if it is not already on.

```

R E A D Y           0 1 : 3 0 P M   1 0 / 0 9 / 0 7
R E A D   D E F I N E   R E P O R T   U T I L
  
```

2. At the Main Menu screen, select **UTIL**.

```

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
  
```

- At the Select Utility Option screen, select **SETUP**.

```

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   F I L T E R   * M O R E

```

- At the Edit Setup Information screen, select ***MORE** to advance to the EDIT SETUP / RS232 option screen.

```

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 access the Select Baud Rate menu. The top line of the display shows the baud rate currently set.

```

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



```

- To change the Baud rate, press the soft key beneath the desired baud rate. The display (top line) automatically updates to reflect the new choice.
- To view the reader's settings for parity, stop bits, and data bits, select **VIEW**.

11: Verify Performance

Before using the ELx808 for the first time, verify that the reader is operating properly by running a System Test and the Absorbance Plate Test. These tests and additional verification procedures are described in *Chapter 4, Instrument Qualification*.

Before Repackaging the Instrument

	<p>Important! Use the instrument's original shipping container and packaging material. 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 BioTek for a new set of shipping materials, and ask for PN 7343000.</p> <p>The instrument's packaging design is subject to change. If the instructions in this section do not appear to apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance.</p>
	<p>Warning! If the reader has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the reader during shipping, handling, and servicing. Decontamination prior to shipping is required by U.S. Department of Transportation regulations</p>

1. Decontaminate the reader prior before shipping. Refer to Decontamination in **Chapter 5, Preventive Maintenance**, for instructions.
2. Once the reader is clean, pack it in its original shipping box, using the original packing materials (see **Figure 1** on page 11).
3. Contact BioTek's Technical Assistance Center for a **Return Materials Authorization (RMA) number** and shipping instructions.

Chapter 3

Operation

This chapter includes instructions for operating the ELx808 Reader and its software.

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Some readers have custom programmed assays installed. Not all features of the software discussed in this chapter are available on custom instruments. Please contact BioTek's Technical Assistance Center (TAC) if you have any questions about the assays on your reader

All users should read ***Recommendations for Optimum Performance*** on page 76.

Getting Started with Gen5

These instructions describe how to create and run an Experiment in Gen5. For more information, or if the instructions below do not match what you see in Gen5, refer to the *Gen5 Getting Started Guide* and help system.

Gen5 version 2.x users:

1. Start Gen5.
2. If the Task Manager appears, select **Read Now > New** and skip to step 4. Otherwise, select **File > New Task** from the main view.
3. Select **Read Now > New**. Gen5 will open the procedure dialog. Go to step 4.

Gen5 version 1.x users:

1. Start Gen5. If the Welcome screen appears, select **Read a Plate** and skip to step 4. Otherwise, select **File > New Experiment** from the main view.
2. Click **Default Protocol** and click **OK**. Gen5 will open the Experiment workspace, which includes the Protocol menu tree and Plate screen.
3. Select **Plate > Read** or click the Read Plate icon. The Procedure dialog will open. Go to step 4.

For any version:

4. Select a **Plate Type**.
5. Click **Read** to open the Read Step dialog.
6. Select a **Read Type**.
7. Select or enter the wavelength(s) at which the plate will be read.
8. Define other reading parameters as desired. Click **Help** for assistance.
9. When complete, click **OK** to return to the Procedure dialog.
10. Click **OK** to save and close the Procedure dialog.
 - Gen5 version 1.x only: The Plate Reading dialog will open. Enter any desired information, place the plate on the carrier, then click **READ** to begin the plate read. If the Save As dialog opens, enter a File name, choose a file location (Save in:) and click **Save**.
11. Click **OK** when the Load Plate dialog appears. The plate will be read.
 - To view the raw data results, use the Data drop-down arrow in the Plate screen to select one wavelength. The results will be displayed for the selected wavelength. Repeat, for other wavelengths.
 - To analyze, manipulate, or print results, Protocol parameters should be defined. Refer to the Gen5 Help system for instructions.

Introduction

The front panel on the ELx808 Reader features a 25-pad keypad and a 2-line x 24-character LCD display as the user interface (see **Figure 4** below). The reader's bidirectional serial port allows computer control of the instrument and provides the means for downloading additional assay protocols to the instrument.

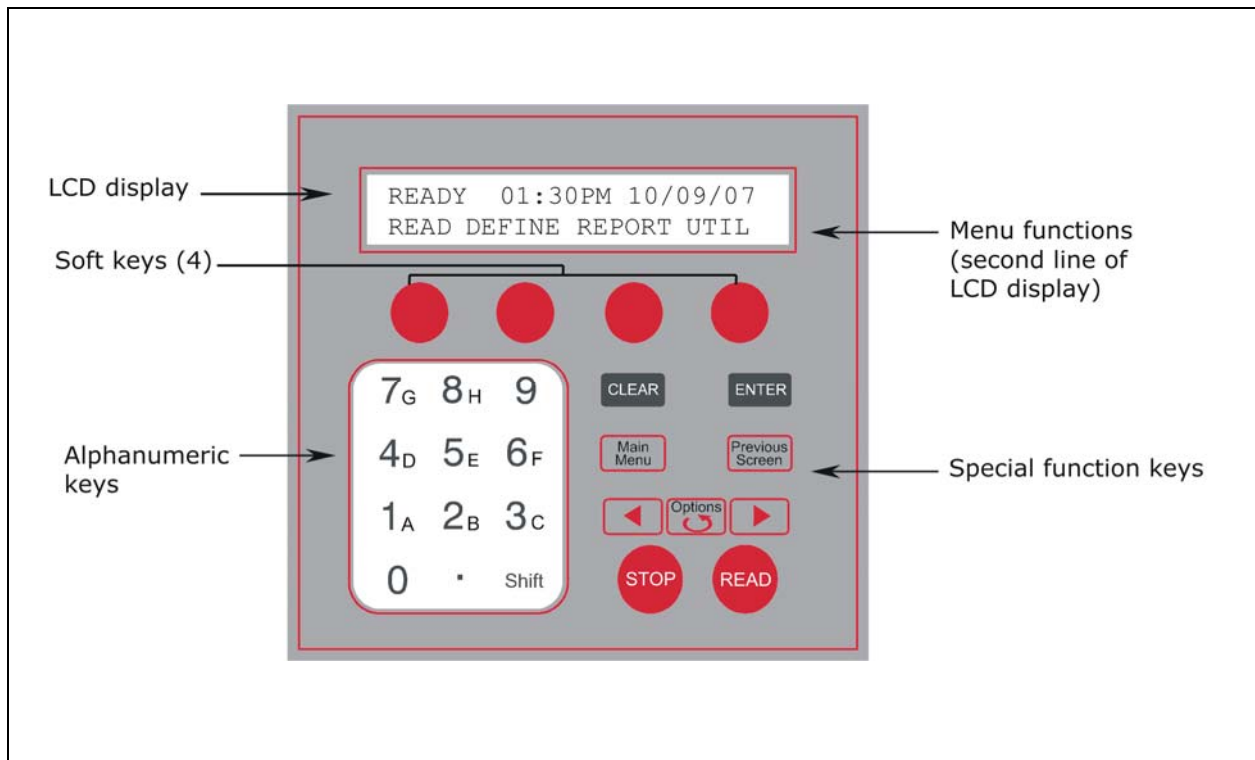
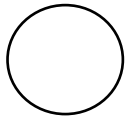


Figure 4: ELx808 Front Panel with LCD and Keypad

The Keypad



Use the four “soft keys,” located directly below the display, to select options presented on the display. For example, from the **Main Menu**, press the leftmost soft key to select **READ**, the rightmost to select **UTIL**.



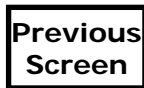
Exit the current screen and return to the Main Menu. After defining a reader program, press the **Main Menu** key, then press **YES** to save the program.



To scroll through the different options within a program, press the **Options** key or the **Shift + Options** key combination. Press the **Enter** key to select the current option.



Pressing **Enter** generally saves the current screen settings and advances to the next screen in a series.



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



Press **Clear** to clear/reset the current value on the reader’s LCD display.



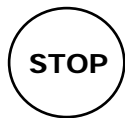
Press the ◀ (reverse) arrow to move the cursor to the left in the LCD display.



Press the ▶ (forward) arrow to move the cursor to the right in the LCD display.



To start running a reader program, press the **Read** key.



To stop running (abort) a reader program, press the **Stop** key.

The Startup Screen

To turn on the ELx808, press the on/off switch on the right side of the reader's base. The ELx808 will perform a system self-test, displaying the screens shown below until initialization is complete. During this time, all keys are inactive.

If the self-test fails, the reader will “beep” and display an error code. Press **Stop** to stop the beep. Refer to **Chapter 6, Error Codes**, to interpret these codes. Contact BioTek Instrument's Technical Assistance Center (see page 7), for further assistance with troubleshooting.

```
P O W E R U P   S E Q U E N C E   V X . X X
I N I T I A L I Z I N G . . .
```

```
B I O T E K       E L X 8 0 8
S E L F - T E S T . . . . .
```

The Main Menu Screen

Following successful power-up of the ELx808, the **Main Menu** screen is displayed. This screen will vary slightly if the instrument has the Incubation/UV option:

Main Menu screen – ELx808

```
R E A D Y           0 1 : 3 0 P M   1 0 / 0 9 / 0 7
R E A D   D E F I N E   R E P O R T   U T I L
```

Main Menu screen – ELx808IU (model with incubation/UV capability)

```
R E A D Y           0 1 : 3 0 P M           3 7 . 0 ° C
R E A D   D E F I N E   R E P O R T   U T I L
```

❖ **Note:** The temperature indicated on the display of incubated models is the actual averaged temperature of the incubator's four zones. The applied setpoint of the last assay is used. To adjust the temperature, a new setpoint value must be assigned to the assay before running.

The keyboard's four "soft keys," located below the on-screen menu options (**READ**, **DEFINE**, **REPORT** and **UTIL**), are activated, and may be selected. Press the soft key that corresponds to a displayed menu option to select that option:

- **READ** option: Initiate a plate read (or, press the key labeled **READ** on the keyboard for plate reading prompts). You will be prompted to select from a list of available assays.
- **DEFINE** option: Create a reading and data reduction protocol. You will be prompted to select/edit an assay and then define its various parameters.
- **REPORT** option: Print results reports and protocol descriptions. For results reports, you will be prompted to select a previously run assay with valid data.
- **UTIL** option: Access various onboard utilities, used for configuring and testing the reader.

Quick Read

On some readers, **Assay 01** has been designed to allow for quick and simple assay programming. It appears as **_Quick Read** on the display. Most of the options available in Assays 2 through 55, and described in this section, are unavailable for programming within Quick Read. You can access the Quick Read assay when **READ** is selected from the main menu.

The Quick Read assay **DEFINE** settings are shown below, and cannot be edited, except where noted:

METHOD

- Single Wavelength 405 nm (editable)

MAP

- 96-well plate geometry
- Blank on Air
- Automap
- Map starting location A1
- Samples only (no blanks, standard, or controls)
- Sample count prompted at runtime (can be turned off in **UTIL > READ** options)

Defining Assays

❖ **Note:** *Appendix B, Instructions for Programming a New Assay*, provides two examples of assay kit instructions and step-by-step directions for programming each assay. The appendix includes two sample assays: one with a ratio transformation calculation and a POS/NEG cutoff determination, and another with a standard curve.

The Main Menu option, **DEFINE**, allows you to customize previously defined assays stored in the reader's memory.

- From the Main Menu, press the soft key beneath the **DEFINE** menu option to advance to the **SELECT ASSAY NUMBER** screen.

Selecting an Assay

At the **SELECT ASSAY NUMBER** screen:

1. Use the **NUMERIC** keys to enter the number of predefined Assay Definition files stored in the reader's memory, or use the **OPTION** 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 (1-55 or more if custom programmed) programmed in the reader's memory.
2. The assay's name and number are displayed on the screen.

```
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
```

3. Press **Enter** to select the assay and advance to the **Name** screen. You may change the default assay name to a more descriptive one (see *Assay Name* on the next page).
4. Press **Enter** to advance to the **Edit Assay Name** screen.

Assay Name

At the **Assay Name** screen, edit the name assigned to the Assay. The assay name can be up to 16 characters.

```
E D I T > H B S - A G 1
-           /           :           S P A C E
```

- Press the **ALPHA** and **NUMERIC** keys to update the assay name. The cursor is positioned at the first editable field.
- Press the **OPTION** key to sequentially advance the character positioned above the cursor. The characters will cycle through the alphabet (A-Z), with a space character following Z.
- Press **Clear** to clear the assay name from the display.
- Use the **Left** and **Right Arrows** to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Press **Soft Keys 1, 2, 3, and 4** when using alphanumeric characters on the display above the soft key in the assay name.

In addition:

- Press the **Main Menu** key to return to the Main Menu screen.
- Press **Previous Screen** to save the contents of the display and return to the previous screen.
- Press **Enter** to save the contents of the display and advance to the next screen.

Defining the Method, Map, Formula, and Curve

The **DEFINE** Option screen allows you to edit the Method, Map, Formula or Curve Fit.

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

Press the soft key beneath the displayed option to access the following functions:

- **SOFT KEY 1: METHOD** is selected, and the user is prompted to select the read method parameters.
- **SOFT KEY 2: MAP** is selected and the user is prompted for plate mapping information.
- **SOFT KEY 3: FORMULA** is selected and the user is prompted to enter a formula.
- **SOFT KEY 4: CURVE FIT** is selected and the user is prompted for curve-fit options.
- In addition, the **MAIN MENU**, **PREVIOUS SCREEN** and **ENTER** keys are active, allowing you to move back and advance through the menu structure.

METHOD

The definition of a method includes selecting:

- Endpoint, Kinetic or Scanning Read Modes
- Delay First Read
- Incubation Parameters
- Filter Wavelengths Applied
- Shake Parameters
- Kinetic Analysis

❖ **Note:** Some screens shown below and on the following pages may not appear on some reader models.

Read Type

This option allows you to enter which read type: Endpoint, Kinetic, or Scan. The following keys are active during this screen:

```

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
  
```

- Press **SOFT KEY 1** to select Endpoint read mode. Press **SOFT KEY 2** to select Kinetic read mode.
- Press **Enter** to save the displayed value and advance to the next screen.

Delay in First Read

Selecting the Delay in First Read option allows you to enter a time delay before the first read.

```

D E L A Y   F I R S T   R E A D
T I M E   :           X X   :   X X
  
```

- Enter the time in minutes and seconds, using the **numeric** keys.
- Press **Enter** to advance to the **INCUBATION TEMPERATURE** screen.

Incubation Temperature

The incubation temperature screen allows you to set the assay incubation 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
  
```

- Press **SOFT KEY 1** or **2** to select ambient incubation.
- Press **SOFT KEY 3** or **4** to select an adjustable temperature.
- Use the **LEFT** and **RIGHT ARROW** keys to move the cursor between the two digits on the input temperature.
- Use **NUMERIC** keys to enter the incubation temperature, up to 50°C.
- Press **Enter** to save and advance to the next screen.

Single or Dual Wavelength

The **WAVELENGTH** selection screen allows you to select **SINGLE** or **DUAL** wavelength for the assay.

```

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

- Press **SOFT KEY 1** to select **SINGLE** wavelength. The reader will measure the optical density of each well with a single filter.
- Press **SOFT KEY 2** to select **DUAL** wavelength. Each well will be read twice, each time with a different filter. **Note:** 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. Dual wavelength readings can significantly reduce optical interference caused by scratched or fingerprinted microplates since the scratches or fingerprints reduce the amount of light on both wavelengths.
- Press **Enter** to save the selection and advance to the next screen.

MEAS Selection

The **MEAS** selection screen allows you to select the filter(s) for the assay. The currently selected filter appears on the top line of the display, and the available options appear on the bottom.

```

M E A S : 4 5 0   R E F : 6 3 0
4 0 5           4 5 0           4 9 0           6 3 0
  
```

- Press **SOFT KEYS 1, 2, 3,** and **4** to select the filter option displayed above the soft key. The display updates to reflect the selection.
- Press the right arrow key to select the **Reference Filter**.
- Press **ENTER** to move to the next screen.

Number of Kinetic Reads/Kinetic Duration Selection

This menu allows you to either select the total number of kinetic reads or the length of time the assay will run (kinetic duration). Any previously defined value is shown on the top line of the display and the options on the second.

```

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
  
```

- Press **SOFT KEY 1** to select the total reads option.
- Press **SOFT KEY 3** to select the duration option.
- Press **Enter** to save the selection and advance to the next screen.

Kinetic Interval

Use this screen to enter the interval of time between each kinetic read.

```

K I N E T I C
I N T E R V A L :   0 1 : 2 3 : 5 6
  
```

- Use the **NUMERIC** keys to enter the time duration. Valid ranges are: 0-1 hours, 0-59 minutes and 0-59 seconds. The number of Reads = Duration/Interval must be less than or equal to 40 and more than or equal to 2.

- Use the **LEFT** and **RIGHT ARROW** keys to move to the next or previous numeric entry fields.
- Press **Enter** to save the value and advance to the next screen.

Kinetic Number of Reads

T O T A L N U M B E R	
O F K I N E T I C R E A D S :	0 6

- Use the **NUMERIC** keys to enter the number of reads required. The range is 2 to 40 reads.
- Press **Enter** to save the entry and advance to the next screen.

Kinetic Duration

Use this screen to enter the duration of the kinetic reaction.

K I N E T I C	
D U R A T I O N :	1 1 : 2 3 : 4 5

- Use the **NUMERIC** keys to enter the time duration in hours, minutes, and seconds. The maximum duration time is 80 hours.
- Use the **LEFT** and **RIGHT ARROW** keys to move between entry fields.
- Press **Enter** to save the entry and advance to the next screen.

Shake Mode Selection

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	

- Press **SOFT KEY 1** to select shaking for the first read only or endpoint read.
- Press **SOFT KEY 2** to select shaking for every read.
- Press **SOFT KEY 3** to select **no** shaking.
- Press **ENTER** to save the selection and advance to the next screen.
- Press **SCREEN** key to save the selection and return to a previous screen.

Shake Time

```
S H A K E   T I M E :   0 0 : 1 2 : 3 4
C O N T I N U O U S
```

- Use the **NUMERIC** keys to enter the shake interval. Valid ranges are: 0-1 hours, 0-59 minutes and 0-59 seconds.
- Use the **LEFT** and **RIGHT ARROW** keys to move the cursor between hours, minutes, and seconds.
- Press **Enter** to save the entry and advance to the next screen.

❖ Note: "Continuous" appears on the display when a kinetic assay with a previously specified shake has been selected.

Shake Speed

The shake movement is a repeated 0.021-inch movement from the shake position and back.

```
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
```

- Press **SOFT KEY 1** to select low-speed shaking.
- Press **SOFT KEY 2** to select medium-speed shaking.
- Press **SOFT KEY 3** to select high-speed shaking.
- Press **SOFT KEY 4** to select variable-speed shaking (1 second of each speed repeated).
- Press **Enter** to save the entry and advance to the next screen.

Kinetic Data Analysis Selection

```
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
```

- Press **SOFT KEY 1** to select the kinetic rate calculation. This method will apply a linear fit to calculate the maximum slope based on the number of kinetic points specified.

- Press **SOFT KEY 2** to select the R-squared rate calculation. This method will calculate the R-squared value at the maximum slope, based on the linear curve fit and the number of kinetic points specified.
- Press **SOFT KEY 3** to select the time calculation, which will calculate the time for each well to reach the onset optical density.
- Press **ENTER** to save the selection and advance.

Number of Kinetic Points Selection

Use this screen to select the number of sequential kinetic points to calculate the steepest Rate, or the R squared at the steepest Rate.

```
K I N E T I C   P O I N T S :   3
A L L   P O I N T S
```

- Use the **NUMERIC** keys to input the number of points. The range is 2 to MAX where max is the total number of reads.
- Press **SOFT KEY 1** or **2** to select **ALL POINTS**.
- Press **Enter** to save the entry and advance to the next screen.

Onset OD Selection

Use this screen to enter the onset OD time.

```
E N T E R
O N S E T   O D :   1 . 2 3 4
```

- Use **NUMERIC** keys to enter the onset OD. 3.000 OD is the maximum value.
- Use the **LEFT** and **RIGHT ARROW** keys to move the cursor within the entered OD field.
- Press the **Enter** key to save the entry and advance.

Linear Scanning

If Scanning is chosen as the Read Type, use the following screen to enter the total number of points to be read in a line across the center of each well.

```
E N T E R   N U M B E R   O F
S C A N   P O I N T S :           1 5
```

The maximum number of points selectable is 31 (odd numbers only). 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.

❖ **Note:** If too many scans are chosen, the reader may be reading the sides of the well.

The reader will read the chosen number of points across the well and report the calculated area under the curve.

MAP

The MAP Definition screen allows you to edit or specify the following options in the assay:

- 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
- Averaging of Samples
- Sample Location

❖ **Note:** Some of the following screens may not appear, depending on the reader model. The valid range of the number of standards is 0-12. The valid range of valid replicate counts for standards is 1-8. The valid range of the number of controls is 0-8. The range of valid replicate counts for controls and samples is 1-12.

```

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

```

- At the **DEFINE** Options screen, press **SOFT KEY 2** to begin the plate **MAP** process.

Map Generation

“Map Generation” represents the method by which blanks, controls, standards, and/or samples are assigned to specific locations on the plate. The currently selected value appears on the top line of the display, and the available options appear on the bottom.

```

M A P   G E N E R A T I O N :   M A N U A L
A U T O       M A N U A L

```

Automatic Plate 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.

Manual Plate Map Generation: Select **MANUAL** to indicate that the well assignments will be performed manually (by the user) at Define and/or Read time.

- Press **SOFT KEY 1** for **automatic** sample plate map generation. The display will update to reflect the selection.
- Press **SOFT KEY 2** for **MANUAL** plate map generation. The display updates to reflect the selection.
- Press **ENTER** to save the selection and move to the next screen.

❖ **Note:** Press **SHIFT-CLEAR** to clear any previously defined manual map.

Mapping Direction

Use this option to select how the wells are mapped on the plate. Any previously defined Mapping Direction appears on the top line of the display; the available options appear on the second line.

```
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
```

- Press **SOFT KEY 1** to map **DOWN** the column.
- Press **SOFT KEY 2** to map **ACROSS** the row.
- Press **ENTER** to save the selection and move to the next screen.

Replication Direction

This option allows you to specify how replicates are mapped on the plate. The currently selected Replication Direction appears on the top line of the display, and the available options appear on the bottom.

```
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
```

- Press **SOFT KEY 1** to map the replicates **DOWN** the column, following the direction of the map listing.
- Press **SOFT KEY 2** to map the replicates **ACROSS** (in a paired format). As an example, two replicates can be placed in A1 and A2 wells. The third replicate would follow in B1. The next standard control, or sample, would follow in B2.
- Press **ENTER** to save the selection and advance.

Examples of mapping and replication directions are shown on the next page.

EXAMPLES OF MAPPING & REPLICATION DIRECTIONS

Map Direction DOWN, Rep Direction DOWN:

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

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	SMP	SMP	SMP	SMP	SMP	SMP				
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										
B	STD2	STD2										
C	STD3	STD3										
D	STD4	STD4										
E	STD5	STD5										
F	PC	PC										
G	NC	NC										
H	SMP	SMP										

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	SMP	SMP			
B	STD1	STD2	STD3	STD4	STD5	PC	NC	SMP	SMP			
C												
D												
E												
F												
G												
H												

Start Mapping at Well Location

The Start Mapping at Well Location screen is only shown if automatic mapping is selected. This option allows you to 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
  
```

- 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 the **NUMERIC** and **ALPHA** keys to enter a letter or number at the cursor location. For all prompts of a well location, only the **ALPHA** keys are active for the first character and **NUMERIC** for the second and third characters.
- Press **ENTER** to save the well location and advance to the next screen.

Selecting a Blank Map

This option allows you to select which blanking method to apply to the assay. The currently selected Blank Map value appears on the top line of the display, and the available options appear on the bottom.

The blanking options, **AIR**, **FULL** and **CONSTANT**, **ROW** and **COLUMN**, and **P-ACROSS** and **P-DOWN** are displayed on three screens.

```

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
  
```

- Press **SOFT KEYS 1, 2, or 3** to select the **BLANK MAP** type above the soft key. The display updates to reflect the selection.
- Press **SOFT KEY 4** to access **MORE** options: **ROW** or **COLUMN**, and **P-ACROSS** or **P-DOWN**.
- Press **ENTER** to save the well location and advance to the next screen.

Blank Map Definitions

- **AIR** performs an initial reading on “air” just prior to the plate read, and uses that value 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 to be subtracted from the whole plate.
- **CONST** (Constant) allows entry of a user-specified absorbance value. This value will be subtracted from each well on the plate. Use a blank value from the first plate, or a blanking plate to save space on subsequent assay plates.
- **ROW** enables a single blank well or an average of blank wells to be selected for each row. The blank (or average) will be subtracted from each well in the row. Use manual mapping to position blanks, controls, standards, and samples.
- **COLUMN** enables a single blank well or an average of blank wells to be selected for each column. The blank (or average) will be subtracted from each well in the column. Use manual mapping to position blanks, controls, standards, and samples.
- **P-DOWN** enables a blank in every even numbered column to be subtracted from the well to the left of it in every odd column. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the odd columns.
- **P-ACROSS** 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. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the A, C, E, and G rows.

Constant Blank Value Entry

This entry screen only appears when a Constant Blank map is selected. Enter the value to 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 . 0 0 0
  
```

- Use the **NUMERIC** keys to enter the value. The range is 0.000 to 3.000. The cursor is positioned at the first editable field and advances automatically.
- Press the **CLEAR** key to clear the value on the display.
- Press the **ENTER** key to save the value and advance.

Number of Blanks

The Number of Blanks field allows you to enter the number of blank wells in the assay. This entry screen is only displayed when Full, Column, or Row blank map is selected.

```

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

- Use the **NUMERIC** keys to enter the number of blanks. The range is 0 to 48.
- Press the **CLEAR** key to clear the Number of Blanks value from the display.
- Press the **ENTER** key to save the value and move to the next screen.

Selecting a Blank Location

The Blank Location screen allows you to define where the blank well is located on the microplate. This screen only appears if manual map generation has been selected. Any previously defined value is displayed.

```

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 1 2
  
```

- Use the **NUMERIC** and **ALPHA** keys to enter a Blank Location, based upon the plate geometry.

- Use the **ARROW** keys to move the cursor to the next or previous editable field. The cursor is positioned beneath the first editable field.
- Press the **ENTER** key to save the value, and move to the next screen.

Number of Standards

Use this option to enter the number of standards that will be used in the assay. Any previously defined value will be displayed on the screen. If the number of standards is altered, the number of replicates for the standard automatically defaults to 1.

E N T E R N U M B E R O F	
S T A N D A R D S :	0 2

- Use the **NUMERIC** keys to enter the Number of Standards. The valid range depends on the selected curve fit method. The maximum number of standards is 12. The minimum is 4 for 4-P fit, cubic, cubic spline, and 2-P; 3 for quadratic; and 2 for linear and point-to-point.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to save the value, and move to the next screen.

Number of Standard Replicates

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

- Use the **NUMERIC** keys to enter the Number of Replicates per Standard. The range is 1 to 8 replicates. The software will verify that the number of replicates, multiplied by the number of standards, does not exceed the number of wells on the plate.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to save the value and move to the next screen.

Average Standards

The Average Standards option allows you to select whether or not to average the Replicates of a Standard. This average is used to calculate the standard curve instead of using the individual replicate of each standard. **Note:** If the replicate selection is 1, this option is not available.

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

- Press **SOFT KEY 1** to select **YES** (average the replicates). The top line of the display updates to reflect the selection.
- Press **SOFT KEY 2** to select **NO** (do **not** average the replicates). The top line of the display updates to reflect the selection.
- Press the **ENTER** key to save the selection, and advance to the next screen.

Standard Concentration

The Standard Concentration field allows you to enter the predicted or expected concentration value for each standard group. Any previously defined value is displayed.

If **Manual Map Generation** is selected, the replicate locations must also be defined.

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

- Use the **NUMERIC** and **ALPHA** keys and the **DECIMAL POINT** key to enter Standard Concentration values. The range is 0.00001 to 999999. The entry cannot exceed six characters including the decimal point. Valid well locations for the defined geometry are listed below.
- Use the **RIGHT ARROW** and **LEFT ARROW** keys to move to the next or previous editable field.
- Press the **CLEAR** key to clear the Standard Concentration value from the display.
- Press the **ENTER** key to save the value on the display and move to the next screen.

Reuse of Standard Curves

The ELx808 has the ability to reuse a standard curve that has already been established.

Limitations of the Reuse of Standard Curves

- The reuse of standard curves can only be done in assay positions 31 through 55. Each of these positions can only store one standard curve.
- Standard curves cannot be reused on panels (see page 67 for *Panel Assays*).
- Standard curves will be stored in memory with the Assay Name, Standard Concentrations, Replicate Counts, and Optical Densities for each standard replicate.
- Stored standard curves can only be reused for the assay that the curve was originally generated on (i.e., the curve for assay #53 cannot be applied to samples on a plate to be run in assay #51).
- To reuse a standard curve, an assay must first be programmed (in positions 31 through 55) and run. During the defining process, you will be prompted to enter the number of standards, the number of standard replicates, and the standard concentrations. The following screen has been added after these prompts:

```

R E U S E   S T A N D A R D   C U R V E ? Y E S
Y E S       N O
  
```

- After the assay has been run, the results have been calculated, and the reports have been generated, the reader will prompt if this standard curve should be stored in memory. The following display will appear:

```

S A V E   S T A N D A R D   C U R V E ?   Y E S
Y E S       N O
  
```

- Select **YES** to store the curve for use at a later time. The next time a plate is to be read using this assay, the instrument will prompt if there are standards on the plate. Select **NO** to discard the curve.

```

S T A N D A R D S   O N   P L A T E ?   N O
Y E S   N O

```

- If **YES** is chosen, a new standard curve will be generated. The plate map is not changed. (If “Prompt for Sample ID” is enabled in the **UTIL** section, you will be prompted to enter the number of samples. See page 18 for more information on the **UTIL** options.)
- If **NO** is chosen, the stored standard curve will be used. If Auto mapping had been used to originally map the standards, blanks, controls, and samples defined for this assay, the map will be automatically regenerated without the standards, beginning in well xxx (where xxx was chosen as the Starting well in the map, usually well A01). If Manual mapping was used to map the plate, the map is **NOT** regenerated - the reader will **NOT** produce results for the well positions that originally were standards. Auto mapping is recommended, if the standards curves will be routinely reused.

Number of Controls

```

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

```

- Use the **NUMERIC** keys to enter the Number of Controls. The range depends on the number of locations on the plate that are undefined. The maximum number of controls is 8.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to save the value and advance to the next screen.

Control Type

```

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

```


- Press the soft keys under the displayed **Control Type** to select the option (**Positive Control**, **Negative Control**, **High Positive Control**, **Low Positive Control**, CTL1, CTL2, CTL3, CTL4).
- Press **CLEAR** to clear the Control Type from the display.
- Press **ENTER** to save the displayed Control Type and advance to the next screen.

Number of Control Replicates

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

- Use the **NUMERIC** keys to enter a value for Number of Control Replicates. The range is 1 to 12 replicates. The software performs a check to ensure the number of replicates does not exceed the number of undefined wells remaining on the plate.
- Press **CLEAR** to clear the displayed Number of Replicates value.
- Press **ENTER** to save the displayed value and advance to the next screen.

Location of Controls

The displayed location field can only be edited if manual map generation was selected.

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 : A 0 1

- Press the **CLEAR** key to clear the value on the display.
- Use the **NUMERIC** and **ALPHA** keys to enter values for well locations on the plate.

Valid Well Locations

For all prompts of a well location, only the **ALPHA** keys are active for the first character and **NUMERIC** for the second and third characters.

- Press the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back and advance through the menu structure.

Number of Samples

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

- Use the **NUMERIC** keys to enter the number of samples in the assay. The range is 0 to the number of undefined well locations remaining on the plate. If there are no controls, blanks, or standards defined on a 96-well plate, the maximum number of samples is 96, and the minimum number of samples is 1.
- Press the **Clear** key to clear values on the display.
- Press **Enter** to save the displayed value and advance to the next screen.

❖ Note: If the number of samples is altered, the number of replicates for the sample reverts to a value of 1.

Number of Sample Replicates

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
--	-----

- Use the **NUMERIC** keys to enter the Number of Sample Replicates. The range is 1 to 12 replicates. The software ensures that the number of replicates multiplied by the number of samples, multiplied again by the number of dilutions, does not exceed the number of undefined wells remaining on the plate.
- Press the **Clear** key to clear the value on the display.
- Press the **Enter** key to save the displayed value and advance to the next screen.

Sample Location

If **Manual Map Generation** is selected, this screen allows you to select the well location of the sample on the plate. Any previously defined Sample Location appears on the display.

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

- Use the **NUMERIC**, **ALPHA**, and **DECIMAL POINT** keys to enter the sample identifier and its location on the plate.

- Press the **CLEAR** key to clear the displayed value.
- Press **ENTER** to save the value and advance to the next screen.

FORMULA

The ELx808 supports three types of formulas (Cutoff, Transformation, and Validation), as well as the ability to define variables for use within formulas. Up to three types of Validation formulas may be defined (Blank, Control, and Assay Validation).

Formulas are processed in the following order, with the number of permitted formulas of each type:

- 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)

Within any given formula type, the order of processing is the order in which the formulas are entered.

Validation Formula Examples

- **Blank Validation:** An assay protocol states that every blank well on a plate should have an OD of less than 0.050. The formula is entered on the reader as a Blank Validation Formula: $BLK < 0.050$.
- **Negative Control Validation:** An assay protocol states that every Negative Control well must have an OD of less than or equal to 0.100. The formula is entered as a Control Validation Formula: $NC \leq 0.100$.
- **Positive Control Validation:** An assay protocol states that every Positive Control well must have an OD higher than 1.000, but less than 2.500. Two Control Validation Formulas can be entered: $PC > 1.000$ AND $PC < 2.500$.
Or, one formula can be used if the formula is 24 characters or less: $PC > 1.000$ AND $PC < 2.500$.
- **Assay Validation:** An assay protocol states that in order for an assay to be valid, the mean of the Negative Control well ODs must be less than 0.100. The Assay Validation formula that should be entered: $NC;x < 0.100$ (the map identifier NC;x indicates the mean of the NCs).

From the assay **DEFINE** Menu, press the arrow corresponding to **FORMULA**.

Formula Type

The ELx808 supports three types of formulas, as well as the ability to program variables for use within Transformation formulas.

- **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 or “call” (POS, NEG, or EQUIV).
- **TRANS**formation formulas are applied to the raw data in preparation for further data reduction and/or curve-fit calculation.
- **VAL**idation 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.
- The **TRANS-VAR** option allows you to define a variable to be used in transformation formulas.

❖ **Note:** **GENERAL** formulas are not used in the ELx808 open assays.

```
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       * M O R E
```

- Press **SOFT KEY 1** to select **CUTOFF** Formula.
- Press **SOFT KEY 2** to select **TRANSFORMATION** Formula.
- Press **SOFT KEY 3** to select **ASSAY VALIDATION** Formula.
- Press ***MORE** to access additional formula types.
- Press **SOFT KEY 3** to select **TRANS-VAR**.
- Press **ENTER** to save the selected formula type and advance to the next screen.

Validation Type Selection

If you selected **VAL**, this option allows you to choose which Validation Formula type (**Control**, **Assay**, or **Blank** Validation formulas) to enter for the assay.

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

- Press **SOFT KEY 1** to select **Control** Validation Formula.
- Press **SOFT KEY 3** to select **Assay** Validation Formula.
- Press **SOFT KEY 4** to select **Blank** Validation Formula.
- Press **ENTER** to save the validation type and advance.

Formula Entry

❖ **Note:** In formulas, "OD" is used to represent the optical density value.

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

- After a moment, the **FORMULA #1:** prompt disappears, and the formula can be entered.
- Each formula can contain a maximum of 24 characters. Spaces are unnecessary.
- Use the **LEFT and RIGHT ARROW** keys to move the cursor to the previous or next editable field.
- Press **SOFT KEY 1** to place the next item on the **MATH** list at the cursor position. The following table shows the order of items on the **MATH** list:

+	Addition sign
-	Subtraction sign
*	Multiplication sign
/	Division sign
%	Percent

=	Equal
>	Greater than
>=	Greater than or equal to
<	Less than
<=	Less than or Equal to

❖ **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.

- Press **SOFT KEY 2** to place the next item on the **OTHER** list at the cursor position. See the table that follows for the order of items on the OTHER list:

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

- Press **SOFT KEY 3** to place the next defined item on the plate map list (i.e., STD, NC, PC, BLK) at the cursor position.
- Press **SOFT KEY 4** to place the next option on the **FUNCTION** list at the cursor position. The available functions are:

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

EXAMPLES:

LOG10: Log Base 10
 $\text{Log10 } 2 = 0.30102995$

ALOG10: Anti Log Base 10
 $\text{ALOG10 } (0.30102995) = 2$

AB: Absolute Value
 $\text{AB } (-1) = 1$

PWR: Power
 $(10 \text{ PWR } 2) = 100$

ALOG: Anti Log
 $\text{ALOG } (0.69314718) = 2$

LOG: Log
 $\text{LOG } 2 = 0.69314718$

SQRT: Square Root
 $\text{SQRT } 2 = 1.4142$

- Well locations, well types, or numbers in parentheses precede functions.
- Press the **Clear** key to clear the item displayed at the cursor position.
- Press the **Shift** and **Clear** keys to clear the entire entry.
- Press **Enter** to save the displayed value and advance to the next screen, or use the **Previous Screen** key to move backward through the menu structure.

Number of Required Controls/Blanks

If a control or blank validation formula is entered, this screen allows you to enter the number of valid controls / blanks for the assay. Any previously defined values will appear on the display.

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

- Use the **Numeric** keys to enter the Number of Required Controls. The range is 1 through the number of defined replicates of a control or blank.
- Press the **Clear** key to clear the displayed value.
- Press the **Enter** key to save the displayed value and advance to the next screen, or use the **Previous Screen** key to move backward through the menu structure.

Cutoff Formulas

A cutoff formula calculates a **cutoff value** that is used for classifying samples.

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. Refer to “FORMULA” on page 53 for the order in which formulas are processed.
- 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 equivocal or indeterminate 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.

After selecting an assay (page 32), define the Cutoff formula as shown below:

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 ↑
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 ↑
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

After the **FORMULA #1:** prompt disappears, enter the formula as described in “Formula Entry” on page 55. Refer to the examples on page 60.

Greyzone Entry

The **greyzone** is a definable area around the cutoff value. Samples falling within an area defined by the greyzone (ex. $\pm 5.0\%$ of the cutoff value) could be considered equivocal (EQUIV).

```
ENTER GREYZONE : 05%
```

- 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** below for information on how calls are assigned.
- Press the **Clear** key to clear the displayed value.
- Press the **Enter** key to save the displayed value and advance.

Positive/Negative Calls for Cutoff

After the greyzone is defined, calls for the sample wells (**POS**itive, **NEG**ative, **EQUIV**ocal) must be defined.

```
SAMPLE > CUTOFF + 05% : POS
POS      NEG
```

- 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 as shown in the above screen, calls will be assigned according to the following equations (SMP represents the sample wells):

EQUIV: $SMP \leq (CUTOFF + (CUTOFF * GREYZONE))$ AND
 $SMP \geq (CUTOFF - (CUTOFF * GREYZONE))$

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 of 0.500. Samples greater than the cutoff should be labeled as positive. No greyzone is required.

- For this example, $NC_{\bar{x}}$ (the mean of the NC wells) equals 1.000 OD
- The cutoff formula is $NC_{\bar{x}} + 0.5$
- The greyzone is 00%
- POS is selected for $SAMP > CUTOFF + 00\%$
- Calls are assigned to sample wells as follows:
 - EQUIV if the sample equals 1.500
 - POS if the sample is greater than 1.500
 - NEG if the sample is less than 1.500

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

- For this example, $STD2_{\bar{x}}$ (the mean of the STD2 wells) equals 2.000 OD
- The cutoff formula is simply $STD2_{\bar{x}}$
- 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 1.800 *and* less than or equal to 2.200
 - POS if the sample is greater than 2.200
 - NEG if the sample is less than 1.800

Transformation Formulas

Transformation formulas change the absorbance data of all wells defined in the Map to another format, in preparation for further data reduction.

Transformation Formula Definition

- From the assay **Define** Menu, press the arrow corresponding to **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
  
```



- This will bring you to a screen asking to **Select Formula Type**. At this screen, select **TRANS** and then enter the formula using the **Math**, **Other**, **Map** and **Function** keys.

```

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
  
```



Example: Divide all ODs on the plate by 2 and multiply by 100.

Enter the formula: **(OD/2)*100**

This formula will be applied to the ODs of all samples, standards, controls, and blanks that are present on the plate map.

Transformation Scope Variable

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

From the assay **Define** Menu, press the arrow corresponding to **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
  
```



This will bring you to a screen prompting you to **Select Formula Type**. Press ***MORE** at this screen.

```

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
  
```



The options displayed now include **TRANS-VAR**. Press **VAR** at this screen.

```

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       * M O R E
  
```



The following screen will appear, asking you to choose the **scope** of this transformation.

```

S C O P E   V A R I A B L E :           O D
S M P           O D
  
```

If **SMP** is chosen, the transformation formula will be applied *only* to the samples defined in the plate map. If **OD** is chosen, the formula definition screen will appear. 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 writing the transformation formula.

Example: An 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) with varying concentrations.

The assay data reduction states:

- Subtract the mean of CTL1 from the mean of the NC. Subtract the difference from all ODs on the plate.
- Divide the result of the above by the means of the NC less the means of CTL1, and then multiply by 100.

On paper, the formula reads:

$$(\text{OD} - (\text{NC}; \text{x} - \text{CTL1}; \text{x})) / (\text{NC}; \text{x} - \text{CTL1}; \text{x}) * 100$$

- On the reader, define the formula **(NC;x-CTL1;x)** as the *Transformation Variable*, since the transformation will apply to all standards, controls and samples on the plate.
- At the SCOPE VARIABLE screen, choose **OD** and press **Enter**. Now enter the formula **(NC;x-CTL1;x)** by using the **MATH, OTHER, MAP** and **FUNCTION** keys. Press the **ENTER** key.
- The formula definition screen is displayed. Choose **TRANS**.
- Enter the following formula: **(OD-(TVar))/(TVar) * 100** using the **MATH, OTHER, MAP**, and **FUNCTION** keys. (TVar is included in Map options on the formula entry screen.) The transformation formula has been added to the assay definition.

Another Transformation Example:

In the case of competitive reactions, converting absorbance data to percent B/B₀ can be: (OD/Std1)*100. This divides all the wells by **STD1** (presumably the 0 standard), and multiplies the results by 100.

- At the **SCOPE VARIABLE** screen, choose OD and press **Enter**.
- Select **STD1** from **MAP** and Press **Enter**.
- Choose **TRANS**.
- Enter **(OD/TVAR) * 100**

CURVE

The **CURVE** entry screens allow editing and entry of:

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

These screens are displayed on the ELx808 in the order in which they appear in the assay. If a closed variable (i.e., an element of the assay definition that you cannot access or modify) is being used in the assay, the entry screen is omitted.

Curve Fit

The **Curve-Fit** screen allows you to select the curve-fit method that will be applied to the assay. Any previously defined curve-fit type appears on the top line of the display, and available options on the second line.

The **Curve-Fit** screen has three sub-menu screens. Each sub-menu screen provides different curve-fit options for selection. These options include C-Spline, Linear, Quadratic, Cubic, 4-P, 2-P (Logit/Log), PT to PT (point to point), and None.

- **Linear curve fit:** A simple best-fit straight line is plotted using the values of the standards.
- **Quadratic or “Quad” curve fit:** A curve fit which uses the Quadratic equation “ $ax^2 + bx + c = y$ ” to plot the standard's values. Utilizing this curve, any data point for a standard that deviates from the ideal value will not affect the entire curve.
- **Cubic curve fit:** A curve fit that uses the equation “ $ax^3 + bx^2 + cx + d = y$ ” to plot the standard's 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.
- **Cubic Spline (C-Spline) curve fit:** 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-Parameter Logistic or “4-P”:** 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.
- **Point to Point or “PT to PT”:** A plot that connects each standard point with a line, with no averaging of the values to “smooth” the curve at each standard.

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

- Press **SOFT KEYS 1, 2, 3,** or **4** to select the curve-fit type that is displayed above the soft key. Select the soft key below the menu option **MORE** to display additional options. The top line of the display updates to reflect this selection.
- Press **ENTER** to save the selection and advance to the next screen, or use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backward through the menu structure.

Edit Standard Outliers

This screen allows you to select which method (None or Manual) will be used to edit Standard Outlier values. After the standard curve has been calculated, one or more standards can be excluded from the recalculation of the curve. Any previously defined edit method is displayed.

```
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
```

- Press **SOFT KEY 1** or **2** to select the edit option displayed above the soft key. The display updates to reflect your selection.
- 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, and then press **ENTER**. The **EDIT STD OUTLIERS? YES/NO** prompt will appear. See *Editing Standard Outliers* on page 74 for further instructions.
- Use the **ENTER** key to save the selection and advance to the next screen, or use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backward through the menu structure.

Axis Selection

This screen allows you to select the X and Y Axis Type. Any previously defined axis type will be displayed. This option screen appears only if Manual Map Generation has been selected.

```

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
  
```

- Press **SOFT KEY 1, 2, 3, or 4** to select the method by which the X and Y-axes will be scaled. The top line of the display updates to reflect the selection.
- 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.
- Press **ENTER** to save the selection and advance to the next screen.

Extrapolation of Unknowns

This screen allows you to choose whether to extrapolate the curve to evaluate samples outside of the absorbance range defined by the standards. Any previously defined decision appears on the screen.

```

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
  
```

- Press **SOFT KEY 1** to select **YES** (extrapolate the unknowns). The top line of the display updates to reflect this selection.
- On the printed reports, extrapolated concentrations (RSLT values) are surrounded by < > (e.g., <44.425>).

- Press **SOFT KEY 2** to select **NO**. The top line of the display updates to reflect this selection.

❖ **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 8 assays to be run on one plate.

- A common reason to use a Panel assay is when one or more samples are tested for more than one antigen. An example is an ENA panel, which could screen dsDNA, Sm, SSA, SSB, Scl-70, and/or Jo-1 on one microplate.
- Only one Panel assay can be defined on the reader at any time.
- The assays specified within the Panel must be predefined 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.
- Panel assays cannot reuse standard curves.
- Only an Auto map is recognized by a Panel assay. Custom locations entered using a Manual map will be overwritten by the Panel assay.
- If the Panel runs in a 1*12(ACROSS) configuration, both the Map Direction and Replication Direction must be set to ACROSS during assay definition. ACROSS would also then be selected as the direction during Panel definition.
- The type and number of controls, blanks, standards, and replicates in the assays chosen for the Panel will be "copied" into the Panel definition. Map or assay parameters must first be changed in the predefined assay before they can change in the Panel.

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

N A M E : P A N E L

```
- / : SPACE
```

- 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.

```
NUMBER OF ASSAYS : 2
```

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

```
MAPPING DIRECTION : DOWN
DOWN ACROSS
```

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

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

```
SELECT ASSAY NUMBER : 2 2
NAME : HBS - AG 1
```

- Press the **Options** key 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 an assay is selected, its starting location must be defined.

```
START MAPPING
AT WELL LOCATION : A 0 1
```

- Use the alpha and numeric keys to choose the well location to begin the assay.
- Repeat this process 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.
 - The Panel Assay results are sorted by Sample (unless a custom assay has been programmed by BioTek).

❖ **Note:** The Interpretation of Results reports for each assay in the Panel will print first, and then the Sample results will print.

Reading a Microplate

Press the **READ** option, found at the Main Menu, to read a microplate.

- From the **MAIN MENU** screen, press the soft key beneath the **READ** menu option to access the **SELECT ASSAY NUMBER** screen.
- Alternately, press the red **READ** key on the lower right of the keyboard.

Select Assay

At the Select Assay Number screens:

- Use the **NUMERIC** keys to enter the number of any predefined Assay Definition Files stored in the reader's memory, or the **OPTION** 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 (1-55) programmed in the reader's memory.

The assay's name and number are displayed on the screen.

S E L E C T A S S A Y N U M B E R : 2 5
N A M E : H B S - A G

- Press **Enter** to advance to the Run-Time prompts.
- **MAIN MENU**: Returns the display to the Main Menu screen.

Run-Time Prompts

After the assay is selected, one or more informational prompts may be presented, depending on preferences selected in **UTIL > READ**, whether or not the assay specifies manual mapping, or if a custom assay database is installed on the reader.

- 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:

- Number of samples
- Standard concentrations
- Assay ID
- Fill pattern
- Blank method
- First well location
- Replicate count for each well type

Wavelength mode
Report preferences

Enter Number of Samples

Use this screen to enter from 00 to the maximum number of samples permitted by the previously created well map. If there are no controls, standards, or blanks defined, the minimum number of samples is 1.

This value controls the number of samples reported if Matrix or Column reports are requested.

E N T E R N U M B E R O F	
S A M P L E S :	9 1

Enter Plate ID

You can enter a 10-character (maximum) identifier to assign to the plate. Since this Plate ID will be stored in the reader's memory, each plate ID should be unique.

- ❖ **Note:** Use caution when creating multiple Plate IDs. The reader does not warn you that you are about to exceed the maximum of 10 plate IDs stored in memory. If an eleventh Plate ID is added, it will overwrite the first Plate ID stored in memory.
- ❖ **Note:** If the internal bar code scanner option is installed, the reader will automatically scan the plate/bar code label and use this as the Plate ID.

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

- Use the **KEYPAD** to enter numbers, and the **LEFT / RIGHT** arrow keys to move the cursor. **Clear** clears the display.

Enter Sample ID

You can start entering a starting sample identification number from 0001 to 9999. The software will automatically increment each subsequent sample identification number by 1. Sample IDs will be assigned according to the previously defined mapping order.

```

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

```

- Use the **KEYPAD** to enter numbers and the **LEFT / RIGHT** arrow keys to move the cursor. **Clear** clears the display.

Prompts for Well Location

Well locations can be changed at run time if a Manual Map has been specified, and you have requested a sample count at run time via the Utilities menu.

```

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

```

- Use the **KEYBOARD** to enter the well location, using the **SHIFT-LETTER** sequence to key in letters, and press **Enter** to specify the desired location.

Beginning the Plate Read

When the following screen appears on the display, the reader is ready to read a plate:

```

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 plate in the carrier and press the **READ** key to initiate the plate read. After the plate has been read, all requested reports will be generated.
- To halt in read in progress, press the **STOP** key.

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

Printing Reports and Assay Lists



Important! The 'OUT' indication on reports means the OD for an individual well, or the average OD for a group of wells, falls outside the minimum/ maximum OD range defined for the assay. For the Quick Read assay on the ELx808, this range is -3.0 OD to +3.0 OD. For assays defined onboard by a user (Assay02-Assay55), it is -4.0 OD to +4.0 OD.

Reports are automatically generated after a plate has been read (see **OUTPUT Options** on page 19 and **REPORT Type** on page 19 in **Chapter 2** for information on selecting reports). To manually regenerate results reports, use the **REPORT** option from the Main Menu. You can also print Map, Assay, and Assay List reports.

❖ See **Appendix A** for sample reports.

```
R E A D Y           9 : 4 5 P M           0 5 / 0 9 / 0 7
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
```

- The eight most recent sets of plate data are stored in memory (see next page). Select **REPORT > RESULT** to print an exact copy of results from the plate reading.
 - 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 selected assay.
- Select **ASSAY** to print a plate map and a listing of all of the 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 ELx808.

❖ **Note:** The reader stores measurement values for the last 8 plates in memory.

Result

```

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

```

- Use the **OPTION** key to select the appropriate **Plate ID** and **Report**. Note that the **Assay ID** will change if the selected **Plate ID** was read with a different assay. Once you have found the correct **Plate ID**, press **Enter**.

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

```

- 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

```

- 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

```

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

❖ **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

```

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

Map

- Select **REPORT** at the Main Menu, and then select **MAP**.

```

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

```

- 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

- Select **REPORT** at the Main Menu, and then select **ASSAY**.

```

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

```

- 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

- Select **REPORT** at the Main Menu, and then select **LIST**. The entire list of assays stored in the ELx808's memory will be sent to the printer.

Recommendations for 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 ELx808 supports standard flat, U-bottom, and V bottom 96-well 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 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 μ L per well.
- 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.
- Although the effect of ambient light is mathematically quantified and subtracted from each absorbance reading, the illumination of the instrument by strong ambient light should be avoided. If interference from ambient light is suspected, read a microplate of high-density solutions under the suspect conditions, and again with all ambient light blocked (in a dark room for example), then compare results. The blocked results will appear as an increase in optical density readings if light is influencing the readings. Because of the mathematical correction, this difference under normal conditions should be slight or nonexistent.
 - A 10-minute warm-up of the instrument is suggested, prior to reading, to achieve the best repeatability from microplate-to-microplate measurements.

Chapter 4

Instrument Qualification

This chapter discusses the tasks and procedures necessary for qualifying instrument performance initially and on an ongoing basis.

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Overview

This chapter contains BioTek Instruments' recommended Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) procedures for all models of the ELx808 Absorbance Microplate Reader.

Every ELx808 reader is fully tested at BioTek prior to shipment and should operate properly upon initial setup. If you suspect that a problem occurred during shipment, if you have received the equipment after returning it to the factory for service, and/or if regulatory requirements dictate that you qualify the equipment on a routine basis, you should perform the procedures outlined in this chapter.

- ❖ An ELx808 Product Qualification Package (PN 7340543) is available for purchase. It contains procedures for performing IQ/OQ/PQ and Preventive Maintenance, with spreadsheets for analyzing results and checklists and logbooks. Contact your BioTek dealer for more information.

Recommended Qualification Schedule

The schedule below defines the factory-recommended intervals for qualifying an ELx808 reader used two to five days a week. The actual frequency, however, may be adjusted depending on your usage of the instrument. This schedule assumes the reader is properly maintained as outlined in the *Preventive Maintenance* chapter.

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

Tests	Installation Qualification	Operational Qualification	Performance Qualification	
	Initially	Initially/ Annually	Monthly	Quarterly
System Self-Test, p. 89	✓	✓	✓	
Checksum Test, p. 89	✓	✓	✓	
Absorbance Plate Test, p. 89	✓	✓	✓	
Empty Carrier Test, p. 91	✓	✓		
Liquid Tests, p. 92 Liquid Test 1*, or Liquid Test 2* Liquid Test 3**, (optional, for 340 nm)		✓		✓

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

Test Descriptions

System Test

The System Test runs automatically whenever the instrument is turned on. It can also be performed manually using Gen5 software, or the keypad (**UTIL > TESTS > SYSTEM**). If the power-up System Test fails, the reader will “beep” repeatedly and display an error code; see Chapter 6.

When run manually, a System Test report is printed (or displayed in Gen5). The report (sample shown on the next page) includes results for the System Optics Test and Autocal Analysis. We recommend saving System Test reports to document periodic testing and assist with troubleshooting.

- The System Test conducts a series of tests at each wavelength defined in the filter table to confirm adequate light levels, low electronic noise, adequate photodiode sensitivity, overall system cleanliness, and (if equipped) proper function of the incubator. The testing is designed to verify that the ELx808 will give in-specification performance for each set wavelength over the specified OD range.
- Autocal is run only at the factory (or by an authorized service engineer) to calibrate the carrier axis of the instrument. The results of this test are included in the System Test Report. In the field, the alignment of the Absorbance Plate Test is used to verify that the carrier positioning is correct.

Terms used on the System Test Report	
Air Blank	A full light reading through a filter with no plate in the light path.
Dark Current	A reading taken with the light blocked to measure background light levels in the reading chamber. Also used as a measure of background electronic noise within the measurement circuit.
Gain	An automatic electronic adjustment to the measurement circuit. The gain adjustment compensates for changing light levels or filter variations. For example, if the lamp output decreases slightly, the gain will increase to make up the difference.
Axis	Refers to a motor for the filter wheel or plate carrier.
Offset	A numerical limit, usually a range. For example, if the gain fails an offset test, it may be too high or too low.

Gen5 System Test Report

Reader: ELx808 (Serial Number: 118943)
 Basecode: P/N 7340201 (v3.15)
 Date and Time: 7/28/2012 3:06:11 PM
 User: Administrator
 Company: BioTek
 Comments: System Test run during the IQ

Test Results

Operator ID: _____

Notes: _____

03:11PM 7/28/12 SYSTEM SELF TEST

Filter: 405	Gain: 15.06								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	24272	36673	54326	49036	37302	41392	37956	46880	42789
Dark:	1627	1790	1738	1983	1922	1969	2155	1978	2041
Delta:	22645	34883	52588	47053	35380	39423	35801	44902	40748

Filter: 450	Gain: 5.12								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	26520	40514	54985	51166	41113	45699	43595	50055	47401
Dark:	587	644	626	708	688	705	767	707	729
Delta:	25933	39870	54359	50458	40425	44994	42828	49348	46672

Filter: 490	Gain: 16.00								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	29585	40785	53183	50389	42324	46927	45225	51112	48181
Dark:	1806	1981	1927	2187	2121	2172	2369	2182	2247
Delta:	27779	38804	51256	48202	40203	44755	42856	48930	45934

Filter: 620	Gain: 8.26								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	28973	40210	53315	49573	42596	47522	46312	50677	49083
Dark:	922	1012	983	1116	1084	1111	1211	1114	1150
Delta:	28051	39198	52332	48457	41512	46411	45101	49563	47933

Filter: 630	Gain: 5.57								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	28127	42576	54097	52142	44139	48634	47638	52007	50305
Dark:	630	691	672	762	739	758	826	760	784
Delta:	27497	41885	53425	51380	43400	47876	46812	51247	49521

Filter: 340	Gain: 64.00								
Channel:	Ref	1	2	3	4	5	6	7	8
Air:	18961	28041	46573	41370	27548	30291	25144	36515	31946
Dark:	7163	7855	7642	8677	8411	8630	9413	8655	8927
Delta:	11798	20186	38931	32693	19137	21661	15731	27860	23019

Channel:	Ref	1	2	3	4	5	6	7	8
Noise Max:	3952	4351	4225	4817	4668	4790	5237	4807	4963
Noise Min:	3951	4346	4220	4814	4664	4785	5233	4802	4957
Delta:	1	5	5	3	4	5	4	5	6


```

03:11PM    7/28/12    INCUBATOR SELF TEST

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

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

AUTOCAL ANALYSIS

Upper Left Corner:  x=5344  y=0
Lower Left Corner:  x=6156  y=0
Lower Right Corner: x=14850 y=0
Upper Right Corner: x=14032 y=0
Delta 1 : 5344-6156=-812
Delta 2 : 14032-14850=-818

SYSTEM TEST PASS
0000

Reviewed/Approved By: _____ Date: _____

For Technical Support

In the U.S.:
BioTek Instruments, Inc.
Tel: 800 242 4685
Fax: 802 655 3399

In Europe:
BioTek Instruments GmbH
Tel: 49 (0) 7136-9680
Fax: 49 (0) 7136-968-111

All Others:
Tel: 802 655 4040
Fax: 802 655 3399

email: TAC@biotek.com
Product support center: www.biotek.com/service
    
```

The **INCUBATOR SELF TEST** shows the PASS or FAIL condition of the four heating zones in the incubator (if equipped). See *Incubator Self-Test* on the next page.

AUTOCAL ANALYSIS provides coordinates of the last Autocal performed on the instrument. AUTOCAL verification is not included in the **SYSTEM TEST** result given below.

The **SYSTEM TEST** result will be either PASS or FAIL. The SYSTEM TEST result refers only to the **System Optics Test** results.

Figure 5: Sample System Test Report (it varies slightly depending on how it was run)

❖ If a System Test shows one or more channels that have air readings falling below 30,000 for filter wavelengths of 405 nm or higher, Liquid Test 1 should be performed. This test is applicable to the eight reading channels only, not the reference channel.

Incubator Self Test

The Incubator Self Test of the ELx808 reader is designed to check for hardware failures (such as open or shorted thermistors, A/D noise) and to verify that each of the four zones in the incubation system are within $\pm 2^\circ$ of the setpoint.

- **Temperature Setpoint:** The desired temperature programmed by the user, either through the reader's keypad or Gen5.
- **Current Average:** Average temperature of the four zones of the incubator at the time the system test is run. This number is displayed on the ELx808 front panel and sent to Gen5 if the reader is being computer controlled.
- **A/D Test:** Checks for allowable noise levels.

03:11PM	7/28/12	INCUBATOR SELF TEST			
Temperature Setpoint: 37.0		Current Average: 36.9		A/D Test: PASS	
Zone 1:	37.0	Min: 36.9	Max: 37.0	Range: PASS	Thermistor: PASS
Zone 2:	37.0	Min: 37.0	Max: 37.1	Range: PASS	Thermistor: PASS
Zone 3:	37.0	Min: 36.9	Max: 37.0	Range: PASS	Thermistor: PASS
Zone 4:	36.9	Min: 36.9	Max: 37.0	Range: PASS	Thermistor: PASS

Zone #: Reports the average temperature of the zone at the time the test is run. Each zone is evaluated independently.

Min: The minimum temperature recorded during the time that the system test is run.

Max: Maximum temperature recorded during the time that the system test is run. *There is no range checking between Min and Max temperatures.*

Range: This test checks that each zone is within $\pm 2^\circ$ of the setpoint.

Thermistor: This test indicates whether the thermistors are functioning or have failed.

Checksum Test

The Checksum Test compares the basecode software to internally recorded checksum values to ensure that the programming has not been corrupted. The test runs automatically when the instrument is turned on, and it can be run manually using the keypad (**UTIL > TESTS > CHKSUM**). If the test fails, the reader will "beep" repeatedly and display an error code; see **Chapter 6**.

When run manually, the software displays the part numbers and versions of software currently loaded on the reader. For example:

Software P/N	Software Version
7340201	Version 3.21
Code Checksum:	(5E35)

The second screen shows:

Configuration P/N

7340203

Configuration Version

Version 3.00

Absorbance Plate Test

This test uses BioTek's Absorbance Test Plate to confirm the Mechanical Alignment, Accuracy and Linearity, Repeatability, and Channel-to-Channel uniformity of the ELx808. The Absorbance Plate Test compares the reader's optical density measurements and mechanical alignment to NIST-traceable values. To run the test, you will need a BioTek Absorbance Test Plate with its accompanying data sheet.

- ❖ The BioTek PN 7260522 Test Plate has a glass filter in location C6, which is used to check the wavelength accuracy of a monochromator. Although the ELx808 does not have a monochromator, you must enter values for the peak wavelength when defining the 7-filter test plate in Gen5 software. Refer to the software help system for more information.

A sample Absorbance Plate Test report is shown on the next page.

Absorbance Test Plate Results						
Reader:	ELx808 (Serial Number: 233820)					
Basecode:	P/N 7340201 (v3.21)					
Date and Time:	5/23/2011 11:29:41					
Absorbance Plate:	7 Filter Test Plate (P/N 7260522) - S/N 233820					
Last Plate Certification:	May 2011					
Next Plate Certification Due:	May 2012					
User:	Administrator					
Comments:						
Alignment Results						
wells	F1	C12	H8			
Read	0.004	0.004	0.003			
Tolerance	0.015	0.015	0.015			
Result	PASS	PASS	PASS			
wavelength = 405 nm						
Accuracy Results						
wells	C1	E2	G3	H6	F5	D4
Reference	0.150	0.642	1.217	1.720	2.097	2.753
Min Limit	0.127	0.609	1.173	1.666	2.035	#N/A
Max Limit	0.173	0.675	1.261	1.774	2.159	#N/A
Read 1	0.143	0.640	1.227	1.719	2.081	2.722
Result	PASS	PASS	PASS	PASS	PASS	#N/A
wells	F12	D11	B10	A7	C8	E9
Reference	0.150	0.642	1.217	1.720	2.097	2.753
Min Limit	0.127	0.609	1.173	1.666	2.035	#N/A
Max Limit	0.173	0.675	1.261	1.774	2.159	#N/A
Read 1	0.144	0.639	1.228	1.719	2.080	2.722
Result	PASS	PASS	PASS	PASS	PASS	#N/A
Repeatability Results						
wells	C1	E2	G3	H6	F5	D4
Read 1	0.143	0.640	1.227	1.719	2.081	2.722
Min Limit	0.137	0.632	1.216	1.705	2.066	#N/A
Max Limit	0.149	0.648	1.238	1.733	2.096	#N/A
Read 2	0.143	0.640	1.228	1.719	2.080	2.721
Result	PASS	PASS	PASS	PASS	PASS	#N/A
wells	F12	D11	B10	A7	C8	E9
Read 1	0.144	0.639	1.228	1.719	2.080	2.722
Min Limit	0.138	0.631	1.217	1.705	2.065	#N/A
Max Limit	0.150	0.647	1.239	1.733	2.095	#N/A
Read 2	0.144	0.639	1.228	1.719	2.080	2.719
Result	PASS	PASS	PASS	PASS	PASS	#N/A
Turnaround Results						
wells	C1	E2	G3	H6	F5	D4
Read 1	0.143	0.640	1.227	1.719	2.081	2.722
Min Limit	0.132	0.624	1.205	1.692	2.050	#N/A
Max Limit	0.154	0.656	1.249	1.746	2.112	#N/A
Read 2	0.144	0.639	1.228	1.719	2.080	2.722
Result	PASS	PASS	PASS	PASS	PASS	#N/A

Figure 6: Sample Absorbance Plate Test Report

❖ The report varies slightly depending on how it was run (via the keypad or Gen5).

❖ **Important!** The Absorbance Plate Test tests the accuracy and repeatability specifications from 0.000 to 2.500 OD only. In the example above, the Analysis report displays the OD values read in well positions D4 and E9 but does not indicate PASS or FAIL, because the values are higher than 2.500 OD and therefore are not within the test range.

If any of the test parameters report as FAIL, confirm that the standard values on the test plate data sheet match the values on the printout. If there are any mismatches, correct them and re-run the test. See also the troubleshooting tips below. If the test continues to fail, contact BioTek TAC. Please have a copy of the test and the reader's serial number ready.

- **Mechanical Alignment:** The Test Plate has several groups of precisely machined holes to confirm the mechanical alignment of different microplate readers. The amount of light that shines through these holes is an indication of how well the reader is aligned. A reading of more than 0.015 OD for any of the designated alignment holes indicates that the light is being "clipped" and the reader may be out of alignment. If the test fails:
 - Examine the microplate carrier to ensure that it is clear of debris.
 - Make sure the Test Plate is properly seated in the microplate carrier.
 - Check the Test Plate's alignment holes to ensure they are clear of debris.
- **Accuracy/Linearity:** The Test Plate contains neutral-density glass filters of known OD values at several wavelengths. Actual measurements are compared against the expected values provided in the Test Plate's data sheet. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is proven that the reader is accurate at these OD values, the reader is also considered to be linear. If the test fails:
 - Check the filters on the test plate to ensure they are clean. If necessary, clean them with lens paper. Do not remove the filters from the test plate, and do not use alcohol or other cleaning agents.
 - Ensure that the filter calibration values entered are the same as those on the Test Plate data sheet.
 - Ensure that the Test Plate is within its calibration certification period.
- **Repeatability:** This test ensures the reader meets its repeatability specification by reading each neutral-density filter on the Test Plate twice with the filter in the same location. Note that there may not be a Pass/Fail indication for filter values that are beyond the specified accuracy (and thus repeatability) range of the instrument. If the test fails:
 - Examine the glass filters on the test plate to see if there are any loose particles that may have shifted between readings and caused changes. If needed, clean the filters with lens paper.

❖ Do not remove the filters from the test plate, and do not use alcohol or other cleaning agents to clean them.

➤ Check the microplate carrier to ensure that it is clear of debris.

- **Channel-to-Channel Variation:** This test ensures that selected channels read the same value for a filter as their paired channel when the plate is “turned around” 180° in the plate carrier. It is a way to verify that each channel’s Delta values are adjusted relative to the signal strength of the reference channel.

Empty Carrier Test

The Empty Carrier Test confirms the ELx808’s read capabilities at the 100% light level, and can help to identify deteriorating interference wavelength filters and other optical problems.

Liquid Tests

Conducting Liquid Tests confirms the ELx808’s ability to perform to specification with liquid samples. Liquid testing differs from testing with the Absorbance Test Plate in that liquid in the wells has a meniscus, while the Test Plate’s neutral density filters do not. The optics characteristics may differ in these two cases, alerting you to different types of problems. The Absorbance Test Plate will indicate the absolute amount of light absorbed, which will effectively test the linearity of the electronics. The liquid tests will help detect optical defects such as dirt on the lenses or other contamination that can contribute to errant readings.

- **Liquid Test 1** tests the alignment, accuracy, repeatability, and channel-to-channel variability of the reader, making evident any problems with the optics of the system. If you have the Absorbance Test Plate, you will only need to run Liquid Test 1 for routine testing.
- If you do not have the Absorbance Test Plate, test the linearity, repeatability, and alignment of the reader by preparing a series of solutions of varying absorbances as described in **Liquid Test 2**.
- **Liquid Test 3** is an optional test at 340 nm, offered for sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test is considered optional because the reader has good “front end” linearity throughout its wavelength range.

Perform the Tests

System Test

❖ If the System Test fails, see *Chapter 6* for troubleshooting tips and error code descriptions.

Using Gen5, select the following in the appropriate software:

- Open the system menu and select **Diagnostics > Run System Test**

Using the instrument keypad:

1. Attach a printer to the instrument.
2. From the Main Menu, select **UTIL > TESTS > SYSTEM**.

Checksum Test

There is no Checksum Test in Gen5. To obtain reader software information:

- Open the system menu and select **Reader Control > ELx808**. To obtain Gen5 software information, select **Help > About Gen5**.

Using the instrument keypad:

- From the main menu, select **UTIL > TESTS > CHKSUM**.
 - The initial checksum test display will show the onboard (base code) software part number, version number, and checksum.
 - After a few moments, a second screen will show the assay configuration software part number and version number.

Absorbance Plate Test

Define the Test Plate Parameters

Using Gen5 (click **Help** for additional instructions):

- Open the system menu and select **Diagnostics > Test Plates > Add/Modify Plates**, and click **Add**.

Using the keypad:

1. From the main menu select **UTIL > SETUP > *MORE > CALPLATE**. The Calibration Filter screen will appear.
2. Select a filter wavelength and press **Enter**. The Wavelength / Calibration Value screen will appear.

3. Enter the values listed on the Test Plate data sheet. After each entry, press **Enter** to advance to the next well location.
4. Repeat for the remaining filters.
5. When all values have been entered, press the Main Menu key.

Run the Plate Test




❖ Before running the test, ensure that the reader is not running in Rapid Mode. To check this, select **UTIL > READ** and then cycle through the prompts until **READ IN RAPID MODE?** is displayed. Choose **NO** for an accurate result.

Using Gen5 (click **Help** for additional instructions):

- Open the system menu and select **Diagnostics > Test Plates > Run**.

Using the keypad:

1. Select Start at the Main Menu and select **UTIL > TESTS > CALPLATE**. The Calibration Filter screen will appear.
2. Select the appropriate wavelength and press **Enter**.
3. When prompted, insert the Test Plate into the plate carrier, and press the **READ** key to begin the test. The Test Plate Analysis Report (sample on page 86) will be sent to a printer when the test is complete.

	Gen5 version 1.07 and earlier users: Gen5's Reader Diagnostics Utility (PN 5320201) must be installed before the Absorbance Plate Test can be performed using Gen5.
	Important! The Absorbance Plate Test and CALPLATE utility in the reader are used only to verify the reader's performance. The Test Plate and utility do not correct or calibrate the instrument.
	The Absorbance Plate Test tests the accuracy and repeatability of the reader to 2.500 OD. It does not indicate a PASS or FAIL above 2.500 OD.

Empty Carrier Test

Perform these steps (do *not* place a microplate on the carrier):

1. For each filter in the filter wheel, run a simple endpoint protocol and then print the results. (Do not apply blanking or transformations.)
2. Examine the OD values on the printouts. Every OD should read 0.000 \pm 0.004 OD.

❖ These limits are required by BioTek and cannot be changed.

3. Sign and date the printouts, and store them with your test documentation.

If the Empty Carrier test fails at more than one wavelength:

- Check the microplate carrier to ensure that it is clear of debris.
- Verify that the lamp is properly aligned, and then run the test again. If the lamp has been in use for approximately 600 hours, it may need to be replaced, and the contacts in the lamp socket may need to be cleaned. Refer to ***Replace the Lamp and Clean the Contacts*** in Chapter 5.
- Follow the steps in ***Inspect and Clean the Wavelength Filters*** in Chapter 5 to remove the filter wheel and clean the filters.

If the Empty Carrier test fails at just one wavelength:

- Remove the filter wheel and examine the filter that exhibited the problem; it may need to be replaced (contact BioTek). Check for spotting or a halo effect.

Liquid Tests

Prepare Test Solutions

BioTek offers a dye solution (PN 7120779, 25 mL; or 7120782, 125 mL) that may be used in the stock solution formulation for Liquid Tests 1 and 2, or, if you prefer, you may use the dye solution described below. The purpose of the formulation is to create a solution that absorbs light at ~2.000 OD full strength when dispensed at 200 μ L/well in a flat-bottom microplate.

Alternatively, any solution that gives a stable color will suffice. (This includes substrates incubated with an enzyme preparation and then stopped with an acidic or basic solution.) Some enzyme/substrate combinations that may be used as alternates to the described dye are shown below:

Typical Enzyme-Substrate Combinations and Stopping Solutions

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

Stock Solution Formulation

The stock solution for Liquid Tests 1 and 2 may be formulated using the materials listed below (Solution A), or by diluting a dye solution available from BioTek (Solution B).

❖ Either solution should result in an absorbance of about 2.000 when using 200 μ L in a flat-bottom microwell at 405 nm. The OD value will be proportional to the volume in the well and the amount of FD&C No. 5 dye or QC Check Solution 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.

Solution A

Required Materials:

- BioTek QC Check Solution No. 1 (PN 7120779, 25 mL; or 7120782, 125 mL)
- Deionized water

- 5-mL Class A volumetric pipette
- 100-mL volumetric flask

Prepare Stock Solution:

1. Pipette a 5-mL aliquot of BioTek QC Check Solution No. 1 into a 100-mL volumetric flask.
2. Add 95 mL of DI water; cap and shake well.

Solution B

Required Materials:

- Deionized (DI) water
- FD&C Yellow No. 5 dye powder (typically 90% pure)
- Tween 20 (polyoxyethylene (20) sorbitan monolaurate) **or** BioTek wetting agent, PN 7773002 (a 10% Tween solution)
- Precision balance with a capacity of 100 g minimum and readability of 0.001 g
- Weigh boat
- 1-liter volumetric flask

Prepare 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, or 5 mL of BioTek's wetting agent.
4. Make up to 1 liter with DI water, cap, and shake well.



96-well, flat-bottom microplates are required for the liquid tests (Corning Costar #3590 is recommended). Use new microplates; any fingerprints or scratches may cause variations in readings.

Before running the tests, ensure that the reader is not running in Rapid Mode. To check this, select **UTIL > READ** and then cycle through the prompts until READ IN RAPID MODE? is displayed. Choose **NO** for an accurate result.

Perform Liquid Test 1

1. Using freshly prepared stock solution A or B (the **concentrated** solution), prepare a 1:2 dilution using DI water (one part stock, one part DI water; the resulting solution is a 1:2 dilution).
2. Pipette 200 μ L of the **concentrated** solution into Column 1 of the plate.
3. Pipette 200 μ L of the **diluted** solution into Column 2 of the plate.

❖ After pipetting the diluted test solution into the microplate and before reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. If a plate shaker is not available, wait 20 minutes after pipetting the diluted test solution before reading the plate.

4. Read the microplate five times at 405 nm using the Normal Read Mode, single wavelength, no blanking (“Normal” plate position).
5. Without delay, rotate the microplate 180° so that well A1 is now in the H12 position. Read the plate five more times (“Turnaround” plate position).
6. Print the ten sets of raw data, or export the data to an Excel spreadsheet using Gen5. The mathematical computations described below may then be performed and the template kept for future tests.

Calculations:

7. Calculate the mean OD value for each well location in columns 1 and 2 for the five plates read in the Normal position, and then again for the five plates read in the Turnaround position. This will result in 32 mean values.
8. Perform a mathematical comparison of the mean values for each microwell in its Normal and Turnaround positions (that is, compare A1 to H12, B1 to G12, H1 to A12, and so on). To pass this test, the differences in the compared mean values must be within the accuracy specification for the instrument. For example:

If the mean value for well A1 in the Normal position is 1.902, where the specified accuracy is $\pm 1.0\% \pm 0.010$ OD, then the expected range for the mean of the same well in its Turnaround (H12) position is 1.873 to 1.931 OD. $1.902 * 0.010 + 0.010 = 0.029$; $1.902 - 0.029 = \mathbf{1.873}$; $1.902 + 0.029 = \mathbf{1.931}$

- ❖ If any set of mean values is out of the expected range, review the other three sets of mean values for the same channel pair. For example, if the A1/H12 comparison fails (the wells are not within the expected range of each other), review the comparisons of A2/H11, H1/A12, and H2/A11. If two or more sets of mean values for a channel pair are out of the expected range, there is a problem with one of the eight read channels. If only one of the four mean values results in a failure, check the well for debris and the plate for scratches or fingerprints.

Accuracy Specification:

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

$\pm 1.0\% \pm 0.010$ OD from 0.000 OD to 2.500 OD at 405 nm

Perform Liquid Test 2

- ❖ The recommended method of testing the instrument performance is to use the Absorbance 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 used for these tests.

Required Materials:

- A new 96-well, flat-bottom microplate (Corning Costar #3590 is recommended)
- Ten test tubes, numbered consecutively, stored in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Stock solution A or B (these are the same solutions as for Liquid Test 1)
- A 0.05% solution of deionized water and Tween 20

Prepare Dilutions:

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

Test Tube Dilutions

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

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

Prepare the plate:

1. Pipette 200 μ L of the concentrated solution from tube 1 into each well of the first column, A1 to H1, of the microplate.
2. Pipette 200 μ L from each of the remaining tubes into the wells of the corresponding column of the microplate (tube 2 into wells A2 to H2, etc.).

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

❖ After pipetting the diluted test solution into the microplate and *before* reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. If a plate shaker is not available, wait 20 minutes after pipetting the diluted test solution before reading the plate.

Perform the Linearity Test (Test A)

1. Read the prepared microplate five times using Normal mode, dual wavelength at 450 nm with 630 nm as the blank.

❖ Do not discard the plate; you will use it again for Test C.

2. Print the ten sets of raw data or export them to an Excel spreadsheet using Gen5. The mathematical computations described below may then be performed and the template kept for future data reduction.

Calculations:

1. Calculate the mean absorbance for each well, and then average the means for each concentration.
2. Perform a regression analysis on the data to determine if there is adequate linearity. Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R Square value of at least 0.99 is considered adequate.

Calculate Repeatability (Test B)

1. Calculate the mean and standard deviation for the five readings taken above at each concentration. Only one data set needs to be analyzed. The well that shows the most variation for any concentration is selected for data reduction.
2. For each mean below 2.500 OD, calculate the allowed deviation using the repeatability specification for a 96-well plate of $\pm 0.5\% \pm 0.005$ OD from 0.000 OD to 2.500 OD at 405 nm.
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 0.5% ($1.951 * 0.005$) = 0.0098, which, when added to the 0.005 ($0.0098 + 0.005$) = 0.0148 OD, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specification:

$\pm 0.5\% \pm 0.005$ OD from 0.000 OD to 2.500 OD at 405 nm

Channel-to-Channel Variation and Alignment (Test C)

1. Using the plate prepared for Test A on the previous page, conduct a Turnaround test by reading the plate with the A1 well in the H12 position five times. This test results in two comparisons of each channel to its corresponding channel.
2. Calculate the means of the wells in column 1 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.
3. Next, 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). 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 $\pm 1.0\% \pm 0.010$ OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. ($1.902 * 1.0\% = 0.019 + 0.010 = 0.029$, 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 set 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 one of the lenses.

If the four corner wells are within the repeatability range, the reader is also in alignment.

Perform Liquid Test 3 (“UV” Models Only)

❖ Liquid Test 3 is an optional test offered for sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test verifies operation of the ELx808 at 340 nm and is optional because the reader has good “front-end” linearity throughout its wavelength range.

Required Materials:

- 340 nm filter installed in the reader
- New 96-well, flat-bottom microplates (Corning Costar #3590 is recommended)
- Calibrated hand pipette(s)
- Beakers and graduated cylinder
- Precision balance with readability to 0.01 g
- Buffer solution as described below

Buffer Solution

- Deionized water
- Phosphate-buffered saline (PBS), pH 7.2-7.6, Sigma tablets #P4417 (or equivalent)
- β -NADH Powder (β -Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma bulk catalog number N 8129, or preweighed 10-mg vials, Sigma number N6785-10VL (or BioTek PN 98233). Store the powder according to the guidelines on its packaging.

1. Prepare a PBS solution using the Sigma tablets.
2. In a beaker, mix 50 mL of the PBS solution with 10 mg of the β -NADH powder and mix thoroughly. This is the **100% Test Solution**.

Perform the Test

1. Check the absorbance of a sample of the 100% test solution 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. If low, adjust up by adding β -NADH powder until the high-level solution is at least at the lower end of this range. Do not adjust if slightly high.
2. Prepare the **75% Test Solution** by mixing 15 mL of the 100% Test Solution with 5 mL of the PBS solution.
3. Prepare the **50% Test Solution** by mixing 10 mL of the 100% Test Solution with 10 mL of the PBS solution.
4. Pipette the three solutions into the *new* 96-well microplate:
 - 150 μ L of the 100% Test Solution into all wells of columns 1 and 2
 - 150 μ L of the 75% Test Solution into all wells of columns 3 and 4
 - 150 μ L of the 50% Test Solution into all wells of columns 5 and 6

❖ After pipetting the diluted test solution into the microplate and before reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. If a plate shaker is not available, wait 20 minutes after pipetting the diluted test solution before reading the plate.

5. Read the microplate five times using Normal mode, single wavelength at 340 nm, no blanking (or blank on air).
6. Print the five sets of data, or export the data to an Excel spreadsheet using Gen5. The mathematical computations described below may then be performed and the template kept for future data reduction.

Calculate Repeatability

1. For each well, calculate the mean and standard deviation of the five readings. Only one data set needs to be analyzed for each concentration. The well that shows the most variation for each concentration is selected for data reduction.
2. For each mean calculated in Step 1, calculate the allowed deviation using the repeatability specification for a 96-well plate of $\pm 1.0\% \pm 0.005$ OD from 0.000 to 2.000 OD at 340 nm.

3. For each well, compare the standard deviation calculation in Step 1 with the allowed deviation calculation in Step 2. The standard deviation should be less than the allowed deviation. For example:

Five readings in well A1 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.

To calculate the allowed deviation for well A1, multiply the mean by 1.0% and add 0.005 ($0.8004 * 0.010 + 0.005$) to get 0.013. Since the standard deviation for well A1 is less than 0.013, the well meets the test criteria.

Repeatability Specification:

$\pm 1.0\% \pm 0.005$ OD from 0.000 to 2.000 OD at 340 nm

Calculate Linearity

1. For each of the three Test Solutions, calculate the mean absorbance for the wells containing that solution (mean of wells A1 to H2, A3 to H4, and A5 to H6).
2. Perform a regression analysis on the data to determine if there is adequate linearity. Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R Square value of at least 0.99 is considered adequate.

Chapter 5

Preventive Maintenance

This chapter provides step-by-step instructions for maintaining the ELx808 in top condition, to ensure that the reader continues to perform to specification.

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Recommended Maintenance Schedule



A general Preventive Maintenance (PM) regimen for all ELx808 models includes periodically cleaning all exposed surfaces, inspecting/cleaning the wavelength filters, replacing the lamp, and decontamination. Robotic models should have certain components lubricated. The table below recommends Preventive Maintenance tasks and the frequency with which each task should be performed.




❖ The risk factors associated with your tests may require that some or all of the procedures be performed more or less frequently than shown below.

Task	Frequency			
	Every three months	Every six months	As needed	Before storage or shipment
Clean Exposed Surfaces, p. 103			✓	
Inspect/Clean Wavelength Filters, p. 104	✓			
(Optional) Lubricate Robotic Components, p. 105		✓		
Replace Lamp and Clean Contacts, p. 107			after ~500 hours	
Decontamination, p. 111				✓


Warnings and Precautions

Please read the following before performing any maintenance procedures.

	Warning! Internal Voltage. Turn off and unplug the instrument for all maintenance and repair operations.
	Warning! Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. Eating and drinking while decontaminating instruments is not advised.

	<p>Warning! Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.</p>
	<p>Important! Do not immerse the instrument, spray it with liquid, or use a “wet” cloth. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact BioTek’s Technical Assistance Center.</p>
	<p>Important! Do not soak the keypad – this will cause damage. Moisten a clean cloth with deionized or distilled water and wipe the keypad. Dry immediately with a clean, dry cloth.</p>

Clean Exposed Surfaces

	<p>Important! Turn off and unplug the ELx808 for the cleaning procedure.</p>
---	---

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

- Mild detergent
- Deionized or distilled water
- Clean cotton cloths

To clean the exposed surfaces:

1. Turn off and unplug the instrument.
2. Moisten a clean cotton cloth with water, or with water and the mild detergent. Do not soak the cloth!
3. Wipe the plate carrier, the inside of the plate carrier door, and all exposed surfaces of the instrument.

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

Inspect and Clean the Wavelength Filters



Important! To properly store interference filters for the ELx808 during extended periods of non-use, package the filters in a light-tight envelope or container, away from high humidity and direct sunlight. This will ensure the longest life for the filters. When handling the filters, keep the surfaces clean from fingerprints and debris by simply wiping with a lens tissue or other lint-free cloth.

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

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

To inspect and clean the wavelength filters:

1. If you have not already done so, turn off and unplug the reader.
2. Temporarily tape the reader's plate access door shut.
3. Using your fingertips, locate the seven perimeter screws around the sides and front where the reader's top shroud meets the base. Remove the screws using a screwdriver.

❖ **Tip:** Bring the reader to the edge of the work surface to access the screws without having to turn the reader upside down.

4. Carefully raise the front of the shroud and tip it toward the back of the instrument.
5. The filter wheel is located in the left-rear quadrant inside the reader. Remove the filter wheel by pulling it off its magnetic hub.
6. Inspect the glass filters for speckled surfaces or a halo effect. This may indicate deterioration due to moisture exposure over a long period of time. If you have any concerns about the quality of the filters, contact your BioTek representative.

7. Clean the filters using lens-cleaning tissue moistened with a small amount of isopropyl, ethyl, or methyl alcohol. Ensure that the filters remain in their current locations.
8. Replace the filter wheel on its magnetic hub. Ensure that the peg on the wheel lines up with the notch on the hub, and that the filter wheel is positioned flat against the hub and rotates freely.
9. Close the top shroud and replace the seven perimeter screws.

- ❖ Store filters in a dry environment, such as a desiccator, if they will not be used for an extended period.
- ❖ If the reader continues to fail tests in *Chapter 4, Instrument Qualification*, after you have cleaned the filters and the lamp contacts, or if one or more filters appear to have deteriorated, contact your BioTek Representative or BioTek.

(Optional) Lubricate Robotic Components

This section applies to ELx808 robotic models only. You will need lubricant, BioTek PN 66039.

1. Remove the top shroud of the instrument (see instructions in *Chapter 2*).
2. Lubricate the components as shown in *Figure 8a* and *Figure 8b* every six months, or after 10,000 cycles, according to the instructions in the table below.

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 described on the next page to work the lubricant into the internal drive nut.
D	Lightly lubricate the surface of the roller.
E	Lightly lubricate the underside of the hook-in bracket.

3. Reattach the top shroud.

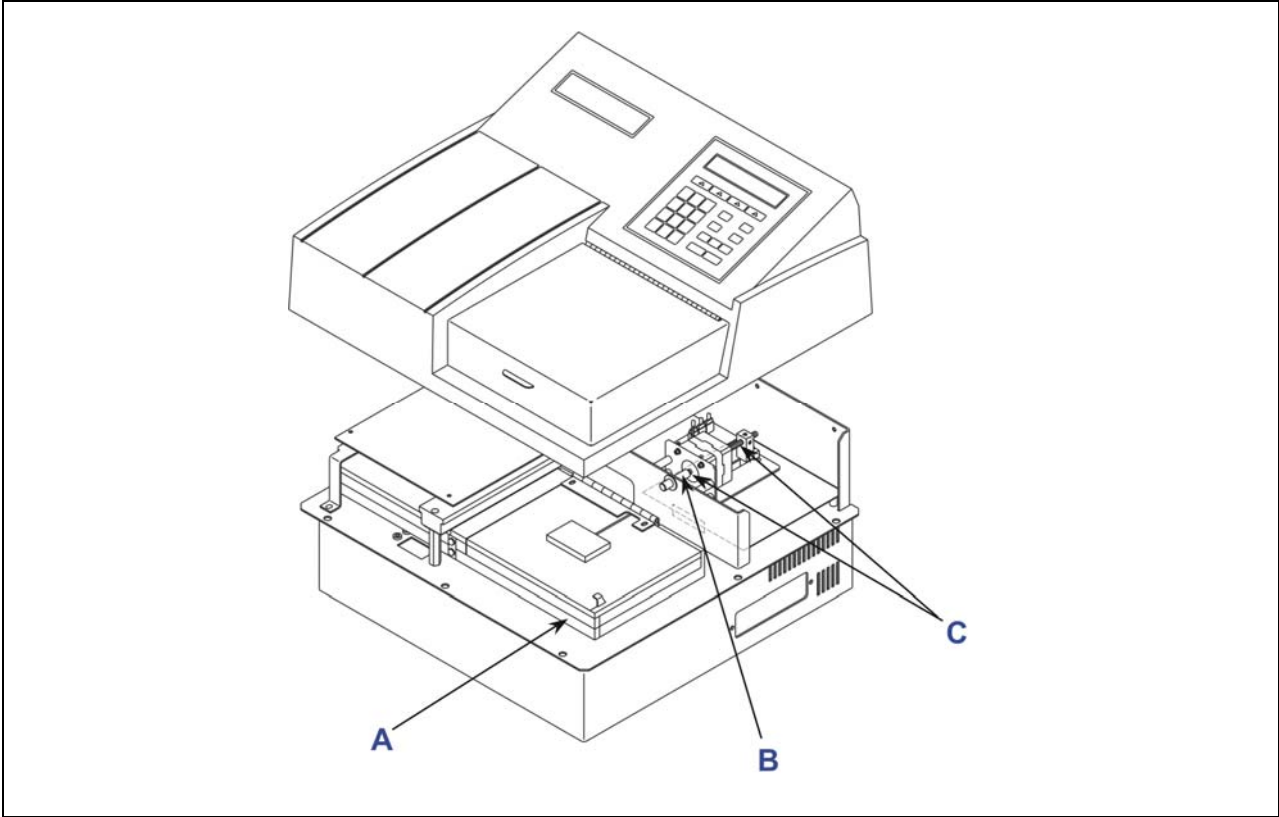


Figure 8a: Location of bracket, motor shaft, and main PCB/motor assembly

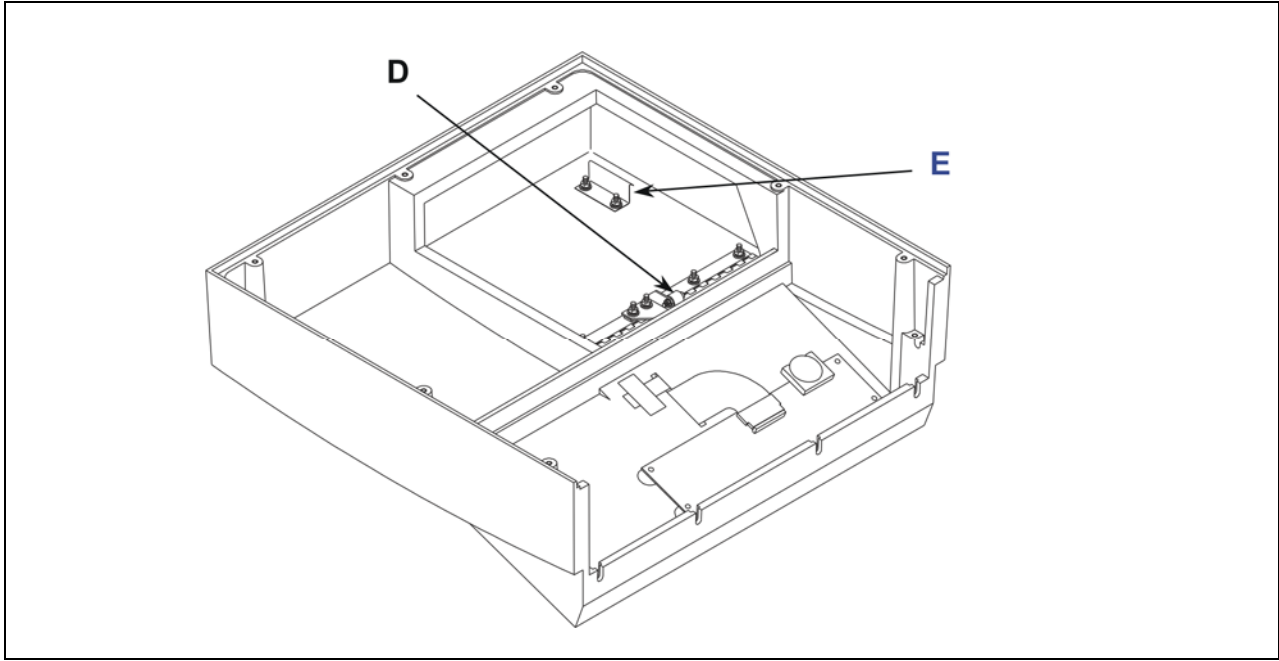


Figure 8b: Roller surface and hook-in bracket

Robotic Motor Adjustment Procedure

After lubrication/reassembly, the robotic door needs to be set to the open and closed positions. Press the following keys to adjust the door.

At the **Main Menu**:

1. Press the **UTIL** soft key.
2. Press the **SETUP** soft key.
3. Press the hidden key between the **Main Menu** and **Previous Screen** keys. You should see the **Door Adjustment** screen.
4. Press the **CLOSE** soft key.
5. 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.
6. Press the **OPEN** soft key.
7. 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.





Important! Do not open the door to its maximum open position.

8. Press the **Main Menu** key.
9. Press the **CLOSE** soft key. This will close the door. The door should rest closed on the top cover.

Replace the Lamp and Clean the Contacts

❖ There are two types of lamp assemblies. Steps 7 and 8 only pertain to the newer assembly. Review the photos below to determine which instructions apply to your reader.

The reader's lamp, on average, should provide 500 hours of total service before it needs to be replaced. This equates to approximately six months to one year of use under routine conditions. The intensity of the lamp will slowly drop over time until the reader's run-time self-check detects a low signal and warns the user with a displayed error code. The bulb should be replaced at this time (PN 3400508), and the electrical contacts in the lamp socket cleaned.

	<p>Warning! Hot surface. The lamp is hot when the instrument is turned on. Turn off and unplug the reader. Allow the lamp to cool down before attempting to replace it.</p>
	<p>Important! Oxide coating on the electrical contacts can reduce lamp output. Clean the contacts in the socket whenever the lamp is replaced (approximately every 500 hours).</p>

You will need:

- Tape
- Screwdriver
- Cotton swabs or pencil eraser
- Isopropyl alcohol
- Lamp, BioTek PN 3400508

To replace the lamp:

1. Turn off and unplug the reader. Allow the lamp to cool for at least ten minutes.
2. Temporarily tape the reader's plate access door shut.
3. Using your fingertips, locate the seven securing (perimeter) screws around the sides and front where the reader's top shroud meets the base. Remove the screws using a screwdriver.

❖ **Tip:** Bring the reader to the edge of the work surface to access the screws without having to turn the reader upside down.

4. Carefully raise the front of the shroud and tip it toward the back of the instrument.
5. The lamp is located in the left rear quadrant inside the reader. Unplug the lamp connector from the circuit board.



Important! Do not touch the glass lens in the lamp bracket.

6. The lamp bracket is attached to the side of the instrument with two screws, which are accessed from the outer left side of the instrument. Using a screwdriver, loosen (do not remove) these two screws. Slide the bracket out of the instrument.

7. **(Lamp Assembly 1 Only)** Carefully unscrew the two thumbscrews on the lamp clamp that holds the bulb in the bracket. Remove the screws, springs, washers, and lamp clamp and set them aside.
8. **(Lamp Assembly 1 Only)** Slide the rubber O-ring down the bulb socket away from the bulb.
9. Remove the bulb from its socket by pushing the socket and the bulb together and rotating the socket counterclockwise.
10. Clean the two contacts in the socket, using a cotton swab moistened with isopropyl alcohol, and allow them to dry for a few minutes. An alternative cleaning technique would be to rub the contacts with a pencil eraser.

To **install** the lamp, reverse steps 1 through 9. Keep your fingers out of the reflector interior and away from the bulb. Ensure that the bulb is set flat against its housing before reinstallation in the instrument.

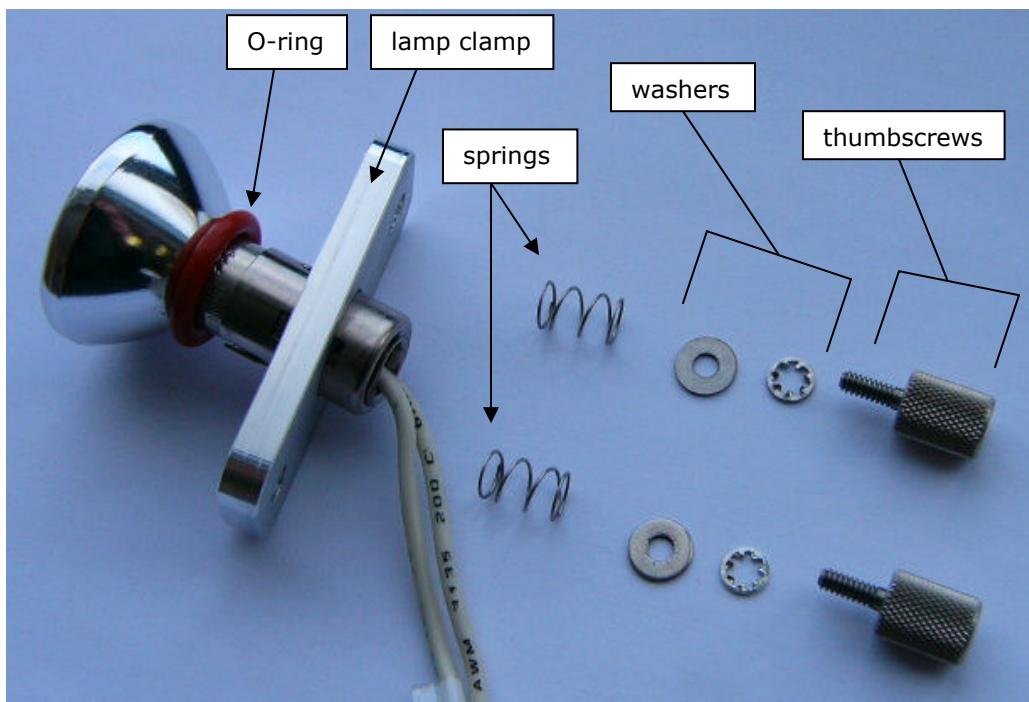


Figure 9: New lamp hardware for the ELx808 (**Lamp Assembly 1**)



Figure 10: New lamp hardware for the ELx808, assembled (Lamp Assembly 1)

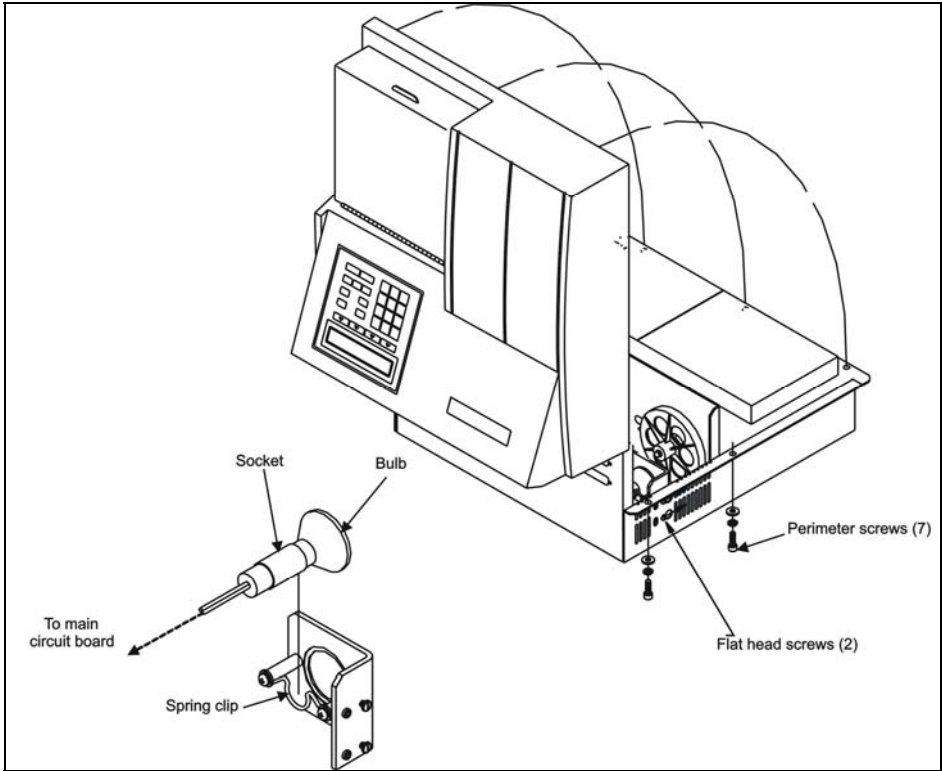


Figure 11: Location of the lamp in the ELx808 (Lamp Assembly 2)





Decontamination

Purpose

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

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



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

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

Tools and Supplies

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

Decontamination Procedure

	<p>Warning! The bleach solution is caustic; wear gloves and eye protection when handling the solution.</p> <p>Do not immerse the instrument, spray it with liquid, or use a “wet” cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department.</p> <p>Do not soak the keypad – this will cause damage.</p>
	<p>Important! Turn off and unplug the instrument for all decontamination and cleaning operations.</p>

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.5% sodium hypochlorite (bleach). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.
 - Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution.

3. Moisten a clean, lint-free cloth with the bleach solution or alcohol. **Do not soak the cloth!** Wipe down the carrier and all exposed instrument surfaces with the bleach solution.
 - Wipe the keypad (do not soak). Wipe again with a clean cloth moistened with deionized or distilled water. Dry immediately with a clean, dry cloth.
 - Wipe the plate carrier, the inside of the carrier door, and all exposed surfaces of the instrument.
4. Wait 20 minutes. Moisten a cloth with deionized or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
5. Use a clean, dry lint-free cloth to dry all wet surfaces.
6. Reassemble the instrument as necessary.
7. Discard the used gloves and cloths, using a Biohazard trash bag and an approved Biohazard container.

Chapter 6

Troubleshooting and Error Codes

This chapter provides a list of Error Codes that may appear on the ELx808 or in Gen5, and their probable causes.

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Terms Used in the Error Codes Tables	116
Error Codes (General)	117
Error Codes (Fatal)	121

Overview

An error code that appears on the display of the ELx808 (during instrument control of the reader), or in Gen5 software (during PC control of the reader) is displayed as a four-digit identifier. The first digit will be 0, 1, 2, or A.

- 0, 1, or 2 denote a noncritical or “General” error, which means that it is still possible for the ELx808 to communicate with the controlling software and run the reader system test. See **Error Codes (General)** beginning on page 117.
- A denotes a more serious error with the memory or processing, which requires that the ELx808 be turned off and then powered up before any diagnostics can be performed. If the error reappears, call BioTek TAC for troubleshooting assistance (see Chapter 1). See **Error Codes (Fatal)** on page 121.

Diagnostics

If an error code is displayed, a System Self-Test should be conducted for diagnostic purposes. Refer to the following *brief* instructions for performing the Self-Test:

- From the Main Menu screen on the ELx808 display, select **UTIL > TESTS > SYSTEM**.
- In Gen5, access the System menu and select **Diagnostics > Run System Test**.

Terms Used in the Error Codes Tables

- **Air Blank:** A full light reading through a filter with no plate in the light path.
- **Dark Current:** A reading taken with the light blocked to measure background light levels in the reading chamber. Also used as a measure of background electronic noise within the measurement circuit.
- **Gain:** An automatic electronic adjustment to the measurement circuit. The gain adjustment compensates for changing light levels or filter variations. For example, if the lamp output decreases slightly, the gain will increase to make up the difference.
- **Axis:** Refers to a motor that positions the filter wheel or plate carrier.
- **Offset:** A numerical limit, usually a range. For example, if the gain fails an offset test, it may be too high or too low.

Error Codes (General)

Code	Probable Cause
0200 0201	<p>Plate carrier did not find the home sensor.</p> <p>Filter wheel motor did not find the home sensor; filter wheel not installed or detached from hub.</p> <p>Errors 0200 and 0201 indicate that a motor was not able to move to its "home" position as registered by feedback from an optical sensor.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Carrier movement may be limited so that the optical sensor cannot be interrupted. Check the carrier path for obstructions. • Filter wheel not installed. The wheel is shipped in a separate envelope and may not be installed or may have broken free of the magnetic hub. • Filter wheel movement may be impeded. Remove the wheel to confirm if a loose filter is impeding the movement of the wheel.
0300 0301	<p>Carrier failed to find light beam.</p> <p>Filter wheel did not find home.</p> <p>Errors 0300 and 0301 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. This error is usually only seen during the service Auto Cal sequence.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Carrier: Lamp may be out or blocked by a faulty filter wheel. A loose belt or a loose motor pulley may cause the carrier to ignore movement instructions. • Filter Wheel Motor: The filter wheel is missing or loose or a loose filter may be impeding filter wheel movement.
0400 0401	<p>Carrier x-axis failed position verify.</p> <p>Filter Wheel motor failed position verify.</p> <p>Errors 0400 and 0401 indicate that an axis failed its Position Verify test. After moving a predefined number of steps from a home position, the motor should return to the home position in the proper amount of time and steps. If the axis moves back to its home position in the wrong amount of time or in too few or too many steps, the test fails.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Mechanical obstruction. • Carrier belt or pulley slipping. • Loose filter wheel hub. • Loose filter may be impeding filter wheel movement. • Defective motor drive circuit or 12-volt power problem.

Code	Probable Cause
0500 0502 to 0506	<p>Filter is not installed.</p> <p>0500 indicates an empty filter location on the filter wheel, or that the filter wheel is not installed.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • All filter locations must have either a filter or a filter blank (BTI PN 3122037) installed. The reader may also display this error if the filter wheel is not installed.
0601 to 0606	<p>Filters 1 through 6 Gain out of range.</p> <p>Errors 0601 through 0606 indicate that the gain for a specific filter is out of range. Gain out of range indicates that the lamp is out, or that a particular filter absorbs or transmits entirely incorrect light levels.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Lamp out. Check that the lamp is lit when the unit is ON. Replace lamp if necessary. • A missing, defective, or misplaced filter or filter blank will cause this error. Observe that the filters in the wheel match the filters programmed in the software. Look for obvious physical defects in the filters.
0700	<p>Reader failed noise test.</p> <p>Error 0700 indicates significant variations in background electronic noise were detected when blocking the light and increasing the Gain to maximum.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened. • Ambient light leak. Check that the plate chamber door is closed to keep out ambient light. • Missing or loose filter allowing unintended lamp light into the measurement chamber. Check filter wheel for empty positions. • Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.
0800	<p>Reader failed offset test.</p> <p>Error 0800 indicates electronic signal detected is outside of acceptable limits at maximum gain when blocking the light.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened. • Ambient light leak. Check that the plate chamber door is closed to keep out ambient light. • Missing or loose filter allowing unintended lamp light into the measurement chamber. Check filter wheel for empty positions. • Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.

Code	Probable Cause
0900	<p>Read time dark value out of range.</p> <p>Error 0900 indicates that the dark current value taken during the current read is significantly different than the same reading during the power-up self-check.</p> <p>Probable cause:</p> <ul style="list-style-type: none"> • Read chamber door has opened or closed relative to when the unit was powered ON. Close door and reboot reader to correct. • Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened. • Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.
0A00, 0A01 to 0A05	<p>Read time air blank out of range.</p> <p>Error 0A00 indicates that the blank (full signal) reading taken during the current read has changed significantly from the same reading taken during the power-up self-check.</p> <p>Probable cause:</p> <ul style="list-style-type: none"> • Lamp has failed since power-up. Check that the lamp is lit and replace if necessary. • Analog PCB failure.
0C00	<p>Printer time out error.</p> <p>Error 0C00 indicates that the time allotted for the instrument to make a valid connection to a printer has expired.</p> <p>Probable cause:</p> <ul style="list-style-type: none"> • Printer out of paper, not connected, or not on-line.
0D00	<p>Reader failed calibration checksum test.</p> <p>Internal software corruption.</p>
0E00, 0E01 to 0E05	<p>Wavelength not detected in reader's filter table.</p> <p>Compare filters' wavelengths to the filter table.</p>
0F00 to 0F86	<p>Reader's filter or channel signal is out of specified range.</p> <p>Check for lamp failure, and/or replace lamp.</p>
1000	<p>Required reader configuration data missing.</p> <p>Re-download software basecode and assays.</p>
1100	<p>Failed configuration checksum test.</p> <p>Re-download software basecode and assays.</p>
1200	<p>Calibration data missing.</p>
1201	<p>Calibration value missing</p> <p>Filter table was updated but System Test was not run. Run a system test.</p>
1300	<p>Motor not homed.</p> <p>Autocal error, see error 0200.</p>

Code	Probable Cause
1400	<p>Assay incubation error.</p> <p>Assay requires incubation but the instrument does not appear to have an incubator.</p>
1500	<p>Incubator failed to hold temperature within tolerances during the assay.</p>
1600	<p>Computer control assay definition error.</p> <p>This error is used only for software development.</p>
1700	<p>Kinetic interval too short for selected options.</p>
1800	<p>Too many kinetic intervals selected.</p>
2311 to 2386	<p>Lamp unstable.</p> <p>Problem with lamp output during power-up or while reading a plate. If a 23xx error is displayed during a System Test or during startup, the last digit indicates the filter wheel position. If a 23xx error is displayed during a read, the last digit indicates the filter number in the list of filters used for the read.</p> <p>Probable causes:</p> <ul style="list-style-type: none"> • Defective or old lamp; replace the lamp. • Lamp not sitting correctly in lamp holder (always remove the holder to install the lamp, then insert the holder with the lamp back into the reader). • Lamp contacts dirty. Clean with ethanol (refer to Chapter 5, Preventive Maintenance). • Lamp cable needs upgrade. • Filter degradation. • Filter not seated properly in filter wheel. • Lower lenses for all or some channels failing and need replacement. • Electrical noise or grounding issue. • Shortened lamp life from performing long kinetic runs every day or leaving the reader on 24 hours a day. • When using 340 nm filters, the analog gain must be increased.

Error Codes (Fatal)

These errors indicate conditions that require immediate attention. If a fatal error is displayed, contact BioTek's Technical Assistance Center for further instructions (refer to the *Product Support & Service* section in *Chapter 1*).

Code	Description
A100	Task control block not available.
A200	Read already in progress.
A300	<Device> not available.
A400	Failed code checksum test on powerup.
A500	DR steps alloc/free error < assay num>.
A600	Data flash write timed out.
A700	Data flash readback did not match write (test) <chip>.
A800	Code flash write timed out.
A900	Memory Manager corruption detected. Power instrument off, then back on.

Appendix A

Sample Reports

The following pages contain examples of reports that can be generated and/or printed from the ELx808 when it is running in standalone mode. For details on how to print these reports, refer to **OUTPUT Options** in Chapter 2, Installation. In addition, an Assay List, Assay Definition, Map, and Result can be printed by choosing REPORT from the Main Menu screen.



Important! The 'OUT' indication on reports means the OD for an individual well, or the average OD for a group of wells, falls outside the minimum/maximum OD range defined for the assay. For the Quick Read assay on the ELx808, this range is -3.0 OD to +3.0 OD. For assays defined onboard by a user (Assay02-Assay55), it is -4.0 OD to +4.0 OD.

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Samples with Calls on Matrix Report

Bio-Tek Instruments

Assay: ASSAY 01

Date: 07/23/96

Lot: _____

Wavelength: 405

Time: 03:05:35AM

Operator: _____

Temp: _____

Plate ID: SONTA

COMMENTS

OUT indicates an out of range value was read.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
CALL		POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	0.182	0.072	0.073	0.077	0.071	0.075	0.073	0.074	0.073	0.075	0.072	0.076
Well	STD1	SMP5	SMP13	SMP21	SMP29	SMP37	SMP45	SMP53	SMP61	SMP69	SMP77	SMP85
RSLT												
B												
CALL		POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	0.529	0.076	0.084	0.085	0.096	0.073	0.073	0.069	0.072	0.070	0.070	0.076
Well	STD2	SMP6	SMP14	SMP22	SMP30	SMP38	SMP46	SMP54	SMP62	SMP70	SMP78	SMP86
RSLT	0.483											
C												
CALL		POS	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS	POS
CalcOD	0.721	0.070	0.351	0.443	0.376	0.076	0.074	0.077	0.071	0.076	0.081	0.066
Well	STD3	SMP7	SMP15	SMP23	SMP31	SMP39	SMP47	SMP55	SMP63	SMP71	SMP79	SMP87
RSLT	0.686		0.294	0.391	0.321							
D												
CALL		POS	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG
CalcOD	0.749	0.081	OUT	OUT	OUT	0.078	0.074	0.079	0.074	0.072	0.076	0.683
Well	STD4	SMP8	SMP16	SMP24	SMP32	SMP40	SMP48	SMP56	SMP64	SMP72	SMP80	SMP88
RSLT	0.715											0.646
E												
CALL	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	0.068	0.070	0.093	0.101	0.081	0.078	0.078	0.074	0.074	0.071	0.074	0.072
Well	SMP1	SMP9	SMP17	SMP25	SMP33	SMP41	SMP49	SMP57	SMP65	SMP73	SMP81	SMP89
RSLT												
F												
CALL	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG
CalcOD	0.083	0.069	0.075	0.070	0.072	0.079	0.082	0.085	0.074	0.069	0.078	0.671
Well	SMP2	SMP10	SMP18	SMP26	SMP34	SMP42	SMP50	SMP58	SMP66	SMP74	SMP82	SMP90
RSLT												0.632
G												
CALL	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	0.073	0.070	0.073	0.072	0.076	0.072	0.074	0.080	0.076	0.072	0.072	0.073
Well	SMP3	SMP11	SMP19	SMP27	SMP35	SMP43	SMP51	SMP59	SMP67	SMP75	SMP83	SMP91
RSLT												
H												
CALL	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS
CalcOD	0.072	0.075	0.073	0.074	0.081	0.069	0.076	0.069	0.738	0.076	0.075	0.072
Well	SMP4	SMP12	SMP20	SMP28	SMP36	SMP44	SMP52	SMP60	SMP68	SMP76	SMP84	SMP92
RSLT									0.704			

Curve Fit Report

Bio-Tek Instruments

Assay: ASSAY 01

Date: 07/23/96

Lot: _____

Wavelength: 405

Time: 03:05:35AM

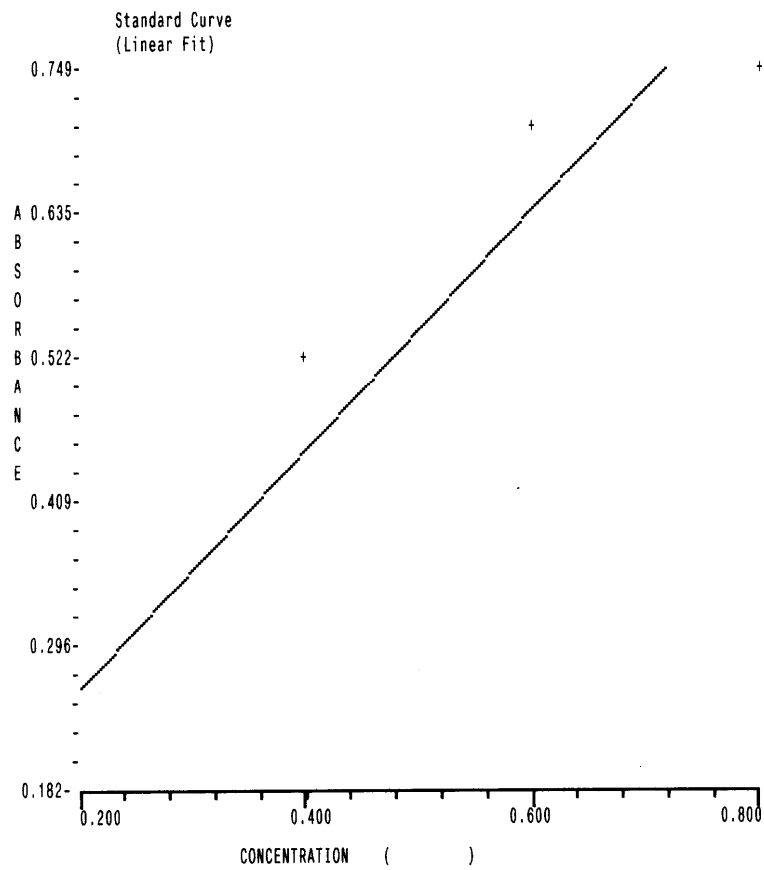
Operator: _____

Temp: _____

Plate ID: SONTA

COMMENTS

OUT indicates an out of range value was read.



Curve: $y = Ax + B$
Coefficients: A=0.946 B=0.073
R-Sqr: 0.8752

Samples with Calls on Column Report

Bio-Tek Instruments

Assay: ASSAY 01

Date: 07/23/96

Lot: _____

Wavelength: 405

Time: 03:05:35AM

Operator: _____

Temp: _____

Plate ID: SONTA

COMMENTS

OUT indicates an out of range value was read.

Interpretation of Results

GREYZONE=0.00 =0.000
CUTOFF=.2 =0.200

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

SMP : SMP>(CUTOFF+(CUTOFF*GREYZONE)) : NEG

SMP : SMP<(CUTOFF-(CUTOFF*GREYZONE)) : POS

Well ID	BldOD	CalcOD	Call	Pred Conc	RSLT	Std Dev	CV%	Notes:
A01 STD1	0.182	0.182		0.200				
B01 STD2	0.529	0.529		0.400	0.483			
C01 STD3	0.721	0.721		0.600	0.686			
D01 STD4	0.749	0.749		0.800	0.715			
E01 SMP0001	0.068	0.068	POS					
F01 SMP0002	0.083	0.083	POS					
G01 SMP0003	0.073	0.073	POS					
H01 SMP0004	0.072	0.072	POS					
A02 SMP0005	0.072	0.072	POS					
B02 SMP0006	0.076	0.076	POS					
C02 SMP0007	0.070	0.070	POS					
D02 SMP0008	0.081	0.081	POS					
E02 SMP0009	0.070	0.070	POS					
F02 SMP0010	0.069	0.069	POS					
G02 SMP0011	0.070	0.070	POS					
H02 SMP0012	0.075	0.075	POS					
A03 SMP0013	0.073	0.073	POS					

Column Report without Samples

Bio-Tek Instruments

Assay: ASSAY 01	Date: 07/29/96	Lot: _____
Wavelength: 405	Time: 09:55:19AM	Operator: _____
	Temp: _____	Plate ID: _____

COMMENTS

OUT indicates an out of range value was read.

Interpretation of Results

GREYZONE=0.25 =0.250
 CUTOFF=.1 =0.100

SMP : $SMP \leq (CUTOFF + (CUTOFF * GREYZONE)) \text{ AND } (SMP \geq (CUTOFF - (CUTOFF * GREYZONE)))$: EQUIV
 SMP : $SMP > (CUTOFF + (CUTOFF * GREYZONE))$: NEG
 SMP : $SMP < (CUTOFF - (CUTOFF * GREYZONE))$: POS

Well ID	B1kd00	Calc00	Call	Pred Conc	RSLT	Std Dev	CV%	Notes:
A01 STD1	0.184	0.184						
B01 STD2	0.529	0.529						
C01 PC	0.723	0.723						
D01 NC	0.746	0.746						

End of Report

Panel Report

Bio-Tek Instruments

Panel

Date:07/31/96

Lot: _____

Time:02:24:01PM

Operator: _____

Temp:

Plate ID: _____

COMMENTS

Specimen	Assay	Well	BlkdOD	CalcOD	Call	RSLT	Std Dev	CV%	Notes:
SMP0001	ASSAY 01	F01	-.006	-.006					
	ASSAY 02	B05	0.000	0.000					
SMP0002	ASSAY 01	G01	0.002	0.002					
	ASSAY 02	C05	0.007	0.007					
SMP0003	ASSAY 01	H01	0.005	0.005					
	ASSAY 02	D05	0.004	0.004					
SMP0004	ASSAY 01	A02	0.002	0.002					
	ASSAY 02	E05	0.012	0.012					
SMP0005	ASSAY 01	B02	-.002	-.002					
	ASSAY 02	F05	0.010	0.010					
SMP0006	ASSAY 01	C02	0.005	0.005					
	ASSAY 02	G05	-.001	-.001					
SMP0007	ASSAY 01	D02	-.001	-.001					
	ASSAY 02	H05	0.002	0.002					
SMP0008	ASSAY 01	E02	0.009	0.009					
	ASSAY 02	A06	0.004	0.004					
SMP0009	ASSAY 01	F02	0.006	0.006					
	ASSAY 02	B06	0.007	0.007					
SMP0010	ASSAY 01	G02	-.006	-.006					
	ASSAY 02	C06	0.010	0.010					
SMP0011	ASSAY 01	H02	-.005	-.005					
	ASSAY 02	D06	0.004	0.004					
SMP0012	ASSAY 01	A03	-.00	-.00					
	ASSAY 02	E06	0.003	0.003					
SMP0013	ASSAY 01	B03	0.002	0.002					
	ASSAY 02	F06	-.001	-.001					
SMP0014	ASSAY 01	C03	-.005	-.005					
	ASSAY 02	G06	0.001	0.001					
SMP0015	ASSAY 01	D03	-.002	-.002					

Assay Detail Report

03:34PM 11/11/03

NAME: ASSAY 55
 READ TYPE: ENDPOINT
 WAVELENGTH: 450
 BLANK MAP: AIR
 AVERAGE STANDARDS? NO
 CONCEN: STD1 6.2
 STD2 12.5

CURVE-FIT TYPE: LINEAR
 EDIT STD OUTLIERS: NONE
 X/Y AXIS TYPE: LIN
 EXTRAPOLATE UNKNOWNNS? NO

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP		
B	STD1	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP		
C	STD2	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP		
D	STD2	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP		
E	PC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP		
F	PC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP			
G	NC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP			
H	NC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP			

Assay List

Assay List**Version: 2.82**

1	_Quick Read
2	ASSAY 02
3	ASSAY 03
4	ASSAY 04
5	ASSAY 05
6	ASSAY 06
7	ASSAY 07
8	ASSAY 08
9	ASSAY 09
10	ASSAY 10
11	ASSAY 11
12	ASSAY 12
13	ASSAY 13
14	ASSAY 14
15	ASSAY 15
16	ASSAY 16
17	ASSAY 17
18	ASSAY 18
19	ASSAY 19
20	ASSAY 20
21	ASSAY 21
22	ASSAY 22
23	ASSAY 23
24	ASSAY 24
25	ASSAY 25
26	ASSAY 26
27	ASSAY 27
28	ASSAY 28
29	ASSAY 29
30	ASSAY 30

Appendix B

Instructions for Programming a New Assay

This appendix provides two sample assay kit instructions with step-by-step instructions for programming each assay: one with a ratio transformation calculation and a POS/NEG cutoff determination, and another with a standard curve.

These examples are based on real assays; however, the results have not been verified on any clinical kits. The kit instructions are provided so that users can see how it is possible to translate the kit wording into an ELx808 assay program. For clarity, only the user menu choices from the reader screens are shown. See Chapter 3 for details.



Important! The user is responsible for programming the reader properly according to their specific kit instructions, and for verifying that the calculations are performed correctly.

Sample ANA Screen Enzyme Immunoassay Kit	132
Sample Anticardiolipin IgG Enzyme Immunoassay Kit	136

Sample ANA Screen Enzyme Immunoassay Kit

This sample demonstrates the use of transformations and cutoffs.

Intended Use

This assay is designed for the in vitro screening of serum for the presence of specific IgG antinuclear antibodies (ANAs), to aid in diagnosing certain systemic rheumatic diseases. Sufficient materials are supplied to allow a maximum of 93 samples to be screened in single, with a positive, cutoff, and negative controls.

Background

Antinuclear antibodies occur in a large number of patients with systemic rheumatic diseases. These diseases are characterized by the presence of one or more ANAs.

Sera positive on this ANA kit should be tested for the specific autoantibodies indicative of the various systemic rheumatic diseases.

Principle of the Assay

Microwells are pre-coated with purified antigens. The prediluted controls and diluted patient samples are added to the wells, and autoantibodies recognizing one or a combination of antigens bind during the first incubation. After washing the wells to remove all unbound proteins, IgG conjugate is added. The conjugate binds to the captured autoantibody, and the excess unbound conjugate is removed by a further wash step. Substrate is added that causes a blue reaction, thereby exposing the bound conjugate, and producing an intensity proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end-point color, which is read at 450 nm.

Materials Supplied

- Instruction Leaflet: Giving full assay details.
- QC Certificate: Indicating the expected performance of the batch.
- ANA Coated Wells: 12 x 8 well strips coated with purified antigens.
- Type III Wash Buffer 20x Concentrate: 1 bottle containing 50 mL of a concentrated buffer for washing the wells.
- Type III Sample Diluent: 2 bottles containing 50 mL of buffer for sample dilution. Ready to use.
- ANA Positive Control: 1 bottle containing 1.8 mL of diluted stabilized serum. Ready to use.

- ANA Cutoff Control: 1 bottle containing 1.8 mL of diluted stabilized serum. Ready to use.
- ANA Negative Control: 1 bottle containing 1.8 mL of diluted stabilized serum. Ready to use.
- ANA Conjugate: 1 bottle containing 12 mL of peroxidase labeled antibody to IgG. Colored red, ready to use.
- TMB Substrate: 1 bottle containing 14 mL TMB substrate. Ready to use.
- Stop Solution: 1 bottle containing 14 mL of 3M Phosphoric acid. Ready to use.

Additional Materials and Equipment – Not Supplied

- Automatic Microplate Plate Washer: This is recommended; however, plate washing can be performed manually.
- Microplate Reader: Capable of measuring optical densities at 450 nm referenced on air.
- Distilled or Deionized Water: This should be of the highest quality available.
- Calibrated Micropipettes: For dispensing 1000, 100, and 10 μ L.
- Multichannel Pipette: Recommended for dispensing 100 μ L volumes of conjugate, substrate, and stop solution.
- Glass/Plastic Tubes: For sample dilution.

Quality Control

For an assay to be valid, all the following criteria must be met:

- Cutoff as well as Positive and Negative controls must be included in each run.
- The OD of the cutoff and the ANA result of the Negative and Positive Controls should be in the ranges specified on the QC Certificate.
- For example: The absorbance of the Positive Control must be greater than 1.200 OD. The absorbance of the Negative Control must be less than 0.300 OD.

❖ If the above criteria are not met, the assay is invalid and the test should be repeated.

Calculation of the Sample Results

Use the following formula to calculate the ANA result for each sample:

$$\frac{\text{Control or sample OD}}{\text{Cutoff control OD}} \times 10 = \text{Control or sample value (U/mL)}$$

Expected Values

The normal range was determined on serum from 200 normal adult blood donors. The cutoff control has been set at a point equivalent to the upper normal limit, or a cutoff level of 10 U/mL.

The ranges are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

ANA Result	Interpretation
< 10.0	Negative
> 10.0	Positive

Programming the Assay on the ELx808

From the Main Menu, press **DEFINE**. Enter the assay number and edit the name if desired. At the **DEFINE** menu:

STEP	COMMENTS
To program the reading method, press:	
METHOD:	
READ TYPE (Endpoint, Kinetic, or Scan): Endpoint	
DELAY FIRST READ:	
If incubator model: INCUBATION TEMP (Ambient or Temperature):	
WAVELENGTH (Single or Dual): Single	
MEASURE (Wavelength[s] to use): 450	
SHAKE (First, Every, or None):	
To program the plate map, press:	
MAP	
AUTO	"Auto" mapping is normally preferred, because it fills in the well IDs logically and automatically after determining which direction to map and how many wells to fill.
DOWN	Maps the wells down the column.
DOWN	Locates replicates in a vertical orientation down the column.
A01	Begins mapping at well location A01.
BLANK MAP: AIR	Choose to blank on "AIR" if no blank wells are required.
NUMBER STDS: 00	

STEP	COMMENTS
<p>REUSE STANDARD CURVE?</p> <p>NUMBER CTLS: 03</p> <p>CONTROL 1: PC</p> <p>CONTROL 2: CTL1</p> <p>CONTROL 3: NC</p> <p>NUMBER OF REPLICATES</p> <p>PC: 01</p> <p>CTL1: 03</p> <p>NC: 01</p> <p>SAMPLES: 91</p> <p>SAMPLE REPLICATES: 01</p> <p>Use the MAP and MATH keys to create the control validation formula:</p> <p>FORMULA</p> <p>VAL</p> <p>CONTROL:</p> <p>PC;x > 1.200</p> <p>NO. OF REPLICATES: 01</p> <p>NC;x < 0.300</p> <p>NO. OF REPLICATES: 01</p>	<p>Can only be programmed in assays 31-55. Cannot be reused on panels.</p> <p>Suggested for the cutoff control.</p> <p>User-defined; recommendation is to fill the entire plate.</p> <p>When defining control validation formulas, use "PC" to indicate the criterion for each of the PC replicates, and "PC;x" to indicate the average of the Positive Control replicates.</p>
To create the plate transformation for CALC, divide the ANA result by a cutoff standard.	
<p>FORMULA</p> <p>*MORE</p> <p>TRANS - VAR</p> <p>SCOPE VARIABLE (SMP or OD)</p> <p>FORMULA</p> <p>CTL1;X</p> <p>TRANS:</p> <p>FORMULA: (OD/TVAR)*10</p>	<p>Select OD to advance to the formula definition screen and define the transformation variable (Tvar)</p> <p>Defining TRANS VAR = CTL1;X isolates the OD value for CTL1;X for use in transformation.</p> <p>Converts all OD values on plate to "ANA Result" per the kit instructions.</p>
To define a cutoff formula for Positive and Negative calls:	
<p>FORMULA</p> <p>CUTOFF: 10.0</p> <p>GREYZONE: 00%</p> <p>SAMPLE > CUTOFF: POS</p>	

Sample Anticardiolipin IgG Enzyme Immunoassay Kit

This sample demonstrates the use of standard curve and cutoffs.

Intended Use

This assay is intended for the in vitro measurement of IgG anticardiolipin antibodies in serum, as an aid in the diagnosis of antiphospholipid syndrome (APS). Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a standard curve and positive and negative controls.

Background

Anticardiolipin antibodies are found in a wide range of conditions either transiently, in some infectious diseases, or more persistently in autoimmune diseases such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS). Anticardiolipin antibodies have also been associated with a range of clinical conditions including fetal loss, endocarditis, stroke, heart attack and autoimmune haemolytic.

Principle of the Assay

Microwells are pre-coated with cardiolipin and cofactor. Standards, controls, and patient samples are added to the wells, and autoantibodies recognizing cardiolipin bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured antibody, and the excess unbound conjugate is removed by a further wash step. Substrate is added that causes a blue reaction, thereby exposing the bound conjugate and producing an intensity proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end-point color, which is read at 450 nm.

Materials Supplied

- Instruction Leaflet: Giving full assay details.
- QC Certificate: Indicating the expected performance of the batch.
- Cardiolipin Coated Wells: 12 break-apart 8-well strips coated with bovine cardiolipin antigen. The plate is packaged in a re-sealable foil bag containing two desiccant pouches.
- Type II Sample Diluent: 2 bottles containing 50 mL of buffer for sample dilution. Colored yellow, ready to use.

- Type II Wash Buffer (20x Concentrate): 1 bottle containing 50 mL of a 20-fold concentrated buffer for washing the wells.
- Cardiolipin IgG Standards: 5 bottles each containing 1.2 mL of diluted serum, with the following concentrations of anticardiolipin autoantibody: 100, 50, 25, 12.5, 6.25 GPL U/mL. Ready to use.
- The standard set is calibrated against the Louisville APL reference preparation (see Assay Calibration, page 138).
- Cardiolipin IgG Positive Control: 1 bottle containing 1.2 mL of diluted serum. The expected value is given on the QC certificate. Ready to use.
- Cardiolipin Negative Control: 1 bottle containing 1.2 mL of diluted serum. The expected value is given on the QC certificate. Ready to use.
- Cardiolipin IgG Conjugate: 1 bottle containing 12 mL of purified peroxidase labeled antibody. Colored red, ready to use.
- TMB Substrate: 1 bottle containing 14 mL TMB substrate. Ready to use.
- Stop Solution: 1 bottle containing 14 mL of 3M phosphoric acid. Ready to use.

Additional Materials and Equipment – Not Supplied

- Automatic Microplate Plate Washer: This is recommended; however, plate washing can be performed manually.
- Microplate Reader: Capable of measuring optical densities at 450 nm referenced on air.
- Distilled or Deionized Water: This should be of the highest quality available.
- Calibrated Micropipettes: For dispensing 1000, 100, and 10 μ L.
- Multichannel Pipette: Recommended for dispensing 100 μ L volumes of conjugate, substrate, and stop solution.
- Glass/Plastic Tubes: For sample dilution.

Quality Control

For an assay to be valid, all the following criteria must be met:

- Standards and the positive and negative controls must be included in each run.
- The values obtained for all the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the standard curve, shown on the QC Certificate.
- If the above criteria are not met, the assay is invalid and the test should be repeated.

Calculate Mean Optical Densities (for assays run in duplicate only)

For each standard, control, and sample, calculate the mean OD of the duplicate readings. The user must verify that the percentage coefficient of variation (%CV) for each duplicate OD is less than 15.0%.

Plot Calibration Curve

The calibration curve can be plotted either automatically or manually as follows by plotting the anticardiolipin autoantibody concentration on the log scale against the OD on the linear scale for each calibrator:

- **Automatic** - Use appropriately validated software, and the curve fit that best fits the data.
- **Manual** - Using log/linear graph paper, draw a smooth curve through the points (not a straight line or point to point).

Treatment of Anomalous Points

If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

Calculation of Autoantibody Levels in Controls and Samples

Read the level of the anticardiolipin autoantibody in the controls and diluted samples directly from the calibration curve. The control values should fall within the range given on the QC Certificate.

❖ The standard values have been adjusted by a factor of 100 to account for a 1:100 sample dilution. No further correction is required.

Assay Calibration

The assays are calibrated against the Louisville reference LAPL-GM-100. One GPL unit is defined as the cardiolipin binding activity of 1 µg/mL of an affinity purified IgG anticardiolipin preparation from a standard serum.

The Louisville reference center recommends the following positive discrimination criteria according to the recommendation of the 2nd International Anticardiolipin Workshop.

Criteria Range	(GPLU/mL)
High Positive	> 80
Medium Positive	≥ 20-80
Low Positive	≥ 10, < 20

Results Interpretation

The association between low positive levels of anticardiolipin antibodies and clinical findings is unclear.

Normal population studies indicate that there is a higher prevalence of IgM positives in the normal population than IgG, 9.4% and 6.5%, respectively. In normal pregnancy, the levels are higher still at 17.0% (IgM) and 10.6% (IgG).

Expected Values

The normal range was determined on serum from 102 normal adult blood donors. The ranges below are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

IgG Anticardiolipin	
< 11 GPL U/mL	Negative result
> 11 GPL U/mL	Positive result

Programming the Assay on the ELx808

From the Main Menu, press **DEFINE**. Enter the assay number and edit the name if desired. At the **DEFINE** menu, follow the steps below:

STEP
To program the reading method, press:
METHOD:
READ TYPE: Endpoint
DELAY FIRST READ:
If incubator model: INCUBATION TEMP (Ambient or Temperature):
DELAY FIRST READ:

STEP
WAVELENGTH (Single or Dual): Single
MEASURE (Wavelength[s] to use): 450 SHAKE (First, Every, or None):
To program the plate map, press:
MAP AUTO DOWN DOWN A01 BLANK MAP: AIR NUMBER STDS: 05 NUMBER STD REPLICATES: 01 CONCENTRATIONS: STD1: 6.25 STD2: 12.5 STD3: 25 STD4: 50 STD5: 100 REUSE STANDARD CURVE? NUMBER CTLS: 02 CONTROL 1: PC CONTROL 2: NC NUMBER OF REPLICATES PC: 01 NC: 01 SAMPLES: 89 SAMPLE REPLICATES: 01
To define a cutoff formula for Positive and Negative calls: Note: Kit instructions specify that samples with concentration values greater than 11 should appear as positive. The ELx808 software calculates the cutoff based on absorbance value or transformed value (see the previous example) and cannot calculate based on concentration. The technician must make the positive or negative determination visually, based on the calculated concentration results.
Curve: 4P Note: As a general guideline, choose "linear" if you expect a straight line result. Choose "4P" for all others, unless otherwise specified by the kit instructions. The reader will automatically calculate the concentrations of the samples when the assay is run.

Adjusting the Power Input Voltage Setting

This appendix does **not** apply to ELx808 readers equipped with an external power supply.

This appendix provides instructions for adjusting the power input voltage setting in ELx808 instruments that are equipped with an **internal** four-voltage range power input module.

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Identifying the Reader's Power Input Module

Previous versions of the ELx808 (models manufactured before December 2005) have an internal, four-voltage range power input module, instead of an external power supply. Located on the right side of the ELx808, the input module can be adjusted for a 100, 120, 230, or 240 V~ voltage setting.

The voltage setting that is currently selected can be determined by observing which of the four possible voltage indicator holes has a white peg in it. Check the peg now to make sure the setting is appropriate for your location.

If you need to adjust the setting, first determine which type of power input module is installed in the reader:

- The **newer style module** has a cover that **can swing back toward the reader**, when opened with small needle-nose pliers. If your reader has this module, follow the instructions starting on page 143 for adjusting the power input voltage setting and reconfiguring/replacing the fuses.
- The **older style module** has a cover that **can be detached from the reader** when opened with a small flat-blade screwdriver. If your reader has this module, follow the instructions starting on page 147 for adjusting the power input voltage setting and reconfiguring/replacing the fuses.

Newer-Style Module

Adjust the Power Input Voltage Setting



Important! Turn off and unplug the reader before adjusting the voltage setting.

1. Unplug the reader and remove the power cord.
2. Insert a small pair of **needle-nose pliers** into the two holes on the cover of the power input module. Lift the cover open and swing it back toward the power cord socket (see **Figure 12** below).
3. As shown below, a small voltage selector card is located to the right of the fuse holder. Using the pliers, carefully pull this card straight out of the compartment.

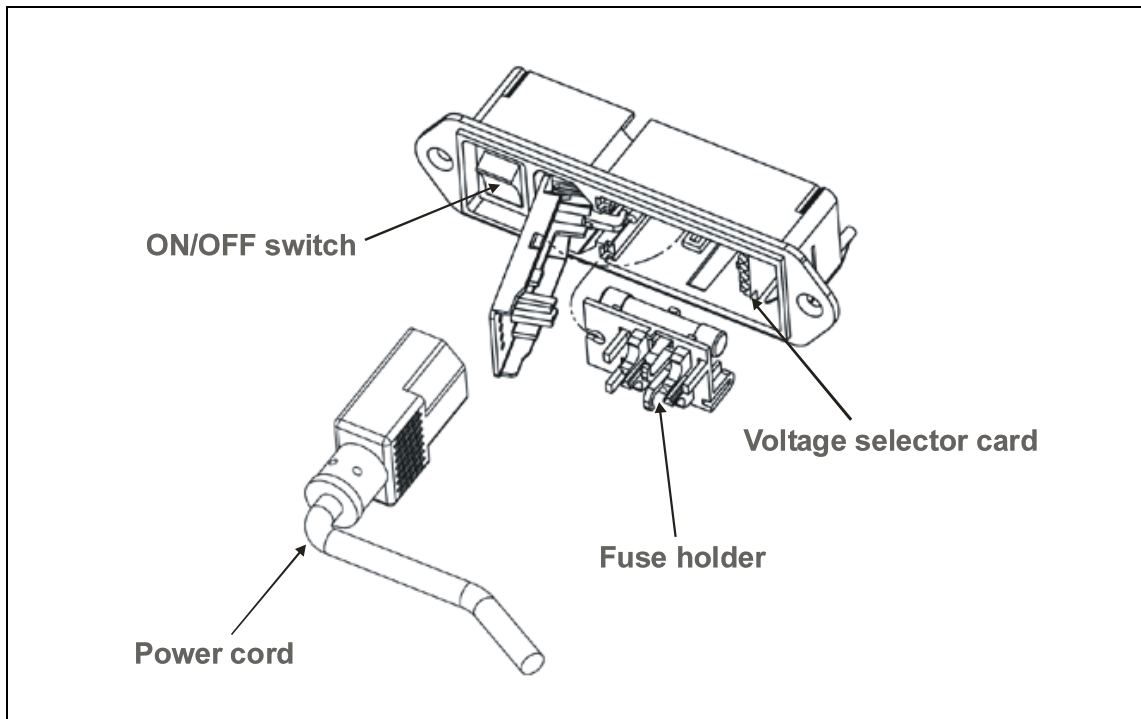


Figure 12: Power input module: accessing the voltage selector card and fuse holder

- As shown in **Figure 13** below, there are four different voltage range positions. Rotate the white plastic indicator peg around the card so that it fits into the correct groove for the desired voltage.

❖ Point the peg upward so that the text matching the desired voltage range is readable at the **bottom** of the card, as shown below.

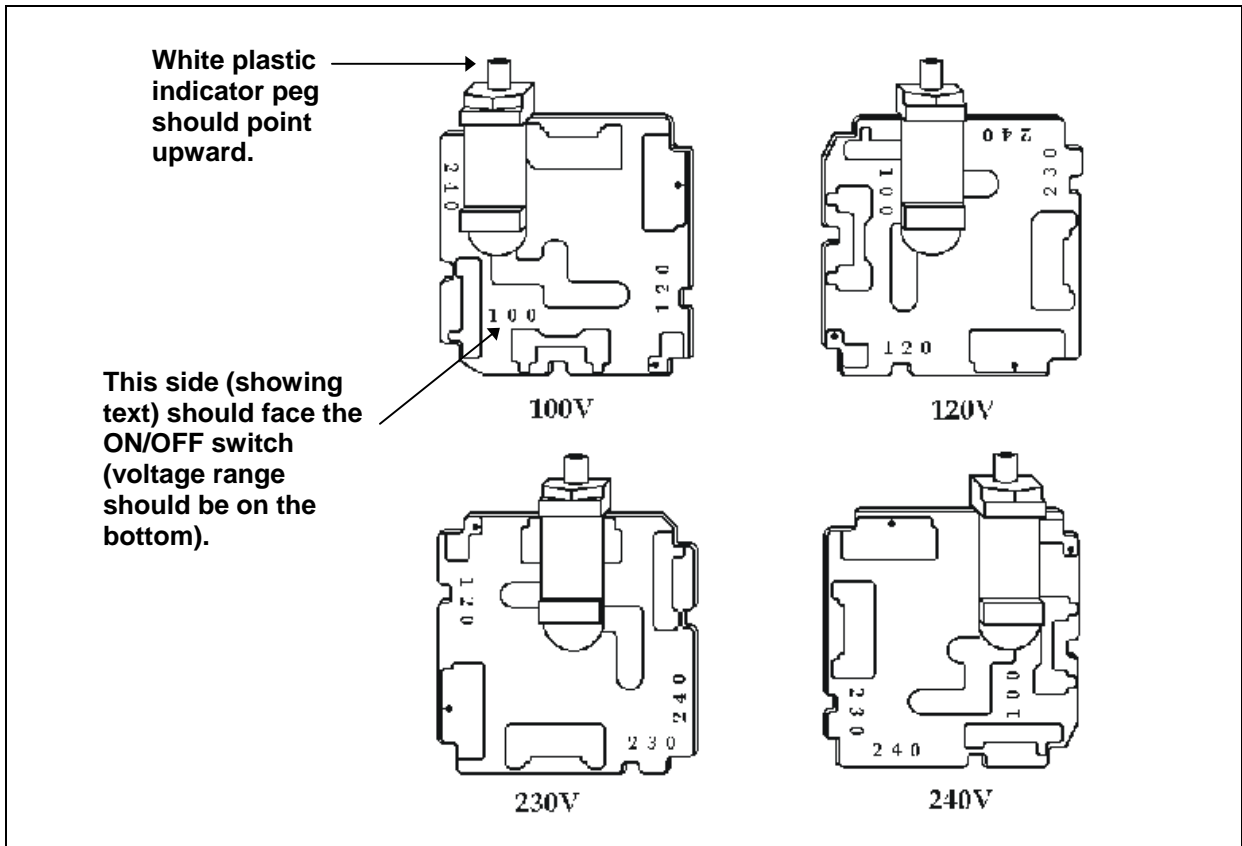


Figure 13: Voltage selector card input range positions

- Reinsert the voltage selector card into the power input module so that the text side faces the ON/OFF switch, and the desired voltage range is on the bottom of the card (see above).
- Reconfigure or replace fuses as needed (see next page).
- Replace the cover, and verify that the white peg lines up with the correct voltage indicator hole.



Important! The peg indicating the intended voltage should protrude through the cover. Do not power up the instrument until the voltage range to be used is indicated correctly by the peg.

Reconfigure or Replace Fuses



Important! Turn off and unplug the reader before reconfiguring or replacing the fuses.

Either USA or International style fuses are installed in the fuse holder that is located inside the power input module. The reader's fuses are configured at the factory prior to shipping. Use the procedure described below if you need to reconfigure or replace fuses.

❖ A failed fuse is usually an indication of another problem that a new fuse is not likely to remedy. Contact BioTek's Technical Assistance Center if the fuse replacement fails to resolve the problem. See **Chapter 1** for contact information.

The fuse holder can have either one of two fuse configurations:

- 100/120 V (USA): A fused hot AG 1.5 A, 250 V Slo Blow (PN 46024), or
- 230/240 V (International): Both hot and neutral fused with a 5 x 20 mm, 0.63 A, 250 V Slo Blow (PN 46038).

To reconfigure fuses:

1. Turn off and unplug the reader.
2. Insert the needle-nose pliers into the two holes on the cover of the power input module. Lift the cover open and swing it back toward the power cord socket.
3. Using the pliers, gently pull the fuse holder out of the power input module and turn it over.

<p>Changing to the 100/120 V Configuration:</p>	<ul style="list-style-type: none"> • Remove the two 0.63 A, 250 V fuses. • Install one AG 1.5 A, 250 V fuse (PN 46024) on the fuse holder on the side opposite the original 0.63 A fuse location. • Reinstall the fuse holder with the fuse toward the back of the power input module.
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	<ul style="list-style-type: none"> • Replace the cover, and verify that the white plastic indicator peg lines up with the correct voltage indicator hole.
Changing to the 230/240 V Configuration:	<ul style="list-style-type: none"> • Remove the single AG 1.5 A, 250 V fuse. • Install two 0.63 A, 250 V fuses (PN 46038) on the fuse holder on the side opposite the original AG 1.5 A fuse location. • Reinstall the fuse holder with the fuses toward the back of the power input module. • Replace the cover, and verify that the white plastic indicator peg lines up with the correct voltage indicator hole.

To replace defective fuses:

1. Follow steps 1 through 3 on the previous page to access the fuse holder.
2. Remove the defective fuse and replace it with the correct new one.
3. Reinstall the fuse holder with the fuse toward the back of the power input module.
4. Replace the cover, and verify that the white plastic indicator peg lines up with the correct voltage indicator hole.


Older-Style Module

Adjust the Power Input Voltage Setting

	<p>Important! Turn off and unplug the reader before adjusting the voltage setting.</p>
	<p>Important! Be careful when removing the cover from the power input module: the fuse holder is attached to the inside of the cover.</p>

Refer to **Figure 14** on the next page for the following instructions:

1. Unplug the reader and remove the power cord.
2. Insert a small **flat-blade screwdriver** into the access hole between the power cord inlet and the power input module cover. Carefully pry the cover off the module. Note that the fuse holder is attached to the inside of the cover.
3. A small voltage selector card is located on the right side of the power input module. Using a small pair of **needle-nose pliers**, carefully pull this card straight out of the module.
4. Text printed on the card identifies the four different voltage positions. Each voltage number has an arrow printed next to it. Rotate the white plastic indicator peg so that it fits into the groove on the card that represents the desired voltage; the peg should point in the *opposite direction* from the arrow printed on the board.
5. Orient the card so that the peg is facing out and the text on the card is facing the ON/OFF switch. The arrow should be pointing into the power input module. Slide the card all the way back into the input module.
6. Reconfigure or replace fuses in the fuse holder, as needed (see the instructions on page 148).
7. Replace the cover on the power input module and verify that the white peg lines up with the correct voltage indicator hole.

	<p>Important! The peg indicating the intended voltage should protrude through the cover. Do not power up the instrument until the voltage range to be used is indicated correctly by the peg.</p>
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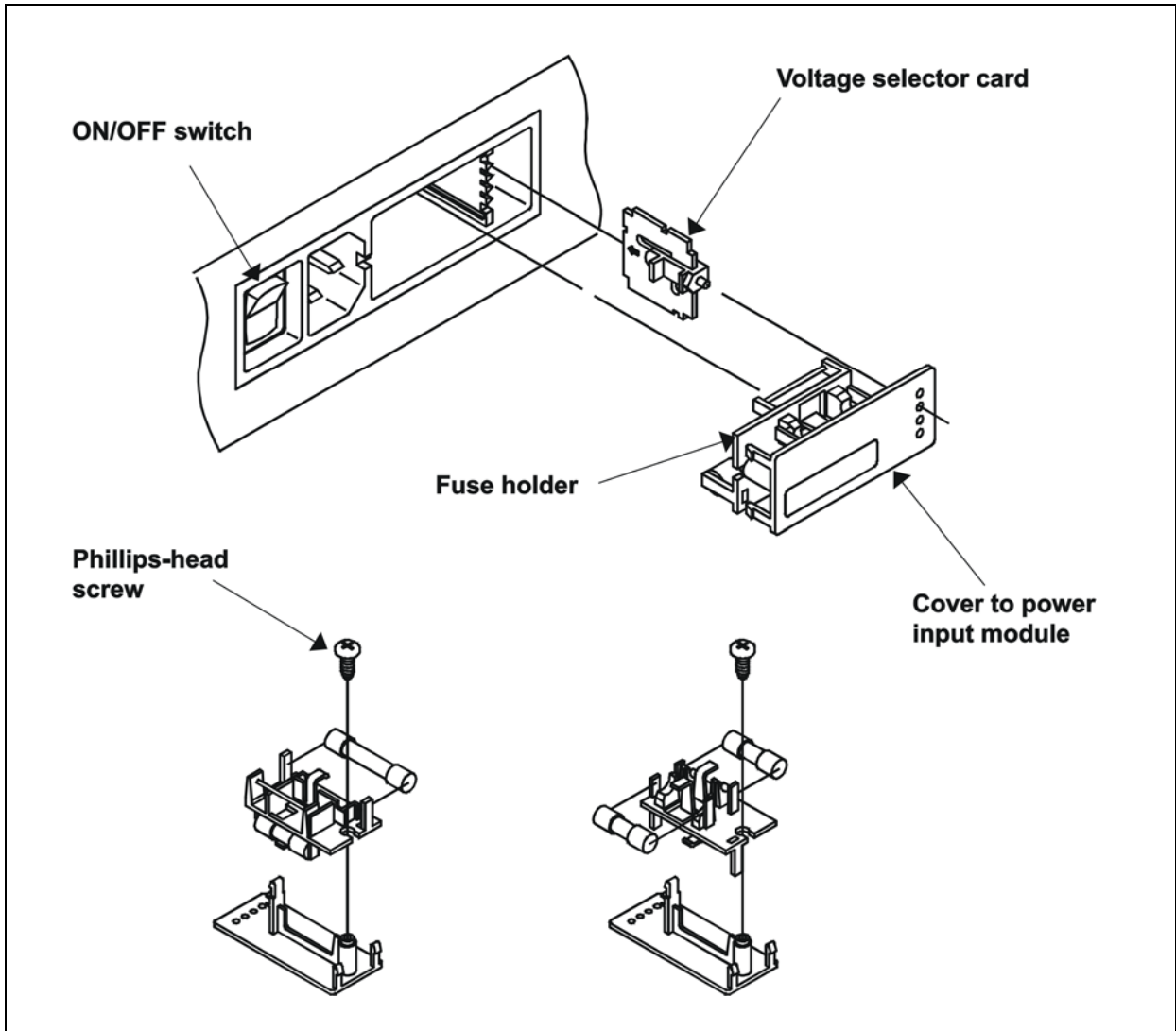


Figure 14: Power input module; accessing the voltage selector card and fuse holder

Reconfigure or Replace the Fuses



Important! Turn off and unplug the reader before reconfiguring or replacing the fuses.

Both USA and International style fuses are installed in the fuse holder that is attached to the inside of the power input module's cover. The reader's fuses are configured at the factory prior to shipping. Use the procedure below if you need to reconfigure or replace fuses.

❖ A failed fuse is usually an indication of another problem that a new fuse is not likely to remedy. Contact BioTek's Technical Assistance Center if the fuse replacement fails to resolve the problem. See **Chapter 1** for contact information.

The fuse holder has two configurations:

- 100/120 V (USA): A fused hot AG 1.5 A, 250 V Slo Blow (PN 46024), and
- 230/240 V (International): Both hot and neutral fused with a 5 x 20 mm, 0.63 A, 250 V Slo Blow (PN 46038).

The fuse configuration (U.S. or International) that is currently being used by the reader is determined by which fusing network is facing the inside of the power input module.

To reconfigure fuses:

1. Turn off and unplug the reader.
2. Insert a **small flat-blade screwdriver** into the access hole between the power cord inlet and the power input module cover. Carefully pry the cover off the module.
3. Use the screwdriver to remove the Phillips-head screw that anchors the fuse holder to the inside of the cover. See **Figure 14**.
4. Turn the fuse holder over.
5. Reattach the fuse holder to the cover with the screw.
6. Replace the cover, and verify that the white plastic indicator peg lines up with the correct voltage indicator hole.

To replace defective fuses:

1. Repeat steps 1 through 3 on the preceding page to access the fuse holder.
2. Remove the defective fuse and replace it with the correct new one. Do *not* turn the fuse holder over, or you will change the configuration.
3. Reattach the fuse holder to the cover with the screw.
4. Replace the cover, and verify that the white plastic indicator peg lines up with the correct voltage indicator hole.

