ELx800™

Operator's Manual

BioTek®

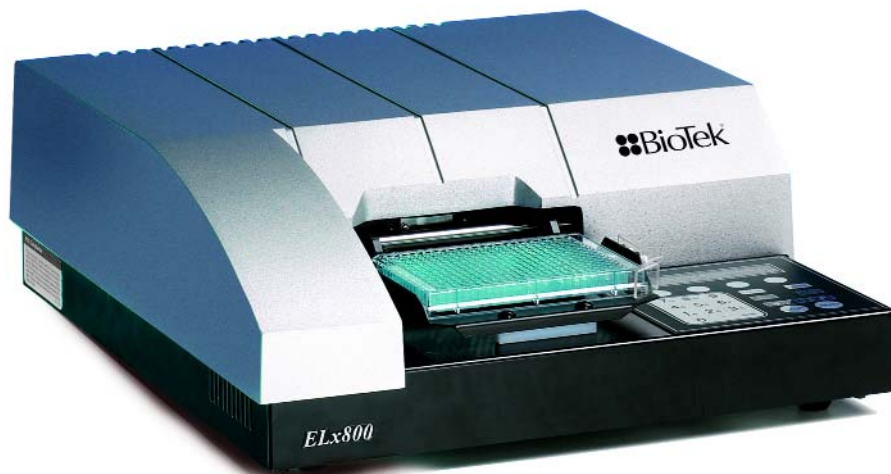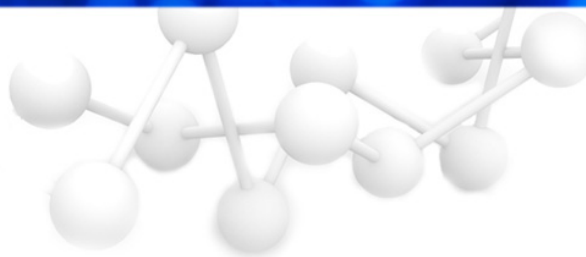**Effective July 2011: This document applies to ELx800 readers equipped with a USB port (see photo below).**

Updated Computer Connectivity: USB / RS232 Ports

The ELx800 is now equipped with a USB port and a 9-pin RS232 serial port for connection to a computer. The ports are located on the back of the instrument, as seen in the photograph below:

Updated package contents:

- Serial cable (PN 75034 replaces former PN 75053)
- USB cable (PN 75108)
- USB driver software (PN 7090204)

Installing the USB driver software

In order for the instrument to interact with the computer via USB cable, the USB driver software must be installed. The software only needs to be installed on the computer one time, regardless of the number of BioTek instruments you are running. However, it is always best to reinstall the drivers if you purchase a new instrument, to make sure you have the latest software. The software on the accompanying CD will scan your computer to determine which USB drivers you need.

> ❖ **Important:** To install software on Windows systems, you must have administrator privileges. Please log onto Windows as "Administrator" or ask your system administrator to install the USB drivers.

1. Plug one end of the USB cable into the instrument and the other end into a USB port on your computer.

2. Turn on both the computer and the instrument.

3. Windows may detect New Hardware and locate drivers. If so, follow the instructions given by the wizard.

If Windows doesn't automatically install the driver:

1. Insert the USB driver software CD into your CD drive.

2. Navigate to your CD ROM drive and run the .exe file. The software will automatically install the drivers.

3. Eject the CD.

Identifying the USB/Virtual COM port

After installing the USB drivers, you can control the connected instrument using Gen5. You must identify the port that the instrument is using to communicate with the computer by selecting its number from a Com Port list. USB/Virtual COM ports are usually the highest number port.
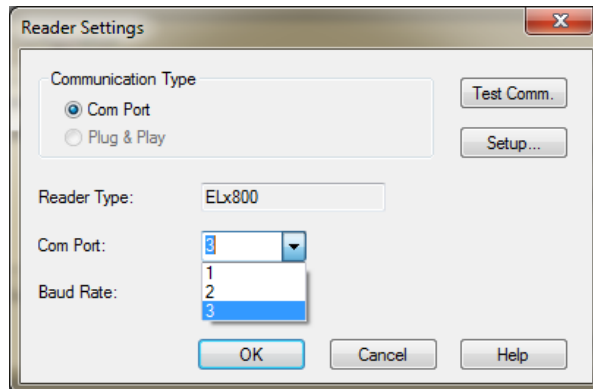
Select a port and click Test Communication. If communication fails, follow the instructions below to identify and select the correct port:

1. Open your computer's Control Panel and go to the system hardware Device Manager.

2. Expand the Ports (COM & LPT) item to locate the USB Serial Port and note the number.

3. Launch BioTek's instrument control software, select the number from the COM Port list, and click Test Communications.

Contact information

If you experience problems, contact BioTek's Technical Assistance Center:

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

E-Mail: tac@biotek.com
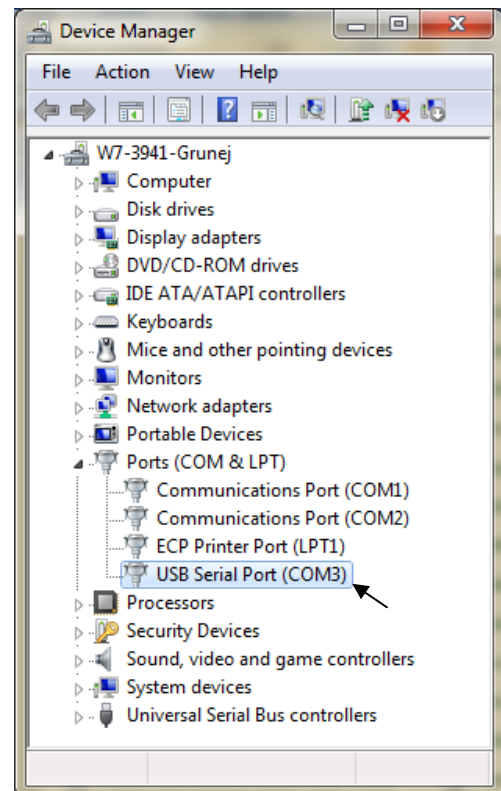
ELx800™

**Absorbance Microplate Reader
Operator's Manual**

Notices

BioTek® Instruments, Inc.
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Document Conventions

Example	Description
⚠️	This icon calls attention to important **safety** notes.
Warning!	A **Warning** indicates the potential for bodily harm and tells you how to avoid the problem.
Caution	A **Caution** indicates potential damage to the instrument and tells you how to avoid the problem.
DEFINE	Text in **COURIER** font represents menu options as they appear on the instrument's display.
Note:	**Bold** text is primarily used for emphasis.
ⓘ	This icon calls attention to important information.

Revision History

Rev	Date	Changes
A	3/95	First Release
B	7/95	Generic
C	9/95	Add Errata sheet
D	10/95	Incorporate Errata: Cal plate PN, cover text to UV.
E	11/96	Added Panel Assay info. Added TVar parameter. Added Appendix D to include all reports.
F	2/97	Added Notes including info on 384-well format. Updated Reuse of Standard Curves and Panel sections. Added Appendix E – KCJr for 384-well format.
G	3/98	Added Liquid Test 1 and 2 to Chapter 4 – Performance Verification. Added Appendix F – Comparison, to show the differences between the ELx800 and the EL800. Added printer information. Changed plate type information to include 384-well and 72-/96-well Terasaki plate formats. Added reference to Quick Read on display.
H	9/98	Modified Appendix B - Computer Control. Updated Elx800 and EL800 model comparison table in Appendix F.
I	2/99	Incorporated Manual Updates. Changed European addresses.

Rev	Date	Changes
J	9/99	Changed printer-compatible description. Changed the number of samples necessary if there aren't controls, standards or blanks defined. Corrected the number of stored plates to 8. Corrected the positions for re-use of standard curves. Removed the "M" command from Appendix B- Computer Control.
K	1/00	Added Liquid Test 3 to test 340 nm filters. Updated screens in Chapter 3. Updated chart in Appendix F.
L	5/03	*Preface*: Updated contact information in Notices (page iii). Added Document Conventions (page v). Corrected errors in Safety Symbol text (page ix).
		Removed "screwdriver" from Package Contents, and added "BioTek QC Check Solutions for 405 nm" to Optional Accessories list (page 1-7).
		Removed About This Manual section (page 1-5).
		Updated contact information in Technical Support (Chapter 1).
		Modified appearance of display screens throughout.
		Revised lamp alignment section in Chapter 2.
		Chapter 4:
		Changed title to "Performance Verification and IQ/PQ/OQ Tests."
		Added IQ/PQ/OQ test procedure information.
		Clarified procedures for liquid tests.
		Replaced outdated Appendix E with instructions for using KCjunior to read 384-well microplates.
		Added new Appendix G with two examples of assay kit instructions and directions for programming an assay.
		Made editorial and formatting changes throughout.
M	9/03	*Preface*:
		Updated Warnings section (pages vii and viii).
		Updated Electromagnetic Compatibility section (page x).
		Added "Consult instructions for use" and "In vitro diagnostic medical device" safety symbols (page xii).
		Expanded Intended Use Statement (page xiii).
		Added Absorbance Test Plate to Optional Accessories list (Chapter 1, page 1-8).
		Changed callout in Figure 2-2 from "Place unit in bag" to "Unit in plastic bag" (Chapter 2, page 2-2).
		Added KC4 startup information to Appendix E.
		Removed references to ELx800UV/EL800UV from Appendix F.
		Standardized the presentation of significant digits throughout.
		Changed "Abs" to "OD" throughout.
N	10/04	Restructured manual according to new template.
		Removed references to "General Formula" throughout.
		Updated Chapter 4, Performance Verification and IQ-OQ-PQ Procedures.
		Added new Chapter 5, Maintenance and Decontamination.
		Added new Chapter 6, Troubleshooting and Error Codes.

Rev	Date	Changes
(N)		Restructured manual according to new template. Removed references to "General Formula" throughout. Updated Chapter 4, Performance Verification and IQ-OQ-PQ Procedures. Added new Chapter 5, Maintenance and Decontamination. Added new Chapter 6, Troubleshooting and Error Codes. Removed Computer Control portion of Appendix A; renamed Appendix to "Controlling the Reader With KCjunior™ or KC4™." Added new Appendix B, Using 384-Well Geometry. Updated sample reports in Appendix C, Report Format (formerly Appendix D). Removed previous Appendix A, Decontamination. Removed previous Appendix C, Error Codes. Added new Appendix E, Instructions for Programming a New Assay.
O	4/06	*General*: Changed "Bio-Tek" to "BioTek," and "ELx800 Automated Microplate Reader" to "ELx800 Absorbance Microplate Reader." Added Gen5 references and instructions wherever KC4 and KCjunior references and instructions were present. *Cover*: Updated BioTek logo to new graphic. Preface: Revised Hazards and Precautions, and removed Warranty and Registration Card. *Chapter 1*: Updated Package Contents, Optional Accessories, and Specifications. Replaced 3-page Technical Support with 1-page Product Support and Service section. *Chapter 2*: Updated unpacking/packing instructions. Added new section, "Installing Software on the Host PC," for Gen5, KC4, and KCjunior users. *Chapter 3*: In System Startup, incorporated information from Manual Update Rev. N2 that not all software features discussed in the manual are available on ELx800 readers with custom programmed software. *Chapter 4*: - Changed title from "Performance Verification and IQ/OQ/PQ Procedures" to "Instrument Qualification." - Add Gen5 instructions for System Test and Absorbance Plate Test. - In Recommended Qualification Schedule, changed PQ frequency from monthly and semiannually to monthly and quarterly, and clarified criteria for running Liquid Tests. - In Absorbance Plate Test section, added a sample test plate report (and explanatory note) illustrating that values higher than the specified 0.000 to 2.000 OD range may not have "PASS"/"FAIL" indications. - For Liquid Tests 1, 2, and 3, incorporated recommendation from Manual Update N2 to shake the plate or wait between pipetting and read steps. *Appendix A*: Changed title of appendix from "Controlling the Reader With KCjunior or KC4" to "Computer Control." Added new section, "Controlling the Reader with Gen5."

Rev	Date	Changes
(O)		*Appendix B*: Added Gen5 instructions for preparing and running a 384-well plate protocol with the ELx800NB. Updated KC4 and KCjunior sections by specifying that the ELx800NB model must be used for running 384-well plates, and added screen shots that illustrate selection of the ELx800NB.
P	10/06	*Ch 3 Operation*, clarified instructions for running Panel assays via the keypad. *Ch 4 Instrument Qualification*, replaced incorrect sample System Test report with a report run using an ELx800. *Ch 5 Maintenance and Decontamination*, added tips for storing and handling filters. *Appx B, Using 384-Well Geometry*, inserted step 13 to select a Read Type when using Gen5.
Q	09/09	*Throughout*: Streamlined content to support reuse. Reduced white space to save paper. *Preface:* Updated Trademarks. Added Warranty and Product Registration section. Added Electromagnetic Environment and Compatibility cautions. Updated Intended Use statement and CE Mark Directives. Removed lists of figures and tables. *Ch 1 Introduction:* Removed sample plates from Package Contents. Added part number disclaimers. Updated optional accessories list. Corrected TAC fax number. *Ch 2 Installation:* Reorganized chapter. Added Product Registration section. Updated repacking instructions and figures. Added part number and packaging design disclaimers. *Ch 3 Operation:* Reorganized some sections. Under Printing Reports, added note explaining 'Out' indication on some reports. Under Reuse of Standard Curves, Limitations, changed assay position range from 31-55 to 32-55. *Ch 4 Instrument Qualification:* Updated System Test by explaining the Optics Test and Autocal Analysis. Under Absorbance Plate Test, removed obsolete PN 9000547 6-Filter Test Plate. Under Liquid Testing, changed "µl" to "µL" and "ml" to "mL"; restructured calculations section in Liquid Test 1; updated information on β-NADH powder in Liquid Test 3. *Ch 5 Preventive Maintenance:* Under Replacing and Aligning the Bulb, replaced previous instructions with reference to bulb replacement kit. *Ch 6 Troubleshooting and Error Codes*: Updated descriptions for error codes 0601-0605, 0800, 0900. *Appendix C Report Format*: Added Overview section. Explained 'Out' indication on reports.

Intended Use Statement

This product may be used for In Vitro Diagnostic, 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 package insert or standard laboratory protocol for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

Warranty and Product Registration

Review the Warranty information that shipped with your product.

Register your product(s) with BioTek to ensure that you receive important information and updates. Contact the **Customer Resource Center (CRC)** at www.biotek.com or by calling 888/451-5171 or 802/655-4740.

Warnings

Operate the instrument on a flat surface and away from excessive humidity.

Bright sunlight 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 and Precautions

Hazards

The following hazards are provided to help avoid injury:

Warning! Power Rating. The instrument's 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.

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

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

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

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. This hazard is noted by the symbol shown here. Always wear safety glasses and appropriate protective equipment, such as chemically resistant rubber gloves and apron.

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

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 read methods. **Failure to conduct quality control checks could result in erroneous test data.**

Warning! Reader Data Reduction Protocol. 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. If the reader is operated via computer control, no limits are applied to the raw absorbance data. All information exported via computer control must be thoroughly analyzed by the operator.

Precautions

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

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

Caution: Environmental Conditions. Do not expose the instrument to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in Chapter 1. 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. Only use the power supply shipped with the instrument. Operate this power supply within the range of line voltages listed on it.

Caution: Shipping Hardware. Shipping straps and a shipping block must be removed before operating the reader. They must be reinstalled before repackaging the reader for shipment. See Chapter 2.

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: Warranty. Failure to follow preventive maintenance protocols may void the warranty. See Chapter 5.

Caution: Electromagnetic Environment. Per EN 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), as these may interfere with the proper orientation.

| CE | **Based on the testing described below and information contained herein, this instrument bears the 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 and EN 61326-2-6: 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 73/23/EEC Low Voltage (Safety)

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

EN 61010-1, "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."

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

- Product registration with competent authorities

- Traceability to the U.S. National Institute of Standards and Technology (NIST): Optical density measurements are traceable to NIST.

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

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

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

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

Canadian Department of Communications Class A

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

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

User Safety

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

North America

- 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"

- UL 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"

International

- EN 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"

Safety Symbols

Some of the following symbols will appear on the instrument.

Alternating current
Courant alternatif
Wechselstrom
Corriente alterna
Corrente alternata

Direct current
Courant continu
Gleichstrom
Corriente continua
Corrente continua

Both direct and alternating current
Courant continu et courant alternatif
Gleich - und Wechselstrom
Corriente continua y corriente alterna
Corrente continua e corrente alternata

Earth ground terminal
Borne de terre
Erde (Betriebserde)
Borne de tierra
Terra (di funzionamento)

Protective conductor terminal
Borne de terre de protection
Schutzleiteranschluss
Borne de tierra de protección
Terra di protezione

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

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

Caution (refer to accompanying documents)
Attention (voir documents d'accompanement)
Achtung siehe Begleitpapiere
Atención (vease los documentos incluidos)
Attenzione, consultare la doc annessa

Warning, risk of electric shock
Attention, risque de choc électrique
Gefährliche elektrische schlag
Precaución, riesgo de sacudida eléctrica
Attenzione, rischio di scossa elettrica

Warning, risk of crushing or pinching
Attention, risque d'écrasement et pincement
Warnen, Gefahr des Zerquetschens und Klemmen
Precaución, riesgo del machacamiento y sejeción
Attenzione, rischio di schiacciare ed intrappolarsi

Warning, hot surface
Attention, surface chaude
Warnen, heiße Oberfläche
Precaución, superficie caliente
Attenzione, superficie calda

Warning, potential biohazards
Attention, risques biologiques potentiels
Warnung! Moegliche biologische Giftstoffe
Atención, riesgos biológicos
Attenzione, rischio biologico

Consult instructions for use
Consulter la notice d'emploi
Gebrauchsanweisung beachten
Consultar las instrucciones de uso
Consultare le istruzioni per uso

***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*

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

Chapter 1

Introduction

This chapter introduces the ELx800 Absorbance Microplate Reader, describes its hardware and software features, and provides contact information for technical assistance.

ELx800 Absorbance Microplate Reader

The ELx800 is a single-channel reader-assay system, designed to automatically perform endpoint analysis for ELISA-based applications. The reader has the following capabilities:

- All models: Measurement of the optical density of solutions in 6-, 12-, 24-, 48-, or 96-well microplates.

- "NB" models: Measurement of 384-well microplates and 60-, 72-, 96-well Terasaki trays as well as 6-, 12, 24-, 48-, and 96-well plates.

- Superior optical specifications, with an extended dynamic range of up to 3.000 absorbance units in some read modes.

- All models: A wavelength range from 400 nm to 750 nm.

- "UV" models: An extended range from 340 nm to 750 nm.

- Kinetic analysis can be performed using computer control (e.g., via Gen5, KC4, or KCjunior running on a host computer).

- Assay definitions (consisting of protocols, templates and formulas) and the data they produce are managed by an onboard processor, via an LCD screen and membrane keypad.

- Data can be stored onboard, printed, and/or uploaded to controlling software on a host computer.

- Designed to serve as a stand-alone system, or as part of a larger laboratory data network, sending, receiving, and manipulating assay data as needed.

Hardware Features

- Single optics channel
- Wavelength range of 400-750 nm ("UV" model range is 340 to 750 nm)
- A 5-position filter wheel
- A 2-line x 24-character LCD display
- A membrane keypad with alphanumeric keys
- X-Y carrier movement
- Capability of reading 6-, 12-, 24-, 48- and 96-well microplates ("NB" model also reads 384-well and 60-/72-/96-well Terasaki microplates)
- External 24-volt power supply, compatible with 100-240 V~ ± 10.0% @ 50 to 60 Hz
- One serial COM port, 25-pin male connector
- One parallel port, 25-pin female connector

Software Features

- An easy-to-use, menu-driven interface

- Endpoint calculations

- Curve Fitting, with 4-parameter, cubic, quadratic, linear, 2-P, cubic-spline, and point-to-point methods

- Transformation and Formula calculations for more complex mathematical operations, including Validations

- Up to 75 assays can be programmed into memory and recalled instantly

- Up to 10 Results can be stored in memory

Package Contents

> ❖ Package contents and part numbers are subject to change over time. Please contact BioTek Customer Care if you have any questions.

- ELx800 Absorbance Microplate Reader

- Power cord (part number varies according to country of use)

- Power supply (PN 61062)

- Filter wheel with 4 standard filters: 405, 450, 490, and 630 nm, and one blank filter. "UV" model includes a 340 nm filter.

- Operator's Manual (PN 7331000)

- Printer cable (PN 71072)

- RS-232 serial cable (PN 75053)

- Dust cover (PN 7332040)

- Documents including Warranty Statement, Certificate of Compliance and Calibration, Declaration of Conformity

Optional Accessories

> ❖ Accessory availability and part numbers are subject to change over time. Please contact BioTek Customer Care if you have any questions, or visit www.biotek.com and use the Accessories search tool.

- Patented BioCell Quartz vessel: to obtain 1 cm fixed pathlength absorbance measurements (PN 7272051)

- BioCell adapter plate assembly: can contain up to 8 BioCells (PN 7270512)

- Replacement lamp for ELx800 (PN 7330513)

- Replacement lamp for ELx800NB, ELx800UV (PN 7330516)

- ELx800 filters (PN 7334 + wavelength): 340 (UV model only), 405, 415, 450, 490, 515, 540, 550, 562, 570, 590, 595, 600, 620, 630, 650, 660, 690, 750 nm

- Terasaki plate adapter (PN 7330531) for 60-, 72-, and 96-well Terasaki plates for ELx800NB and ELx800RNB

- BioTek 7-filter absorbance test plate (PN 7260522)

- Absorbance Liquid Test Solutions:

  ➢ BioTek Wetting Agent Solution (PN 7773002)

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

- Adapter to connect the reader to a USB-only printer (75135)

- HP DeskJet printer (PN 97152)

- Gen5 software (visit biotek.com or contact your local dealer for details)

- ELx800 IQ/OQ/PQ Package (PN 7330538)

Specifications

All Models

Light Source:	Tungsten gas-filled bulb
Dimensions:	42 cm x 38 cm x 18 cm (16.5" deep x 15" wide x 7" tall)
Weight:	8 kg (18.5 lb. maximum)
Environment:	Operating temperature 18° to 40°C
Humidity:	10% to 85% noncondensing
Power Supply:	Input 100 to 240 V~ ± 10.0% @ 50 to 60 Hz Output +24 VDC, 2.1 A

Standard Model

Wavelength Range:	400 to 750 nm
Filters:	10 nm half-bandwidth interference filters.
	User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time.
	Filters supplied: 405, 450, 490, and 630 nm.

The following specifications apply to 96-well, flat- or round-bottom plates, single-wavelength measurements with a 50-second read (normal read mode):

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD at 405 nm ± 3.0% from 2.000 OD to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

The following specifications apply to 96-well, flat- or round-bottom plates, single-wavelength measurements with a 30-second read (rapid read mode):

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

Ultraviolet/UV Model

Wavelength Range:	340 to 750 nm
Filters:	10 nm half-bandwidth interference filters.
	User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time.
	Filters supplied: 340, 405, 450, 490, and 630 nm.

The following specifications apply to 96-well, flat- or round-bottom plates:

Optical specifications for 400-750 nm range (50-second read in normal read mode):

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD @ 405 nm ± 3.0% from 2.000 to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

Optical specifications for 340-400 nm range (50-second read in normal read mode):

Absorbance Measurement Range: 0.000 to 2.000 OD

Accuracy:	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 340 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 340 nm
Repeatability (STD):	± 1.5% ± 0.005 OD from 0.000 to 2.000 OD @ 340 nm

Optical specifications for 400-750 nm range (30-second read in rapid read mode):

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

Optical specifications for 340-400 nm range (30-second read):

Absorbance Measurement Range: 0.000 to 2.000 OD

Accuracy:	± 2.5% ± 0.020 OD from 0.000 to 2.000 OD @ 340 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 340 nm
Repeatability (STD):	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 340 nm

Narrow Beam/NB Model

Filters: 10 nm half-bandwidth interference filters.

User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time.

Filters supplied: 405, 450, 490, and 630 nm.

The following specifications apply to 96-well, flat- or round-bottom plates with a 50-second read (normal read mode).

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD @ 405 nm ± 3.0% from 2.000 to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

The following specifications apply to 96-well, flat- or round-bottom plates with a 30-second read (rapid read mode).

Absorbance Measurement Range: 0.000 to 3.000 OD

Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

The following specifications apply to a 384-well plate. The minimum volume of fluid in a well to obtain the specified performance is 80 microliters. The specifications apply to the dual wavelength mode of read only.

Absorbance Measurement Range: 0.000 to 3.000 OD

Normal Read Mode:	Dual wavelength (4 minutes, 45 seconds)
Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.5% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Rapid Read Mode:	Dual wavelength (3 minutes, 25 seconds)
Accuracy:	± 2.5% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

Product Support & Service

A superior support staff backs all of BioTek's products. If your instrument(s) 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

The TAC is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays.

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

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 software part number and basecode version (available via the keypad by selecting **UTIL > TESTS > CHKSUM**)
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed (see also Chapter 6)

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 before shipping the instrument. Repackage the instrument properly (see Chapter 2), write the RMA number on the shipping box, and ship to this address:

BioTek Instruments, Inc.
ATTN: RMA# xxxxx
100 Tigan Street
Highland Park
Winooski, Vermont 05404 USA

Chapter 2

Installation

This chapter provides instructions for unpacking and setting up the ELx800 and for connecting printers and/or serial devices.

Product Registration

Please register your product with BioTek to ensure that you receive important information and updates about the products you have purchased. Contact the Customer Resource Center (CRC) at www.biotek.com or by calling 888-451-5171 or 802-655-4740.

Unpacking and Inspecting the ELx800

(i) **Important! Save all packing materials.** If you are sending the reader to BioTek for repair or replacement, use the original packing materials. Using other forms of commercially available packing materials, or failure to follow the packaging instructions at the end of this chapter, may **void your warranty**. If the original materials have been damaged or lost, replacements are available from BioTek (PN 7332062).

Carefully unpack the reader and retain the packing materials for future use.

Inspect the shipping box(es), reader, and accessories for signs of damage.

If the reader is damaged, notify the carrier and your manufacturer's representative. Keep the shipping cartons and packing material for the carrier's inspection. The manufacturer will arrange for repair or replacement of your reader immediately.

See *Preparing the ELx800 for Shipment* at the end of this chapter for shipping instructions.

Remove the Shipping Hardware

1. Carefully turn the reader upside down on a level surface.

2. Remove four mounting screws to detach the top cover from the base. Set the screws aside.

3. Unhook the shipping straps from the post in the middle of the base.

Mounting screws

Post

Shipping straps

Top cover
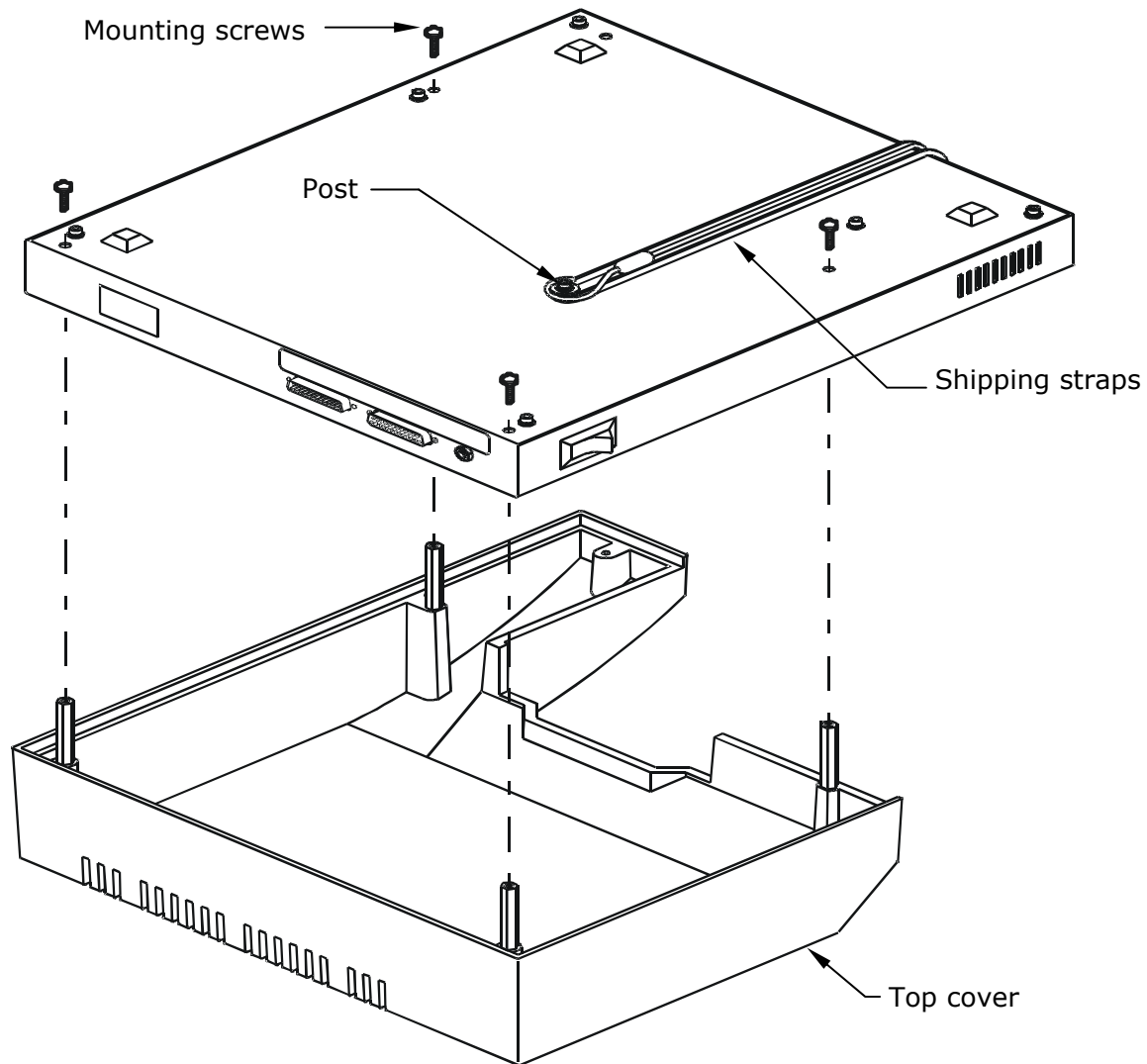
Figure 1: Remove the mounting screws (4)

4. While holding the carrier, turn the reader's base right side up.

5. Remove two mounting screws to detach the shipping block from the base. Store the screws and block with the packaging materials.

Shipping block

Carrier
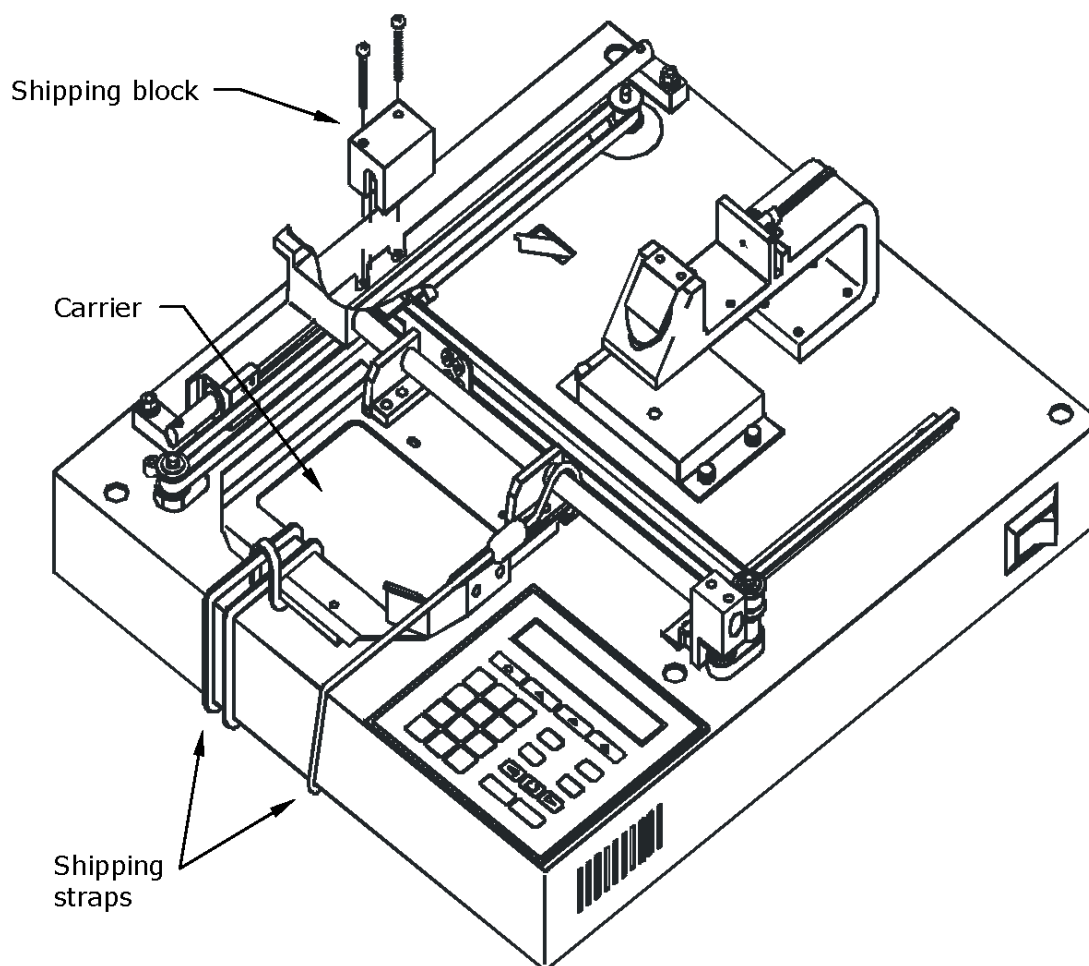
Shipping straps

Figure 2: Remove the shipping block

6. Remove the shipping straps from the top of the carrier and store them with the packaging materials.

7. If you need to install additional filters, do so now (see page 16).

8. Place the top cover on the base. While holding the carrier, turn the reader upside down and replace the four mounting screws. When finished, carefully turn the reader right side up.

Setting Up the ELx800

Select an Appropriate Location

Install the reader on a level surface in an area where ambient temperatures remain between 18°C (64°F) and 40°C (104°F). The reader is sensitive to extreme environmental conditions; avoid these conditions:

- **Excessive humidity:** Condensation directly on the sensitive electronic circuitry can cause the reader to fail internal self-checks.

- **Excessive ambient light:** Bright sunlight or strong incandescent light can reduce the linear performance range of the reader.

- **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.

Install the Power Supply

⚠️	**Caution! Power Supply.** Use only the specified power supply to ensure proper operation of the unit. The ELx800 has a universal 24-VDC, 2.1 A power supply that functions from 100 to 240 V~ (± 10.0%) @ 50 to 60 Hz without external switching.
	Never use a two-prong plug adapter to connect primary power to the ELx800 power supply. Use of a two-prong 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.

1. Connect the power cord to the external power supply.

2. Plug the cord into the **Power In** port on the back on the reader and tighten the nut to secure it.

3. Plug the end of the power cord into an appropriate power receptacle.

⚡	**Warning! Power Rating.** The 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.

Power-Up and Run System Test

After you have installed the ELx800 and connected the power supply, turn on the reader to run a System Test. The on/off switch is located on the lower right side.

If the test passes, a brief **SYSTEM TEST PASS** message will display and then the main menu will appear (see below). If the test fails, the reader will "beep" and display an error code. If this happens, turn to Chapter 6 for a list of error codes.

ELx800 Main Menu

Following successful power-up, the **Main Menu** appears:

R E A D Y	0 1 : 2 2 P M	0 7 / 2 3 / 0 9
R E A D	D E F I N E R E P O R T	U T I L

●   ●   ●   ●

The keypad has four buttons (called **soft-keys**) below the selectable options. To select a menu option, press its corresponding soft-key. See *Keypad and Main Menu* in Chapter 3 for additional instructions.

Change Global Default Settings (if needed)

BioTek defines certain settings onboard the reader before shipping it. Brief instructions for modifying the default settings are provided below. See *Using the Utility Options* in Chapter 3 for more information.

To change the Date/format:

1. Select **UTIL > SETUP > DATE**.
2. Select the desired format: MM/DD/YY or DD/MM/YY.
3. Use the arrow and number keys to change the date, if necessary.

To change the Time/format:

1. Select **UTIL > SETUP > TIME**.
2. Select the desired format: 12 Hour or 24 Hour.
3. Use the arrow and number keys to change the time, if necessary. When using the 12 hour format, use AM/PM option to change the setting.

To change the Output option:

1. Select **UTIL > SETUP > OUTPUT**.

2. Select Print, Computer or Both to specify where to send the results (only applicable when using the reader in standalone mode).

To change the read-time prompt and read mode options:

1. Select **UTIL > SETUP > READ**.

2. Select Yes or No and press Enter to proceed through each of the options.

Connect a Printer to the ELx800 (for keypad control)

> (i) Connect a printer to the ELx800 *only* if you are running the reader in standalone mode (using the keypad). If you are using BioTek's Gen5, KCjunior, or KC4 software, skip this step.
>
> To avoid system instability, turn off the reader before connecting the printer.

The ELx800 has a printer port to connect to parallel (Centronics) printers, such as HP Deskjet or Inkjet printers. (See *Using the Utility Options* in Chapter 3 for more information.) The port is shown in **Figure 3** on the next page. Use the parallel cable (PN 71072) provided with the reader.

1. Turn the reader off.

2. Place the printer next to the reader.

3. Attach one end of the cable to the port on the printer and the other end to the parallel port on the reader.

4. Tighten the screws on both ends of the cable.

5. Turn on the reader, and then turn on the printer.

❖ BioTek offers an adapter to connect the reader to a USB-only printer. See "Optional Accessories" in Chapter 1.

❖ For a list of compatible printers, visit www.biotek.com, or call BioTek's Technical Assistance Center.

Serial port

Parallel port
(printer port)
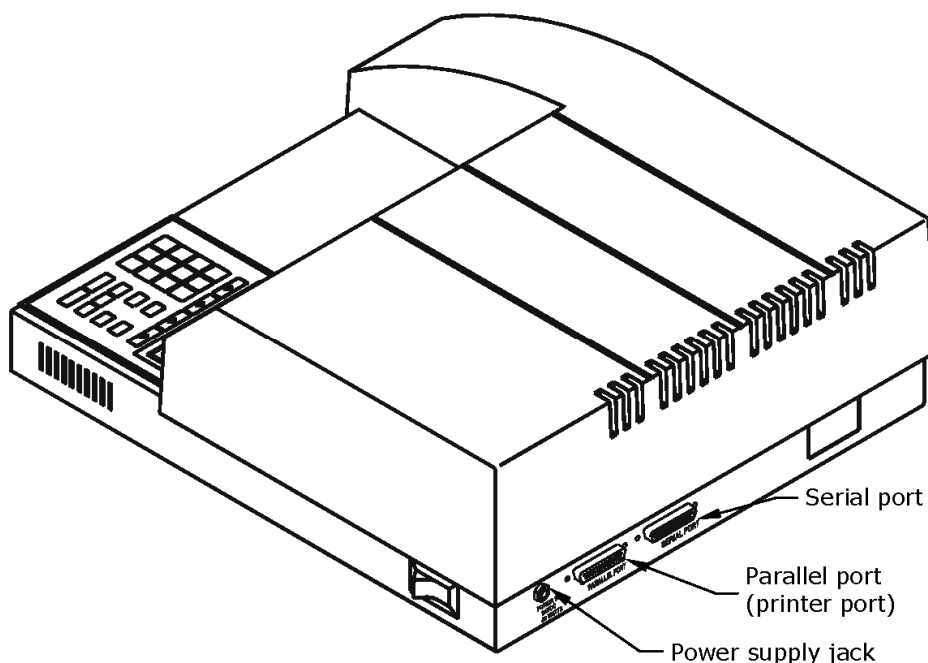
Power supply jack

Figure 3: Connectors for printer (parallel), computer (serial), and power supply

Install Additional Filters (if needed)

The filters that come with the reader are already installed in the internal, five-position filter wheel. Standard models have 405, 450, 490, and 630 nm filters. The UV model has an additional 340 nm filter.

> ⓘ Keep track of all filter locations. The physical locations 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 light direction arrow pointing downward.**
>
> Store unused filters in a cool, dry place away from direct sunlight. The filters can be wrapped in a piece of lens paper to protect them from scratches and dust .

To install additional filters or change the filter locations:

1. Turn off the reader and disconnect the power cord.

2. If you have not already done so, remove the reader's top cover as described on page 11.

3. The filter wheel is inside a small black box (filter wheel cover) directly under the end of the optics arm (see below). Remove the four thumbscrews from the filter wheel cover.

4. The filter wheel sits on a pin and can be lifted off. Remove the filters by turning the wheel upside down over a lint-free cloth. The filters, which are labeled with the **wavelength** and **light direction arrow**, should easily slide out. Handle the filters from the edges. Do not touch the glass portion of the filter. Clean any filters that appear dirty with lens paper and isopropyl alcohol.

5. Once the filters are installed in the wheel, place the wheel back on the pin, making sure it sits flat and meshes with the filter wheel drive gear.

6. Install the filter wheel cover with the four thumbscrews.

7. Reinstall the top cover as described on page 11.

8. Reconnect the power supply and cable.
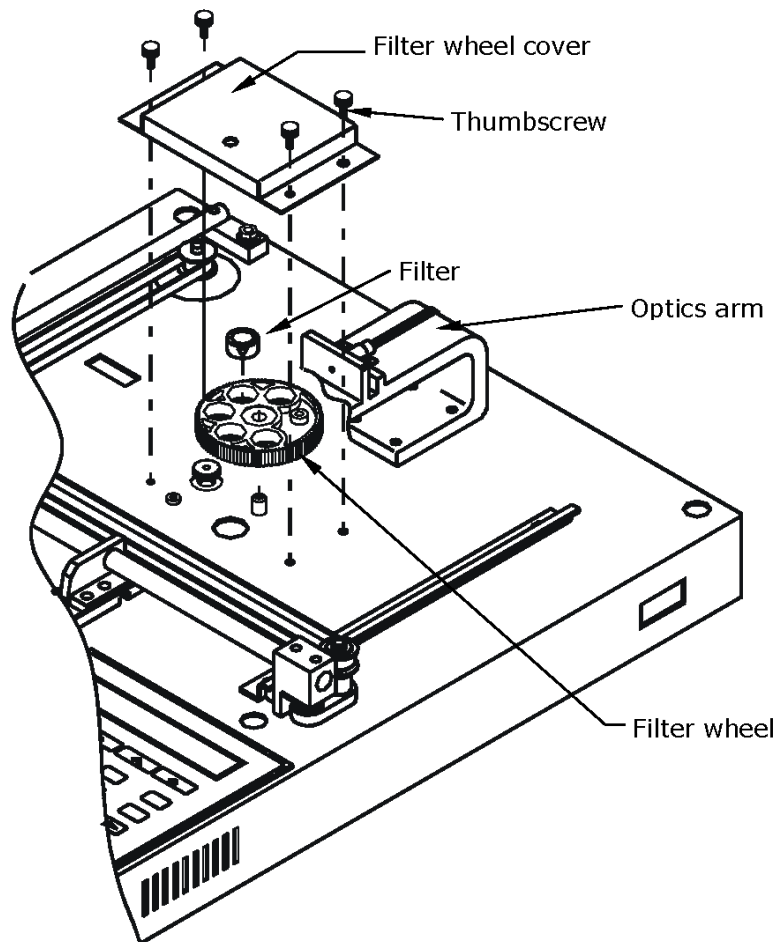
Figure 4: Accessing the filter wheel

Check the Reader's Filter Table

If you installed or moved filters, ensure that the ELx800's filter table (the reader's software reference for filter locations) matches the physical location of the filters.

To check or change the software filter table:

1. Turn on the reader.

2. From the Main Menu, select **UTIL > SETUP > FILTER**.

3. The wavelength for Filter #1 will be displayed. To change a filter wavelength value, use the keypad to enter a number at the cursor location. The cursor will automatically advance to the next editable location. Press the **Enter** key to save the value and move to the next filter.

```
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
```

4. After entering the last filter, press the **Main Menu** key to return to the main menu.

Install Software/Connect to Computer (Optional)

The ELx800 has a 25-pin serial (RS-232) port on its rear panel. This port allows the reader to communicate with a computer, using BioTek's Gen5, KC4, or KCjunior software. It also allows for upgrades of the ELx800 basecode software.

Attach the Cable

1. Turn off the reader and the computer.

2. Connect the appropriate serial cable to both machines.

3. Turn on the reader and the computer.

Install Software on the Host Computer

See Appendix A for basic software installation and configuration instructions. Refer to the Gen5 Getting Started Guide or to the KC4 or KCjunior User Guide for complete instructions.

Communication Parameters

The reader's default communication parameters are: 9600 Baud Rate, 8 Data Bits, 2 Stop Bits, No Parity. The baud rate can be changed to 1200 or 2400 if necessary, however, Gen5 requires 9600. The Data Bits, Stop Bits, and Parity settings cannot be changed. See *Using the Utility Options* in Chapter 3 for more information.

Preparing the ELx800 for Shipment

(i)	**Important!** Failure to properly package the reader increases the likelihood of damage to the reader during shipping. The shipping system stabilizes the reader's mechanical components, which would otherwise be free to move around during shipping.
⚠	**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.

If you need to send the reader to BioTek for service or repair, use the original packing materials. Other forms of commercially available packing materials are not recommended and can void the warranty. If the original materials have been damaged or lost, contact BioTek for replacements (PN 7332062; see Chapter 1 for contact information).

> ❖ Packing materials are subject to change over time. If the instructions in this section do not apply to the materials you are using, please contact BioTek's Technical Assistance Center for guidance.

1. Decontaminate the reader before repackaging it. See Chapter 5 for instructions.
2. Move the carrier to its full-out position, if not already there.
3. Turn off the instrument and unplug the power supply.
4. Wrap the shipping straps around the carrier (p. 20).
5. While holding the carrier, carefully turn the instrument over and hook the shipping straps over the post in the middle of the base (p. 21).
6. Remove the four mounting screws (p. 21).
7. Lift the base off the cover and turn it right side up.
8. Install the shipping block: place the groove in the block over the shaft and attach the two mounting screws (p. 22).
9. Turn the reader over and install the top cover.
10. Turn the reader right side up, place it in the plastic bag, and attach the end caps (p. 22).
11. Place the reader with the end caps into the box. Place the accessories tray onto the end caps, and the power supply into a bubble bag. Place the power supply on the tray (p. 22).

12. Obtain a Return Materials Authorization (RMA) number from BioTek's Technical Assistance Center (see Chapter 1). Explain whether the reader requires calibration, cleaning, periodic maintenance, warranty work, and/or repair. Describe any error messages displayed and (if possible) the steps that produced them. Provide BioTek with the name and contact information of a person who may be contacted if questions arise.

13. Close the box and tape it shut. Write "RMA" and the RMA number in large, clear letters on the outside of the shipping container, and ship the instrument to BioTek.
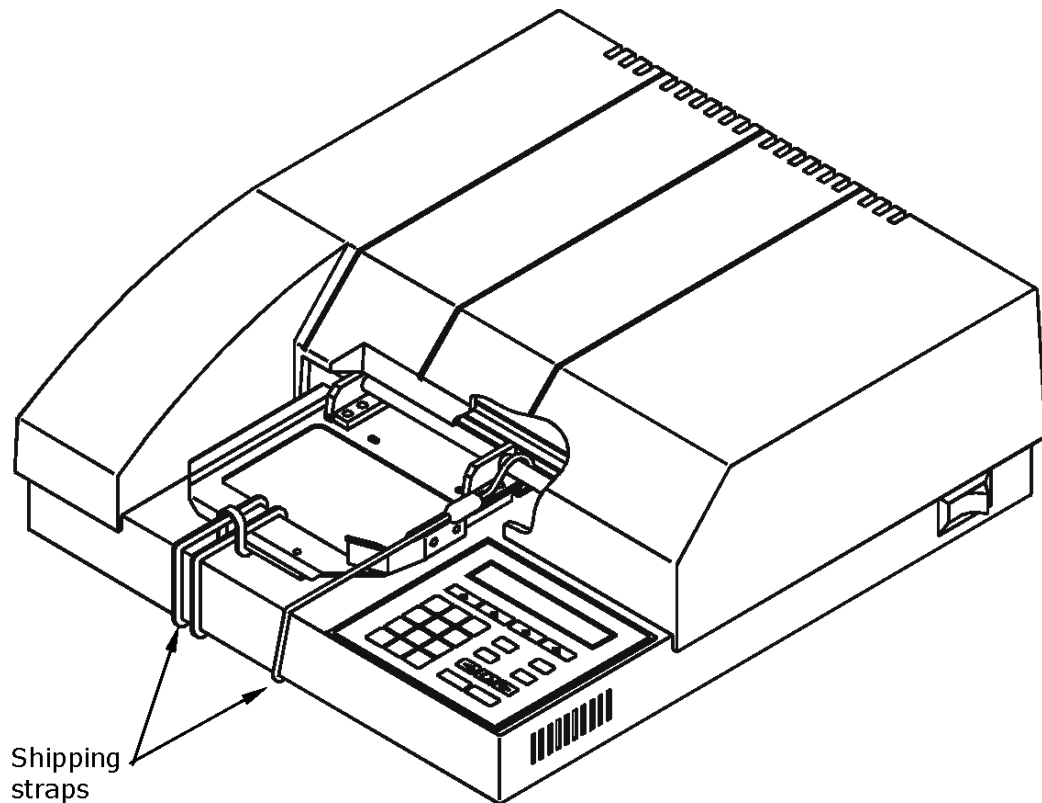
Shipping straps

Figure 5: Secure the carrier
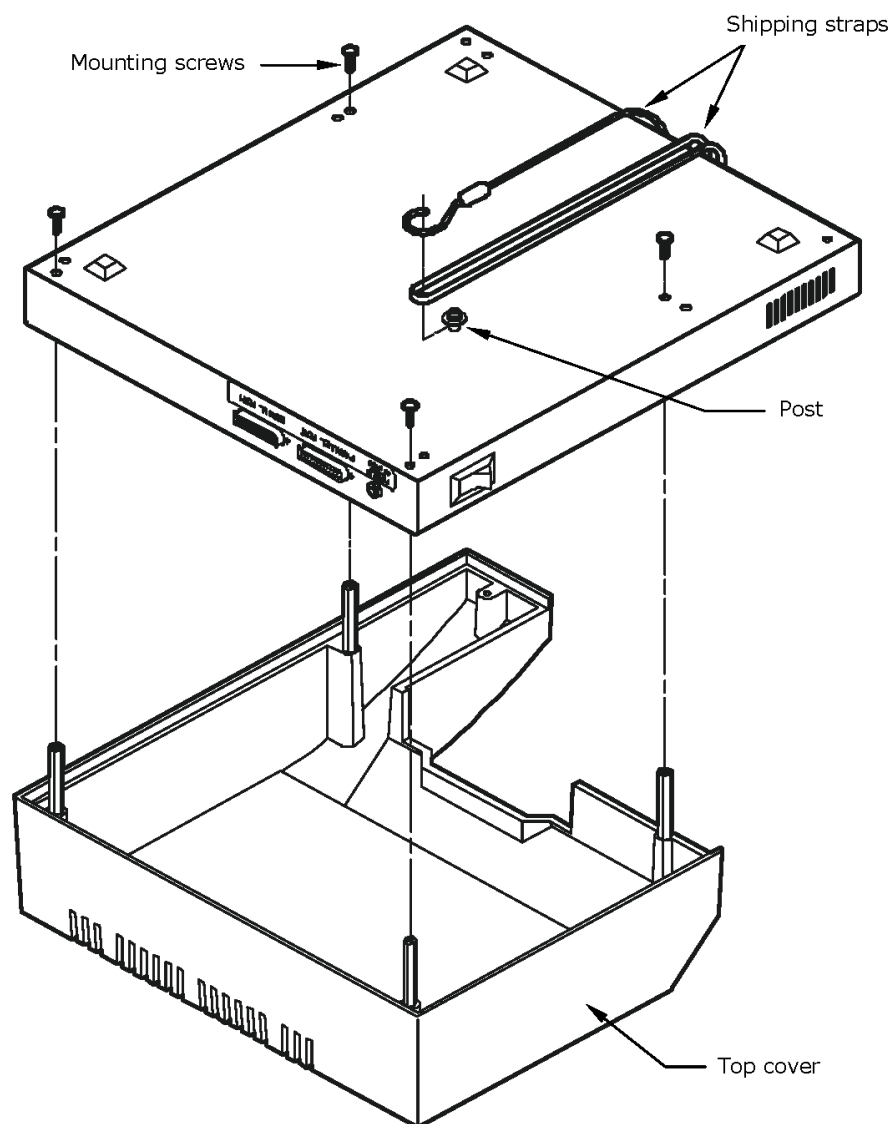
Figure 6: Attach shipping straps, remove mounting screws

Shipping block

Shaft
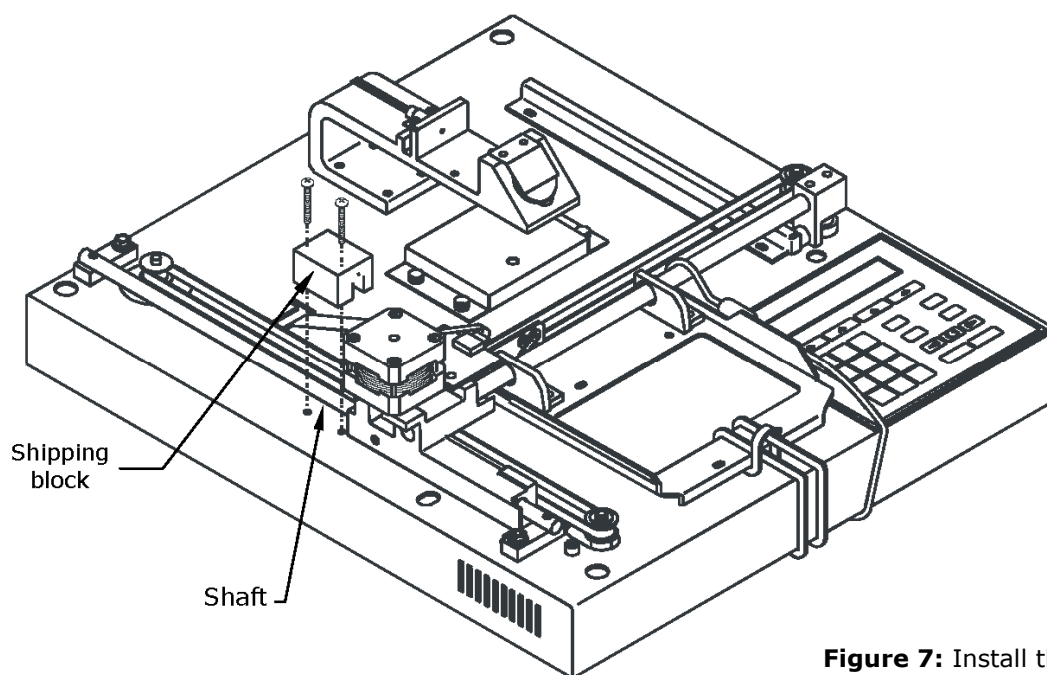
Figure 7: Install the shipping block and then replace the cover

Accessories tray
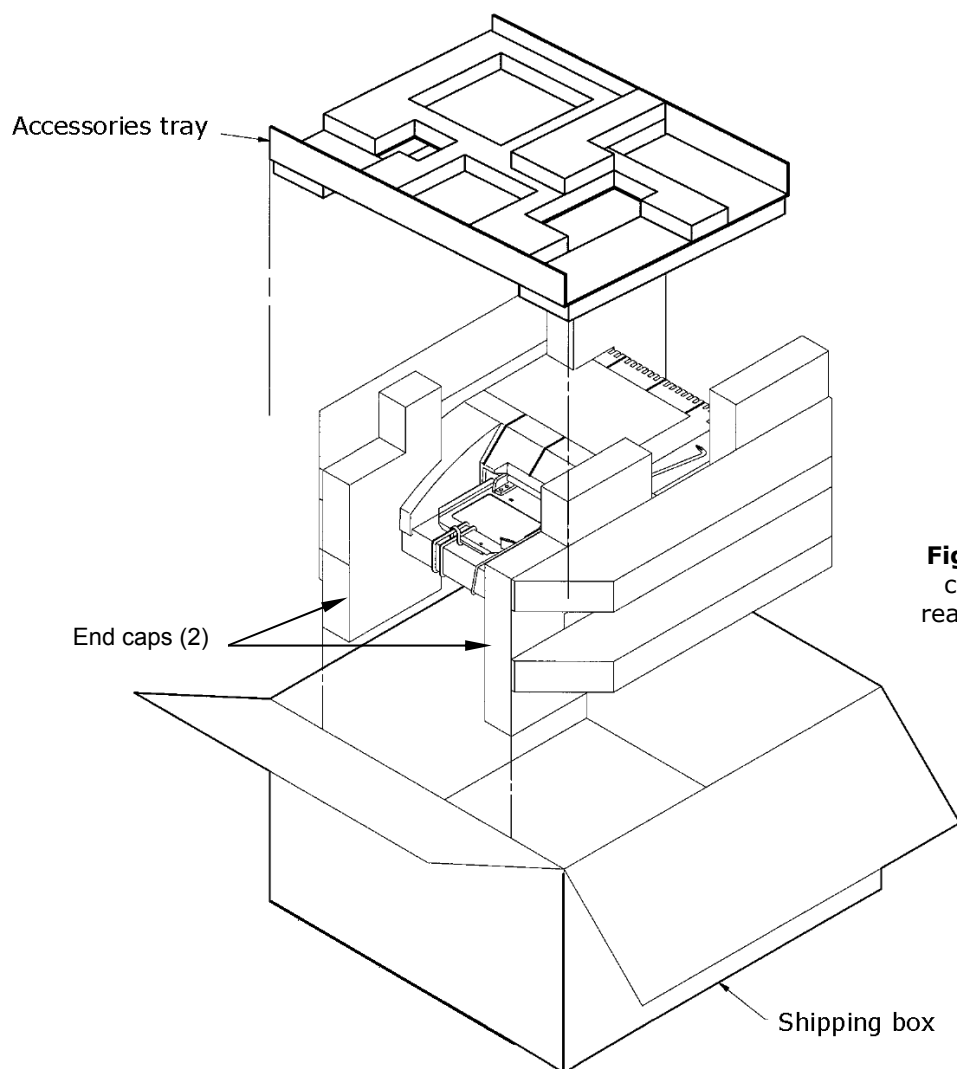
End caps (2)

Figure 8: Attach the end caps (2) and place the reader in the shipping box

Shipping box

Chapter 3

Operation

This chapter describes how to operate the reader using its keypad. Gen5, KC4, and KCjunior users should refer to *Appendix A, Computer Control* and the documentation that came with the software.

All users should read *Recommendations for Optimum Performance* at the end of this chapter.

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.

Keypad and Main Menu

The ELx800 features a keypad and 2-line x 24-character LCD display:
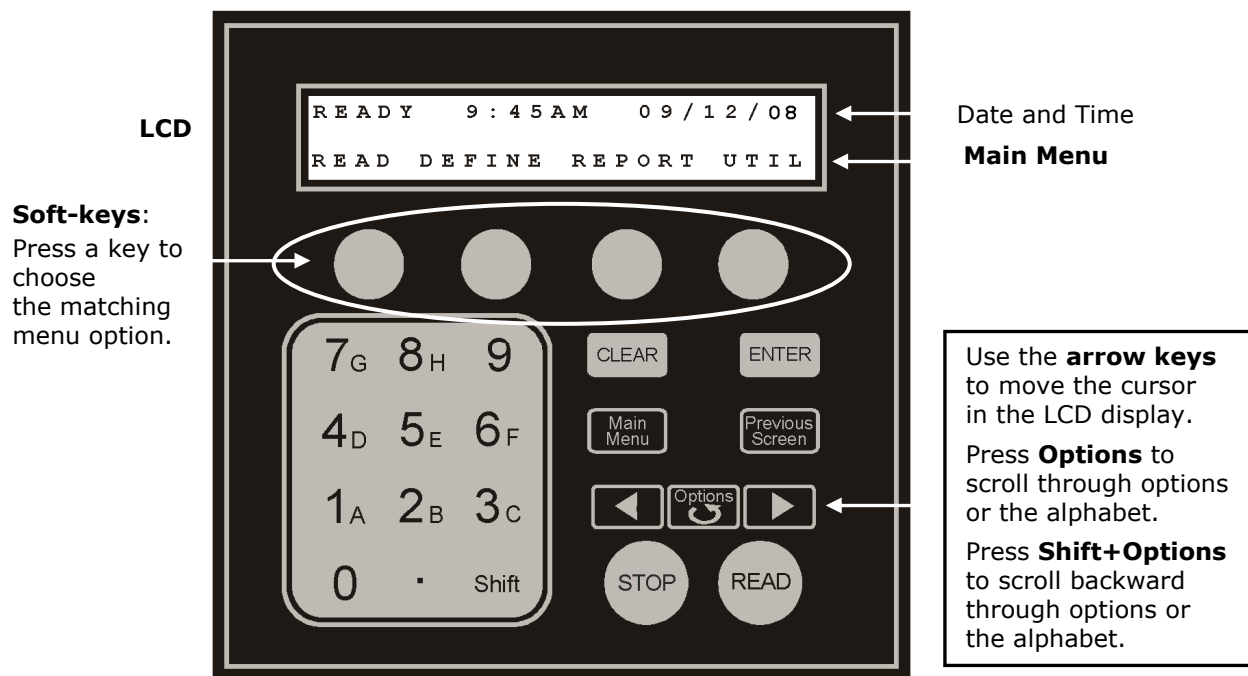
The Main Menu offers these options:

- **READ** (or, press the key labeled READ) to read a plate. You must select a preprogrammed assay or previously defined protocol.

- **DEFINE** to create or edit a reading and data reduction protocol. You will be prompted to select an existing assay. Assign a new name to create a new protocol or modify the parameters, as needed, to fine-tune the selected assay.

- **REPORT** to print stored protocol results; requires a previously run assay with valid data.

- **UTIL** to view or change settings or to run onboard utilities.

Performing a "Quick Read"

> ⓘ On some readers, assay number 01 allows for quick reading and simple programming. If available on your reader, it appears as "_Quick Read" on the display.
>
> Most of the options available in assays 02 through 55 (and described in this chapter), are unavailable for programming within the _Quick Read assay.

The default Quick Read assay settings are shown below. You can edit the wavelength(s) but the Map is fixed. Read-time prompts can be disabled via **UTIL > READ** (see page 65).

Method: Single Wavelength 405 nm (editable)

Map:

- 96-well plate geometry
- Blank on Air
- Auto mapping
- Map starting location A1
- Samples only (no blanks, standards or controls)
- Sample count prompted at runtime

To run the Quick Read assay:

1. Select **READ** at the main menu.
2. Select assay number **01_Quick Read** and press Enter.
3. Choose Single or Dual wavelengths and press Enter.
4. Select the measurement wavelength. For Dual readings, use the arrow key to move the cursor to the reference wavelength and select it. Press Enter.
5. If prompted, enter the number of samples on the plate and press Enter.
6. If prompted, enter a Plate ID and press Enter.
7. If prompted, enter a Sample ID and press Enter.
8. When the plate read is complete, **Generating Report Results** will display for a few moments and then the results report will print.

Creating or Editing an Assay

Use the **DEFINE** main menu option to specify the data acquisition and reduction parameters for a new assay, or to modify a previously defined assay. Assay parameters are organized into these categories:

Option	Description	Turn to:
METHOD	Type of reading: single or dual, wavelengths, and plate geometry.	page 28
MAP	Plate layout: the arrangement of samples, standards, controls, and blanks on the plate. Map the plate manually, or let the reader define the map automatically.	page 30
FORMULA	Data reduction steps: the cutoff, transformation, and validation formulas. Create variables to be used within formulas.	page 41
CURVE	Plotting a curve: the curve fit and x/y axis types; lin/log. Specify how to handle standard outliers. Enable or disable the extrapolation feature.	page 51

"NB" models using 384-well geometry do not have access to map, formula, or curve options. The 384-well plate type is preset for the map to have 384 samples. This does not include any blanking, controls, or standards. (Selecting 6-, 12-, 24-, 48-, or 96-well geometries enables data reduction capabilities.)

❖ The ELx800 has 55 "open" assays and Panel assay 99 available. The EL800 has 10 open assays and does not have Panel assay 99.

❖ If you are creating/editing a PANEL assay (#99), see page 54 for special instructions.
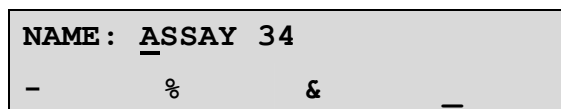
Create a New Assay

Select **DEFINE** at the Main Menu and enter the number of an unused or unneeded assay (or press the Options key until it appears). Use the keypad to assign a **unique** name (see the next page). Follow the instructions for each of the parameter categories shown above to define the assay parameters.

Edit an Existing Assay

Select **DEFINE** at the Main Menu and enter the number of the assay you wish to edit (or press the Options key until it appears). Use the keypad to edit the name, if necessary (see below). Follow the instructions for each of the parameter categories shown above to edit the assay parameters.

How to Name an Assay

```
NAME: ASSAY 34
      _
–        %        &              _
```

At the **NAME** screen when you are creating or editing an assay, you can enter up to 16 alphanumeric characters. Each assay must have a **unique** name.

- Press **Shift** + the number key for **A-H**, or scroll through the alphabet with the **Options** key for **A-Z**.

- Press **Shift + Options** to reverse direction.

- Use the arrow keys ◄ ► on either side of the **Options** key to move the cursor within the display.

- To add one of the four symbols (- % & _) in the display to the protocol name, press its Soft-key.

- Press **Enter** when you are finished to store the protocol name.

Programming Note

Assays with certain criteria may "lock up" during the "Calculating Results…" phase of data reduction (see *Beginning the Plate Read* on page 59). This can be corrected by modifying assay criteria as follows:

- Specify at least one (01) sample when mapping the plate, and/or change Prompt Sample Count to **YES**.

- Set Curve Fit to **NONE** if the assay does not use a standard curve.

1. From the Main Menu, select **DEFINE**. Select the desired assay and then select **MAP**. Press **Enter** until Enter Number of Samples is displayed. Define at least one sample. The software will lock up if zero (00) samples are selected.

2. From the Main Menu, select **UTIL > READ**, and press **Enter** until Prompt Sample Count is displayed. Select **YES**.

3. From the Main Menu, select **DEFINE > SELECT ASSAY NUMBER: > NAME > ENTER**. At the DEFINE menu, select **CURVE** to display Curve-Fit Type and select **NONE**.

Define (Method, Map, Formula, Curve)

DEFINE → METHOD

METHOD assay parameters include single or dual wavelength, wavelengths (filters), and plate geometry.

At the DEFINE options screen, select **METHOD** to begin. Assay parameter screens appear in the order that they were defined in the assay. If the assay has a closed variable (i.e., an element of the assay definition that you cannot access or modify), the screen for this variable is not displayed.

```
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
```

Single or Dual Wavelength

1. Select **METHOD** and then select **SINGLE** or **DUAL** wavelength.

2. Press **Enter** to continue. The Wavelength selection screen allows you to select **SINGLE** or **DUAL** wavelength for the assay:

   • Single measures the optical density of each well with a single filter.

   • Dual wavelength reads each well twice, each time with a different filter. Dual-wavelength readings can significantly reduce optical interference caused by scratched or dirty microplates. The microplate carrier does not exit the reading chamber between the two measurements. The final reported optical density is the difference between the two readings: measuring wavelength - reference wavelength = delta OD.

```
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
```

3. Select the measurement wavelength.

4. If applicable, press the right arrow key to move the cursor to **REF** and then select the reference wavelength.

5. Press **Enter** to move to the next screen.

Plate Type

For 6- to 384-well standard plates, the plate types and sizes included in the ELx800 software are based on the brands listed below. For best measurement results, use these brands when operating the ELx800 via its onboard software.

Plate Type	Brand
6-well	Costar
12-well	Corning
24-well	Corning
48-well	Costar
96-well	Costar
384-well*	Nunc square
96 T*	Terasaki
72 T*	Terasaki
60 T*	Terasaki

*Used only on ELx800NB models

The Plate Type selection screen allows you to select the geometry of the plate that will be used for the assay. Press ***MORE** to cycle through the available options.

6 :	6-well (2 x 3)	**96:**	96-well (8 x 12)
12:	12-well (3 x 4)	**96H:**	96-well Hellma Quartz (8 x 12)
24:	24-well (4 x 6)	**96M:**	96-well Metric (8 x 12, 9 mm well
48:	48-well (6 x 8)		spacing)

❖ "NB" models include choices for 384-well, or 72- and 96-well Terasaki plate formats. An adapter (PN 7330531) is available from BioTek if Terasaki plates are to be used.

DEFINE → MAP

MAP assay parameters include the following:

- Automatic or manual map generation
- Mapping direction
- Replication direction
- Blank map selection
- Blanking constant
- Number of blanks
- Location of blanks
- Number of standards
- Number of standard replicates

- Averaging of standards
- Concentration and location of standards
- Number of controls
- Control type definition
- Number of control replicates
- Control location
- Number of samples
- Number of sample replicates
- Sample location

At the DEFINE options screen, select **MAP** to begin the plate mapping process. Assay parameter screens appear in the order that they were defined in the assay. If the assay has a closed variable (i.e., an element of the assay definition that you cannot access or modify), the screen for this variable is not displayed.

```
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
```

Map Generation

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

```
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
```

- Select **AUTO** to instruct the software to automatically generate a plate map after the blanks, controls, standards, and/or samples have been defined.

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

- Press **Enter** to save the selection and continue.

❖ Press **SHIFT+CLEAR** to remove a previously defined manual map.

Mapping Direction

Specify how the blank, control, standard, or sample **groups** will be mapped on the plate. The well types can be listed in column format (down) or in row format (across).

```
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
```

- Select **DOWN** to map down the column.

- Select **ACROSS** to map across the row.

- Press **Enter** to save the selection and continue.

Replicate Direction

Specify how **replicates** are mapped on the plate.

```
R E P   D I R E C T I O N : A C R O S S

D O W N     A C R O S S
```

- Select **DOWN** to map the replicates down the column, following the direction of the map listing.

- Select **ACROSS** 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 continue.

Example: 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										

Example: 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												

Example: 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										

Example: 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												

Start Mapping at Well Location

This screen appears if **automatic** mapping is selected. Enter the location of the starting well for automatic mapping.

```
START   MAPPING
AT   WELL   LOCATION: A01
```

- Use the **numeric** and **alpha** keys to enter a letter or number at the cursor location. For any well location, only the **alpha** keys are active for the first character and **numeric** for the second and third characters. The valid entry range is from A01 to the last well on the plate, depending on the plate type and the number of blanks, standards, controls, and/or samples defined in the assay.

- Press **Enter** to save the well location and continue.

Blank Map

Select which blanking method to apply to the assay.

- The available blanking methods are displayed on three screens (select ***MORE** to see additional methods):

```
BLANK   MAP:        FULL
  AIR     FULL    CONST   * MORE
```

```
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
```

- Select the Blank Map method (see the descriptions below).

- Select ***MORE** to cycle through the available options: **ROW** or **COLUMN**, and **P-ACROSS** or **P-DOWN**.

- Press **Enter** to save the method and continue.

Blank Map Methods

- **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.

❖ Tip for using CONST: 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 maximum number of blanks is 48. 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 placed in each column. Since the maximum number of blanks is 12, and if all 12 columns are used, each column can have only one blank. Manual mapping is recommended in this case. Replicates follow in the same column as the first well of each sample, even if the ACROSS direction has been specified for replicates.

- **P-ACROSS** enables a blank in every even-numbered column to be subtracted from the well to the left of it in every odd column. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the odd columns.

- **P-DOWN** enables a blank in the B, D, F and H rows to be subtracted from the well above in the A, C, E and G rows. 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

This screen appears if the **Constant** blank map method 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  :     1  .  2  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 **CLEAR** to clear the value on the display.
- Press **Enter** to continue.

Number of Blanks

This screen appears when the **Full**, **Column**, or **Row** blank map method is selected. Enter the number of blank wells in the assay.

```
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, 0 to 48.
- Press **CLEAR** to clear the value on the display.
- Press **Enter** to continue.

Blank Location

This screen appears if **manual** mapping was selected. Define where the blank well or wells occur on the microplate.

```
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 **Enter** to continue.

Number of Standards

Enter the number of standard **groups** that will be used in the assay. If the number of standards is changed, the number of replicates for the standard automatically resets to 01.

```
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 logit-log; 3 for quadratic; and 2 for linear and point-to-point.
- Press **CLEAR** to clear the value on the display.
- Press **Enter** to continue.

Number of Standard Replicates

Enter the number of **replicates** per standard group in the assay.

```
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 standard replicates, from 1 to 8. 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 continue.

Average Standards

Select whether or not to calculate the average of the replicates of each standard group. This option is not available if the number of standard replicates is 01.

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

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

Standard Concentrations

Enter the predicted or expected concentration value for each standard group. If manual mapping was 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 : 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.

- If automatic mapping is selected, each replicate's location is available for viewing only. Pressing **Enter** advances to the concentration value entry for the next standard.

- If manual mapping is selected, the location must be defined. Pressing **Enter** from the standard concentration entry moves the cursor to the Location field. Pressing **Enter** from the Location field advances to the concentration value entry for the next standard.

Valid Well Locations

When defining the replicate locations, only the **alpha** keys are active for the first character and **numeric** for the second and third characters. Valid characters and numeric entries are based on the selected plate geometry.

Acceptable entries for well locations based on plate geometry:

Plate Type	Range
6-Well	A01-B03
12-Well	A01-C04
24-Well	A01-D06
48-Well	A01-F08
96-Well	A01-H12
96H	A01-H12
96M	A01-H12

Reuse of Standard Curves

The ELx800 has the ability to reuse a standard curve that has already been established.

Limitations:

- Standard curves can only be reused in assay positions 32 through 55. Each of these positions can only store one standard curve.

- Standard curves cannot be reused on panels (see page 54).

- Standard curves will be stored in memory with the Assay Name, Standard Concentrations, Replicate Counts, and Optical Density values for each standard replicate.

- Stored standard curves can only be reused for the assay that the curve was originally generated on (e.g., the curve for Assay 53 cannot be applied to samples on a plate to be run in Assay 51).

- To reuse a standard curve, you must first program an assay (in positions 32 through 55) and then run the assay. 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 will appear 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 65 for more information.)

- 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 standard's curves will be routinely reused.

Number of Controls

Enter the number of control **groups** that will be used in the assay.

```
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 control groups in the assay. For example, if the assay requires one or more positive control wells and one or more negative control wells, enter 02.

- The valid entry range depends on the number of locations on the plate that are undefined. The maximum number of control groups is 8.

Control Identifiers

Select an identifier for each control group used in the assay.

```
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
```

- Choose one control identifier for each type of control in your assay. The available options are: **P**ositive **C**ontrol**, N**egative **C**ontrol, **H**igh **P**ositive **C**ontrol, **L**ow **P**ositive **C**ontrol, **CTL1**, **CTL2**, **CTL3**, **CTL4**.

- After choosing an identifier for Control #1, press **Enter** to choose the identifier for the next control.

Number of Control Replicates

Enter the number of replicates for each control group.

```
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
```

- The well ID associated with Control #1 appears first. Press **Enter** to advance to the next control.

- Use the **numeric** keys to enter a value for the number of replicates.

- The valid entry range is from 1 to 12 replicates. The software automatically performs a check to ensure the number of replicates, multiplied by the number of controls, does not exceed the number of undefined wells remaining on the plate.

Location of Controls

Enter the location of controls in the assay.

```
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 2
```

> ❖ The location can only be edited if manual mapping was selected (see page 30).

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

Number of Samples

(Optional) Enter the number of sample **groups** on the plate. This information can also be entered at run-time (see *Selecting Read Options* on page 65).

```
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 sample **groups** on the plate. The range is 0 to the number of undefined well locations remaining on the plate. For example, 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.

Number of Sample Replicates

Enter the number of replicates for each sample group.

```
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 valid range is from 1 to 12 replicates. The software automatically performs a check to ensure that the number of replicates multiplied by the number of samples does not exceed the number of undefined wells remaining on the plate.

Sample Location

If **manual** map generation was selected and samples are defined, specify the location for each sample replicate.

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

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

DEFINE → FORMULA

❖   This feature is not available on the EL800.

The ELx800 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).

At the DEFINE options screen, select **FORMULA**. The Select Formula Type screen will appear (described on the next page).

```
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
```

Calculation Structure

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

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

ⓘ   When used in standalone mode, the ELx800NB does not have access to data reduction options for 384-well plates. To capture and manipulate the raw data, you must use Gen5, KC4, or KCjunior software. See Appendix B for more information.

Formula Type

The ELx800 supports three types of formulas, as well as the ability to define variables for use within Transformation formulas. **Note:** GENERAL formulas are not used.

```
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
```

- **CUTOFF** formulas are used to classify results. During data reduction, results are evaluated against the cutoff formulas, and each well is assigned a user-specified label (**POS**, **NEG**, or **EQUIV**).

- **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.

Formula Entry

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

❖ 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
```

```
P C ; X > 1 . 2 0 0
  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. Use the options found under Math, Other, Map, and Functn to "build" the formula.

  ➢ To cycle through the available Math, Other, Map, and Functn options, continue to press the appropriate soft key. For example, press the Math soft key several times to see +, -, *, /, %, =, and so

on. When the desired option appears, press the **right arrow** key to select it and advance to the next editable field.

> ➢ Press the **left arrow** key to move the cursor to the left.

> ➢ Press **CLEAR** to delete the item above the cursor.

> ➢ When a formula is complete, press **Enter** to continue.

- Select **MATH** to insert a mathematical symbol such as +, %, or <=.

- Select **OTHER** to insert an opening or closing parenthesis "(" or ")", or logical operators AND or OR.

- Select **MAP** to insert a well ID such as BLK;x or NC;1.

- Select **FUNCTN** to insert a mathematical function such as LOG or SQRT.

> (i) The reader software checks the formulas for errors during data reduction. A syntax error in a formula will result in a "Token Error" message on results reports.

MATH

The following mathematical symbols can be used in formulas:

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

OTHER

The following additional symbols can be used in formulas:

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

MAP

The available MAP options depend on the formula type and the current plate map. MAP options resemble **BLK;x** (mean of the blank wells), **NC;1** (the first NC well), or **OD** (every well).

FUNCTION

The following functions can be used in formulas:

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

Examples

Log10 2 = 0.301029995	ALOG (0.69314718) = 2
ALOG10 (0.30102995) = 2	LOG 2 = 0.69314718
AB (-1) = 1	SQRT 2 = 1.4142
(10 PWR 2) = 100	

Validation Formulas

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

See *Formula Type* on page 42 for instructions on selecting an assay and accessing the Select Validation Type screen.

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

Control and Blank Validation Formulas

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

- One blank validation formula can be defined.

- Up to four control validation formulas can be defined.

- Define the plate map (via **DEFINE > MAP**) before creating blank or control validation formulas.

- Blank/Control validation can be performed on individual replicates (BLK, PC), or on the group mean (BLK;x, NC;x).

Examples

If an assay protocol states that

- Each blank well on a plate should have an OD of less than 0.050, the formula is: `BLK < 0.050`

- Each Positive Control replicate must have an OD higher than 1.000, but less than 2.500, this can be accomplished with one formula: `PC>1.000ANDPC<2.500`

  Or with two separate formulas: `PC > 1.000` *and* `PC < 2.500`

- Negative Control mean must have an OD of less than 0.100, the formula is: `NC;x < 0.100`

Number of Required Controls/Blanks

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

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

- Use the **numeric** keys to enter the number of valid controls required. 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 **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.

Assay Validation Formulas

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

- Up to 4 assay validation formulas can be defined.

- Define the plate map (via **DEFINE > MAP**) before creating assay validation formulas.

Examples

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

- The mean of the negative controls must be less than 0.100. The formula is: `NC;x<0.100`

- The mean of the positive controls must be greater than the mean of the negative controls. The formula is: `PC;x>NC;x`

Transformation Formulas

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

See *Formula Type* on page 42 for instructions on selecting an assay and accessing the Transformation Formula definition screen.

- If a blanking method is selected in the assay, transformation formulas are applied to the blanked absorbance values; otherwise, they are applied to the raw data. Turn to page 41 to review the results calculation structure.

- One transformation formula may be defined per assay.

- A transformation formula can be simple (ex. **(OD/2)*100** to multiply all wells on the plate by 100), or more complex with the inclusion of a predefined Transformation Variable (see *TVAR*, below).

Simple Transformation Formulas

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

- To divide the OD in each well on the plate by 2 and then multiply by 100, the formula is: **(OD/2)*100**

Transformation Variable (TVAR)

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

```
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 to the *sample* wells only.

  SMP and any other well identifiers (**BLK**, **PC**, **NC**, **STD**, etc.) defined will become available as MAP options when building the transformation formula.

  <u>Example:</u> The assay plate map contains 2 NC wells and 2 PC wells. The remainder of the map is filled with samples. The assay data reduction requires that the mean of the NC be subtracted from all the samples on the plate. The transformation formula is: **SMP-NC;x**

- If **OD** is chosen:

  The formula definition screen will appear so that you can define a formula for use *within* the transformation formula.

  Use the formula keys (Math, Other, Map and Function) to define the Transformation Variable (TVAR). Once the variable has been defined, it can be used in a transformation formula. The TVAR will be available as a MAP option when building the transformation formula.

  <u>Example:</u>

  The assay plate map has 2 blanks, 1 control well in duplicate (**CTL1**), 1 negative control well in triplicate **(NC)**, and 5 standards in duplicate **(STD1-STD5)**. 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 mean of the **NC** less the mean of **CTL1**, and then multiply by 100.

  On paper, the formula reads:
  (OD-(NC;x-CTL1;x))/(NC;x-CTL1;x)*100

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

  1. At the **SCOPE VARIABLE** selection screen, choose **OD** and press **Enter**.

  2. Enter the formula **(NC;x-CTL1;x)** by using the **MATH, OTHER, MAP** and **FUNCTION** keys. Press **Enter**.

  3. The formula definition screen is displayed. Choose **TRANS**.

  4. Enter the formula **(OD-(TVAR)/(TVAR)*100)** using the **MATH**, **OTHER**, **MAP**, and **FUNCTION** keys. ("TVAR" is a MAP option.)

Example:

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

1. At the **SCOPE VARIABLE** selection screen, choose **OD** and press **Enter**.

2. Enter **STD1** as the TVAR formula. Press **Enter**.

3. The formula selection screen is displayed. Choose **TRANS**.

4. Enter the formula **(OD/TVAR)*100** using the **MATH**, **OTHER**, **MAP**, and **FUNCTION** keys. "TVAR" is a MAP option.

Cutoff Formulas

A cutoff formula calculates a **cutoff value** that is used for classifying samples. See *Formula Type* on page 42 for instructions on selecting an assay and accessing the Cutoff formula definition screen.

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

- One cutoff formula may be defined per assay.

- If Transformation Formulas are defined, cutoffs are based on the transformed results. Refer to "Defining Formula" on page 41 for the order in which formulas are processed.

- A cutoff formula can consist of a simple numeric value (**1.500**); a well identifier (**PC** to indicate the criterion for each of the PC replicates, or **PC;x** to indicate the average of the Positive Control replicates); 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.

Greyzone Entry

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

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

- 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 the next section for information on how calls are assigned.

Positive / Negative Calls for Cutoff

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

```
S A M P L E > C U T O F F + 0 5 % :       P O S
  P O S       N E G
```

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

Example:

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;x** (the mean of the NC wells) equals 1.000 OD
- The cutoff formula is **NC;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

<u>Example:</u>

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;x** (the mean of the STD2 wells) equals 2.000 OD
- The cutoff formula is simply **STD2;x**
- The greyzone is **10%**
- POS is selected for **SAMP>CUTOFF+10%**
- Calls are assigned to sample wells as follows:
  - ➢ **EQUIV** if the sample is greater than or equal to 1.800 *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

DEFINE → CURVE

> ❖ This feature is not available on the EL800.

CURVE assay parameters include curve fit type, editing of outliers, axis identification, and extrapolation of unknowns.

At the DEFINE options screen, select **CURVE**. Assay parameter screens appear in the order that they were defined in the assay. If the assay has a closed variable (i.e., an element of the assay definition that you cannot access or modify), the screen for this variable is not displayed.

```
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
```

Curve-Fit Type

The ELx800 supports several curve-fitting methods.

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

```
C U R V E - F I T   T Y P E :   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
```

- **LINEAR**: A simple best-fit straight line is plotted using the values of the standards.

- **QUAD** (Quadratic): A curve fit that 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**: 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. **Note:** Uses linear axis type only.

- **C-SPLINE** (Cubic Spline): A piecewise polynomial approximation consisting of joining a set of data points by a series of straight lines, which is then smoothed by using a cubic fit.

- **4-P** (4-Parameter Logistic): A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that 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. Note: Uses linear axis type only.

- **PT-PT** (Point to Point): A plot that connects each standard point with a line, with no averaging of the values to "smooth" the curve at each standard.

Edit Standard Outliers

Choose to enable or suppress the ability to edit standard outliers after the standard curve has been calculated. If enabled, the user can exclude one or more standards from the recalculation of the curve.

```
EDIT   STD   OUTLIERS:MANUAL
 NONE   MANUAL
```

- Select **NONE** to suppress the ability to edit standard outliers for this assay.

- Select **MANUAL** to enable the capability.

  - ➢ If Average Standards is set to **NO**, the 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, select **REPORT > RESULT**, select the assay, and then press **Enter**. The **EDIT STD OUTLIERS? YES/NO** prompt will appear. See *Editing Standard Outliers* on page 60 for further instructions.

X/Y Axis Type

Select the X/Y Axis Type.

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

- Select the method by which the X- and Y-axes will be scaled.

- This option is not available for the 2-P and 4-P curve-fit types; the X/Y scaling for these curves is always LIN/LIN.

- LIN/LIN axes are recommended when using the Cubic Spline fit.

Extrapolation of Unknowns

Specify whether to extrapolate the curve to evaluate samples outside of the absorbance range defined by the standards.

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

- Select **YES** to enable extrapolation; otherwise, select **NO**.

- On the printed reports, extrapolated concentrations (RSLT values) are surrounded by **< >** (e.g., **<44.425>**).

If Extrapolate Unknowns is enabled 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 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 and 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 should 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:

1. Start at the Main Menu, select **DEFINE** and choose assay number **99**.

2. The default assay name is "PANEL". Use the **alpha** and **numeric** keys to update the assay name, if desired.

```
N A M E :          P A N E L

   –              /            :        S P A C E
```

3. Press **Enter** to continue. The Number of Assays screen appears.

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

```

4. Specify the number of assays to include in the panel (1 to 8).

5. Press **Enter** to continue. The Mapping Direction screen appears. This option ensures that all assays will be mapped in the same direction.

```
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
```

6. Select **DOWN** or **ACROSS**.

7. After selecting the mapping direction of the assays, choose which assays to include in the panel. Press **Options** to cycle through the assay numbers and names, or use the **numeric** keys to enter an assay number. Press **Enter** to make a selection.

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

8. After an assay is selected, define its starting location. Use the **alpha** and **numeric** keys to choose the well location to begin the assay.

```
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
```

9. 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 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).

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

Reading a Microplate

Use the **READ** option, found at the Main Menu, to select an assay to run, define any required run-time options, and then begin a plate read.

> ❖ Before reading a plate, ensure that the reporting options are set correctly under **UTIL > OUTPUT**.

1. Start at the Main Menu and select **READ**. The Select Assay Number screen will appear.

2. Select an assay, and then press **Enter**. (See *Selecting an Assay to Run* below for detailed instructions.)

3. If required, enter the number of Samples, Plate ID, and/or Sample ID.

> ❖ The options to present these screens are configurable by selecting **UTIL > READ**. See *Selecting Read Options* on page 65 for more information.

```
ENTER    NUMBER    OF
SAMPLES:                        20
```

```
PLATE    ID:    1234A
 -            /         :        SPACE
```

```
ENTER
SAMPLE    ID:    0001
```

4. Place the plate in the carrier, and then press the **READ** key to continue.

Selecting an Assay to Run

1. Start at the Main Menu and select **READ**. The Select Assay Number screen will appear:

```
SELECT    ASSAY    NUMBER:      65
NAME: HBS-AG1
```

2. Use the **numeric** keys to enter the number of any predefined assay stored in the reader's memory, or the **Options** key to advance one assay at a time.

3. Press **Enter** to select the assay and continue.

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

If prompted, enter the number of sample **groups** on the plate. (The number of sample replicates is typically predefined in the assay, but if this is a custom assay, you may also be prompted to enter the replicate count.)

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

- Use the numeric keys to enter the number of sample groups.

- The valid entry range is from 01 to the maximum number of wells remaining on the plate after any blank, control, or standard wells are mapped.

- If you enter a value greater than the number of empty wells remaining on the plate, the reader will "beep" and automatically change the value to the maximum permissible number of samples.

Enter Plate ID

If prompted, enter a unique plate identifier to be stored in memory with the assay name and absorbance data.

```
PLATE   ID:   1 2 3 4 A
 –              /           :        S P A C E
```

- Use up to 10 alphanumeric characters. See page 39 for instructions on using the keypad.

- Press **Enter** to advance to the Enter Sample ID screen.

> ❖ Use caution when creating multiple Plate IDs. The reader does not warn you that you are about to exceed the maximum of 8 plate IDs stored in memory. If a ninth Plate ID is added, it will overwrite the first Plate ID stored in memory.

Enter Sample ID

If prompted, enter a **starting** sample identification number.

```
ENTER
SAMPLE   ID:   0 0 0 1
```

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

Prompts for Well Location

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

```
SAMPLE#   1             LOCATION
                        REP#   1 : G 0 1
```

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

- Press **Enter** again to advance to the next replicate.

Beginning the Plate Read

When the following screen appears, 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
```

- Ensure that the printer is connected, turned on, and full of paper.

- Place the plate in the carrier and press the **READ** key.

- After the read is complete, data reduction will be performed ("Calculating Results…"), and then the reports will print ("Generating Reports…").

- To halt the read in progress, press the **STOP** key.

Printing Reports

<table>
<tr>
<td>ⓘ</td>
<td>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 ELx800, this range is –3.0 OD to +3.0 OD. For assays defined onboard by a user (Assay 2 – Assay 55), it is -4.0 OD to +4.0 OD.</td>
</tr>
</table>

Reports are automatically generated after a plate has been read (see *Specifying Data Output and Reporting Options* on page 64 for information on selecting reports). Results reports also can be regenerated manually by using the REPORT option from the Main Menu. In addition, Map, Assay, and Assay List reports can be printed.

❖ See Appendix C for sample reports.

```
R E A D Y       0 1 : 2 2 P M       0 1 / 2 3 / 0 8
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 particular 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 reader.

Results Report

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

```
R E P O R T : H B S - A G
I D :   0 0 1        0 1 / 2 3 / 0 8
```

- The most recently read plate is presented first, showing the assay name, the plate ID (if one was entered), and the date the plate was read.

- Press **Options** to see the next plate in memory.

- Press **Enter** to select a plate and continue.

- If a standard curve was generated and Edit Standard Outliers was set to **manual** in the assay definition, the Edit Standard Outliers? prompt is presented; otherwise, the Print Results? prompt is presented.

Editing Standard Outliers

If a standard curve was generated and if Edit Standard Outliers was set to **MANUAL** in the assay definition, the option to edit outliers is presented.

```
E D I T   S T D   O U T L I E R S :
  Y E S      N O
```

- 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.

> (i) 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.
- Select **YES** to print reports, or **NO** to return to the Main Menu.

Map Report

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

```
S E L E C T    A S S A Y    N U M B E R : 0 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 Report

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

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

- Press **Options** to cycle through the list of available assays, or enter the number of the desired assay.

- Press **Enter** to print the report.

List Report

The List Report lists the all of the assays (name and number), currently programmed on the reader. Select **REPORT** from the Main Menu, and then select **LIST** to print the report.

Using the Utility Options

The **UTILITY** option allows you to set up the date and time, specify your data output and report options, and select your read options.

Setting the Date and Time

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

1. From the Main Menu, select **UTIL > SETUP.** The Edit Setup Information menu appears:

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

2. Select **DATE**. The Date entry screen appears:

```
D A T E :     0 8 / 2 3 / 0 9           M D Y
M M D D Y Y             D D M M Y Y
```

- Enter the new date using the **numeric** keys on the keypad. The cursor is positioned under the first editable field and advances automatically.

- To change the date format, press the soft key beneath **MMDDYY** or **DDMMYY**. The display automatically updates to reflect the new format.

- Press **Enter** to return to the Edit Setup Information menu.

3. To change the current time and/or the time format, select **TIME** from the Edit Setup Information menu. The Time entry screen will appear:

```
T I M E :       1 2 : 4 7 P M     1 2 H O U R
1 2 H O U R         2 4 H O U R   A M / P M
```

- Enter the time using the **numeric** keys on the keypad. The cursor is positioned under the first editable field and advances automatically.

- To change the time format, select **12HOUR** or **24HOUR**, then **AM** or **PM**. The display automatically updates to reflect the new format.

- Press **Enter** to return to the Select Utility Option menu.

Viewing/Editing the Filter Table

After installing new filters, ensure that the ELx800's filter table (the reader's software reference for filter locations) matches the physical location of the filters in the filter wheel.

1. From the Main Menu, select **UTIL > SETUP > FILTER**.

2. The wavelength for Filter #1 will be displayed. To change a filter wavelength value, use the keypad to enter a number at the cursor location. The cursor will automatically advance to the next editable location. Press the **Enter** key to save the value and move to the next filter.

```
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
```

3. After entering the last filter, press the Main Menu key.

Specifying Data Output and Reporting Options

❖ These options have no effect on data output if the reader is being controlled by software (such as Gen5) running on a host computer.

Plate data can be sent to an attached printer or external computer.

- The onboard software provides several options for report format and content.

- Data sent to an external computer has no data reduction applied to it, with the exception of dual-wavelength subtraction (if defined in the assay). Any reporting options selected through the onboard software have no effect on serial output.

1. To specify data output and reporting options, start at the Main Menu and select **UTIL > OUTPUT.** The Report Output screen will appear.

```
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
```

- Select **PRINT** to send reports directly to a printer, **COMPUTER** to send data out through the serial port, or **BOTH.**

2. Press **Enter** to continue. The Select Printer screen will appear.

```
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
```

❖ The ELx800 supports printers using HP's PCL3 language, such as the HP DeskJet series. For the latest list of compatible printers, consult the BioTek website, or call BioTek TAC.

3. Select **HP** and press **Enter** to continue. The Report Type screen will appear.

```
R E P O R T    T Y P E :        C O L U M N
C O L U M N        M A T R I X    B O T H
```

4. The currently selected report type is displayed in the top line. Select **COLUMN** to print information in a list (columnar) format, **MATRIX** to print in a format that resembles the plate type (ex. 8 x 12 matrix), or **BOTH.**

> ❖ See Appendix C for sample reports.

5. Press **Enter** to continue. The Samples on Column Report screen will appear.

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

6. Select **YES** to print results for all wells on the plate, including samples, or **NO** to limit the results information to blanks, controls, and standards.

7. Press **Enter** to continue. If applicable, the Print Curve-Fit screen will appear.

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

8. Select **YES** to print the standard curve (only applies to quantitative assays).

9. Press **Enter** to return to the Select Utility Option screen.

Selecting Read Options

The software can be configured to present the user with a series of prompts at plate-read time, to enter information such as Plate ID, Sample ID, or Sample Count.

1. To specify various read-time options, start at the Main Menu and select **UTIL > READ.**

- The Prompt for Plate ID screen will appear. Select **YES** or **NO** and press **Enter** to cycle through the remaining prompts.

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

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

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

If selected, at read-time:

- **PLATE ID** prompts for microplate identification.

- **SAMPLE ID** prompts for identification for each sample group.

- **SAMPLE COUNT** prompts for the number of samples on the plate.

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

- **RAPID MODE** reads a 96-well plate in approximately 30 seconds (single-wavelength assay). If set to NO, the plate will be read in Normal Mode (approximately 50 seconds). See Chapter 1 for specifications.

Changing the Baud Rate

To change the baud rate from the instrument keypad:

1. Select **UTIL > SETUP > RS232.**

2. The Select Baud Rate screen will appear, showing the currently defined Baud Rate:

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

- Select the desired baud rate.

- Select **VIEW,** if you wish, to see the reader's other communication settings.

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

To change the baud rate (or other communications settings) in KC4 or KCjunior software, refer to the KC4 or KCjunior User Guides, or to *Appendix A, Computer Control*.

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 reader supports standard flat, U-bottom, and V-bottom microplates, optimum performance is achieved with optically clear, flat-bottomed wells.

- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual-wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.

- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µL per well in a 96-well plate and 25 µL in a 384-well plate (EL800NB model).

- ELx800NB model only: Dispensing solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies; however, for best results, remove the air bubbles by degassing the plate in a vacuum chamber before reading.

- The inclination of the meniscus can reduce reading 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.

Chapter 4

Instrument Qualification

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

Overview

This chapter contains BioTek Instruments' recommended Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) procedures for all models of the ELx800 Absorbance Microplate Reader.

Every ELx800 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 ELx800 Product Qualification Package (PN 7330538) 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 ELx800 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 Performance and Operational 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. 76	✓	✓	✓	
Checksum Test, p. 76	✓			
Absorbance Plate Test, p. 77		✓	✓	
Liquid Test 1, p. 79*		✓		✓
Liquid Test 2, p. 81*		✓		✓
Liquid Test 3, p. 84** (Optional, for 340 nm)		✓		✓

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

** Liquid Test 3 is optional. Run it if your worksite requires qualification 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, KC4, or KCjunior 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, KC4, or KCjunior). 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 Optics Test confirms that the light levels and electronic noise at all set wavelengths fall within factory accepted criteria, and accomplishes this by measuring the air and dark readings and evaluating them to ensure they fall within specified ranges. The Optics test also confirms adequate photodiode sensitivity, and overall system cleanliness.

- The Autocal Analysis is run only at the factory 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.

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**). When run manually, the software displays the part numbers and versions of software currently loaded on the reader. If the test fails, the reader will "beep" repeatedly and display an error code; see Chapter 6.

```
                   Gen5 System Test Report

Reader:             ELx800  (Serial Number: 203828)
Basecode:           P/N 7330202  (v3.07)
Date and Time:      4/23/2009 4:20:15 PM
User:               Administrator
Company:
Comments:


Test Results
Operator ID:_____
Notes:_____

04:21PM   04/23/09    SYSTEM SELF TEST

Filter: 405        Gain:  5.33
Air:               54279
Dark:               1055
Delta:             53224


Filter: 450        Gain:  2.19
Air:               54802
Dark:               1053
Delta:             53749


Filter: 490        Gain:  1.80
Air:               54703
Dark:               1053
Delta:             53650


Filter: 630        Gain:  2.25
Air:               54626
Dark:               1053
Delta:             53573


Filter: 340        Gain: 85.33
Air:               41582
Dark:               1091
Delta:             40491


Noise Max:         1071
Noise Min:         1069
Delta:                  2

AUTOCAL ANALYSIS

Upper Left Corner:    x=
Lower Left Corner:    x=
Lower Right Corner:   x=
Upper Right Corner:   x=        514     y=10806
Delta 1:   9198   - 9200=       -2
Delta 2:    514   -  516=       -2
Delta 3:  10806   -10814=       -8
Delta 4:  16340   -16346=       -6

SYSTEM TEST PASS
-000
```

System Optics Test

AUTOCAL ANALYSIS provides coordinates of the last Autocal performed on the instrument. AUTOCAL 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 9: Sample System Test report (it varies slightly depending on how it was run)

Absorbance Plate Test

This test uses BioTek's Absorbance Test Plate to confirm the Mechanical Alignment, Accuracy and Linearity, and Repeatability of the ELx800. 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 BioTek's 7-Filter (PN 7260522) Absorbance Test Plate with its accompanying Standards Certificate, shown below.

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

WAVELENGTH (nm)

Well	405nm	450nm	490nm	550nm	620nm	630nm	690nm	750nm
C1	0.147	0.140	0.135	0.130	0.136	0.136	0.127	0.134
E2	0.618	0.575	S A M P L E			.668	0.485	0.434
G3	1.133	1.052				.040	0.881	0.783
H6	1.701	1.578				.560	1.323	1.179
F5	2.279	2.024	1.976	1.956	1.893	1.865	1.537	1.272
D4	2.945	2.604	2.545	2.513	2.437	2.400	1.972	1.632

Set # 2453   **Serial # 161259**

Figure 10: Sample Standards Certificate for the Absorbance Test Plate

❖ The Test Plate has a glass filter in location C6, which is used to check the wavelength accuracy of a monochromator. Although the ELx800 does not have a monochromator, you must enter values for the peak wavelength when defining the 7-filter test plate in Gen5, KC4, or KCjunior software. Refer to the software help system for more information.

```
Alignment Results:
     WELLS        B2       B12       G1       G11
     DATA       0.000     0.000    0.000    0.000
     TOLERANCE  0.015     0.015    0.015    0.015
     RESULT      PASS      PASS     PASS     PASS

Wavelength = 405 nm

Accuracy Results:
     WELLS        C1       E2       G3       H6       F5       D4
     STANDARD   0.147    0.618    1.133    1.701    2.279    2.945
     MIN. VALUE 0.124    0.586    1.090    1.647    #N/A     #N/A
     MAX. VALUE 0.170    0.650    1.176    1.755    #N/A     #N/A
     DATA       0.148    0.616    1.129    1.695    2.243    2.850
     RESULT      PASS     PASS     PASS     PASS    #N/A     #N/A

Repeatability Results:
     WELLS        C1       E2       G3       H6       F5       D4
     READ1      0.148    0.616    1.129    1.695    2.243    2.850
     MIN. VALUE 0.137    0.600    1.108    1.668    #N/A     #N/A
     MAX. VALUE 0.159    0.632    1.150    1.722    #N/A     #N/A
     READ2      0.149    0.616    1.130    1.695    2.242    2.853
     RESULT      PASS     PASS     PASS     PASS    #N/A     #N/A
```

Figure 11: Sample Test Plate Analysis Report

> ❖ The report varies slightly depending on how it was run (via the keypad, Gen5, KC4, or KCjunior).
>
> ❖ **Important!** The Absorbance Plate Test tests the accuracy and repeatability specifications from 0.000 to 2.000 OD only. In the example above, the Analysis report displays the OD values read in well positions D04 and F05 but does not indicate PASS or FAIL, because the values are higher than 2.000 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 Standards Certificate 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 TACenter. Please have a copy of the test and the reader's serial number ready.

- **Mechanical Alignment:** The Test Plate has precisely machined holes to confirm the mechanical alignment of different microplate readers. The amount of light that shines through these holes indicates whether the reader is properly aligned. A reading of more than 0.015 OD for any of the designated alignment wells indicates that the light is being "clipped" and the reader may be out of alignment. If the test fails:

  ➢ Ensure that the Test Plate is correctly seated in the microplate carrier, with "A1" in the upper left corner. Check the four alignment holes to ensure that they are clear of debris.

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

- **Accuracy and Linearity:** The Test Plate contains neutral density glass filters that have assigned OD values at several wavelengths. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is shown that the device is accurate at these OD values then, by definition, it is linear. **Note:** There may not be a Pass/Fail indication for filter values that are beyond the specified accuracy range of the instrument. 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 Standards Certificate.

  ➢ Ensure that the Test Plate is within its calibration certification period.

- **Repeatability:** This test ensures that the instrument meets its repeatability specification by reading each Test Plate neutral density filter twice with the filter in the same location. **Note:** There may not be a Pass/Fail indication for filter values that are beyond the specified accuracy (and, thus, repeatability) range of the device. If the test fails:

  ➢ Check the filters on the test plate to ensure that there is no debris that may have shifted between readings and caused changes.

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

Liquid Tests

Conducting Liquid Tests confirms the reader'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, whereas the test plate's neutral density glass filters do not. The optics characteristics may differ in these two cases, thus alerting the operator to different types of problems. The liquid tests will help to detect optical defects or contamination that can contribute to errant readings.

- **Liquid Test 1** confirms repeatability and consistency of the reader when a solution is used in the microplate. If these tests pass, then the lens placement and system cleanliness are proven.

- **Liquid Test 2** can be used to test the linearity, repeatability, and alignment of the reader if you do not have an Absorbance Test Plate.

- **Liquid Test 3** is an optional test, and can only be performed with the "UV" model. It is offered for those sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test is optional since the ELx800 has good "front end" linearity throughout the specified wavelength range.

Perform the Tests

Run the System Test

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

Using Gen5, KC4, or KCjunior, select the following in the appropriate software:

- Gen5: **System > Diagnostics > Run System Test**
- KC4: **System > Diagnostics > Run Optics Test**
- KCjunior: **Utilities > Diagnostics > Reader System Test**

Using the instrument keypad:

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

Run the Checksum Test

There is no Checksum Test in Gen5, KC4, or KCjunior. To obtain reader software information select the following in the appropriate software:

- Gen5: **System > Reader Control > ELx800**. To obtain Gen5 software information, from the main screen select **Help > About Gen5**.
- KC4: **Reader Control > Information**. To obtain KC4 software information, from the main screen select **About > About KC4**.
- KCjunior: **Help > About KCjunior**. For the Reader Control version, click the **Reader Control Version** button.

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.

Run the Absorbance Plate Test

Define the Test Plate Parameters

Using Gen5, KC4, or KCjunior (click the Help button for additional instructions):

- Gen5: **System** > **Diagnostics** > **Test Plates** > **Add/Modify Plates**, and click **Add**.

- KC4: **System** > **Diagnostics** > **Define Universal Plates** > **Add**.

- KCjunior: **Utilities** > **Diagnostics** > **Universal Plate Test**. Click **New Data Sheet**.

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 Standards Certificate. 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, KC4, or KCjunior (click the Help button for additional instructions):

- Gen5: **System** > **Diagnostics** > **Test Plates** > **Run**.

- KC4: **System** > **Diagnostics** > **Run Universal Plate Test**.

- KCjunior: **Utilities** > **Diagnostics** > **Universal Plate Test**. Select the **Universal Plate ID (Wavelength)**.

Using the keypad:

1. From the main menu 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 73) will be sent to a printer when the test is complete.

Perform the Liquid Tests

Prepare Test Solutions

BioTek offers a dye solution (PN 7120779, 25 mL; or 7120782, 125 mL) for use with 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 µL in a flat-bottom microplate well.

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

- 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

- Precision balance with 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.

Solution B

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. Make up to 100 mL with DI water; cap and shake well.

> (i) 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 µL of the **concentrated** solution into Column 1 of the plate.
3. Pipette 200 µL 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 (Chapter 3), single wavelength, no blanking.

5. Print the five sets of raw data or export it to an Excel spreadsheet using Gen5, KC4, or KCjunior.

> ❖ If you are exporting the data to your own Excel spreadsheet, perform the calculations described below and keep the spreadsheet for future tests.

Repeatability Specification:

For comparison in this test, the following repeatability specification is applied, using the Normal Read Mode and a 96-well microplate.

± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

Calculations:

1. Calculate the mean and standard deviation for each well location in columns 1 and 2 for the five plates read.

2. For each mean below 2.000 OD, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 0.5% ± 0.005 OD.

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.

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:

- New 96-well, flat-bottom microplates (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)

Prepare Dilutions:

1. Set up a rack containing 10 tubes, numbered consecutively.
2. If you have not already done so, prepare concentrated Stock Solution A or B.

   Refer to the table below when executing steps 3 and 4.

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 µL	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

3. Create a percentage dilution series, beginning with 100% of the concentrated stock solution in tube 1, 90% of the concentrated solution in tube 2, 80% in tube 3, and so on to 10% in tube 10.
4. Dilute using amounts of the remaining 0.05% solution of deionized water and Tween® 20, as shown in the table above.

Prepare the plate:

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

> ❖ 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 Test

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

> ❖ Do not discard the plate; you will use it for the Alignment Test.

2. Print the raw data or export it to an Excel spreadsheet using Gen5, KC4, or KCjunior.

> ❖ If you are exporting the data to an Excel spreadsheet, perform the calculations described below and on the following page, and keep the spreadsheet for future tests.

Linearity Test:

1. Calculate the mean absorbance for each well, and average the means for each concentration.

2. Perform a regression analysis on the data to evaluate linearity.

   ### Expected Results

   Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Square value greater than or equal to 0.990 is considered adequate.

Repeatability Test:

1. Calculate the mean and standard deviation for the five readings taken above at each concentration. 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 below 2.000 OD, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 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

For comparison in this test, the following repeatability spec is applied, using Normal mode, 96-well microplate:

± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @405 nm

Alignment Test:

1. Using the prepared plate, conduct a turnaround test by reading the plate with the A1 well in the H12 position five times. This test results in values for the four corner wells that can be used to determine alignment.

2. Calculate the means of wells A1 and H1 in the normal plate position (data is from Linearity Test) and in the turnaround position (from Step 1 above). Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the accuracy specification for the instrument.

   For example: If the mean of well A1 in the normal position is 1.902, where the specified accuracy is ± 1.0% ± 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% = 0.019 + 0.010 = 0.029, which is added and subtracted from 1.902 for the range.)

3. If the four corner wells are within the accuracy range, the reader is in alignment.

Accuracy Specifications:

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

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

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 ELx800 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 A **or** B

Solution A: 10x Concentrate Phosphate Buffered Saline (PBS)

Required Materials:

- Deionized water
- Ingredients shown in the table below
- 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. **Note:** Manufacturer part numbers are subject to change over time.

1. Prepare the stock buffer solution using the ingredients below:

PBS 10X Concentrate Solution

KH_2PO_4 anhydrous	0.2 grams
NaCl	8.0 grams
Na_2HPO_4 anhydrous	1.15 grams
KCl	0.2 grams
Tween® 20	0.5 mL
Add DI water to bring to	100 mL

2. Mix 5 mL of the concentrated PBS solution with 45 mL of DI water.

3. Add 10 mg of the β-NADH powder and mix thoroughly. This is the **10x Concentrate PBS Solution**.

Solution B: Sigma PBS

Required Materials:

- Deionized water

- Tween® 20 (polyoxyethylene (20) sorbitan monolaurate)

- Sigma® PBS 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 (prepared from the Sigma tablets) with 10 mg of the β-NADH powder and mix thoroughly. This is the **Sigma PBS Solution**.

Perform the Test

1. Check the absorbance of a sample of buffer solution A or B at 340 nm on the microplate reader. This solution, which will be referred to as the **100% Test 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 solution is at least at the lower end of this range. Do not adjust if slightly high.

2. Carefully prepare a **75% Test Solution** by diluting 15 mL of the <u>100% Test Solution</u>:

   - If using the <u>Sigma PBS Solution</u>, use 5 mL as the diluent.

   - If using the <u>10x Concentrate PBS Solution</u>, mix one part of the concentrate with nine parts of DI water. Use 5 mL of this solution as the diluent.

3. Carefully prepare a **50% Test Solution** by diluting 10 mL of the <u>100% Test Solution</u>:

   - If using the Sigma PBS Solution, use 10 mL as the diluent.

   - If using the 10x Concentrate PBS Solution, mix one part of the concentrate with nine parts of DI water. Use 10 mL of this solution as the diluent.

4. Pipette the three solutions into a new 96-well microplate:

   - 150 µL of the 100% Test Solution into all wells of columns 1 and 2

   - 150 µL of the 75% Test Solution into all wells of columns 3 and 4

   - 150 µL of the 50% Test Solution into all wells of column 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 raw data or export it to an Excel spreadsheet using Gen5, KC4, or KCjunior.

> ❖ If you are exporting the data to an Excel spreadsheet, perform the calculations described below and keep the spreadsheet for future tests.

Repeatability Test

1. For each well, calculate the mean and standard deviation of the five readings.

2. For each mean calculated in step 1, calculate the allowed deviation using the repeatability specification for a 96-well plate in the Normal Read Mode, which is ± 1.5% ± 0.005 OD from 0.000 to 2.000 OD @ 340 nm (mean * 0.015 + 0.005).

3. For each well, compare the standard deviation calculated in step 1 with the allowed deviation calculated in step 2. The standard deviation should be less than the allowed deviation.

   For example: Absorbance readings of 0.802, 0.802, 0.799, 0.798, and 0.801 will result in a mean of 0.8004 and a standard deviation of 0.0018. The mean multiplied by 1.5% (0.8004 * 0.015) equals 0.012, and when added to the 0.005 (0.012 + 0.005) equals **0.017**, which is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.017, the well meets the test criteria.

Linearity Test

1. For each of the three dye concentrations, 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.

Expected Results:

Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Square value greater than or equal to 0.990 is considered adequate.

Chapter 5

Preventive Maintenance

This chapter contains procedures for maintaining and decontaminating the ELx800.

Overview

A daily preventive maintenance routine is the best way to ensure accurate performance and a long life for your instrument. Frequent cleaning of the microplate carrier and all exposed surfaces of the instrument will help to reduce the amount of particulates or dust that can cause erroneous readings.

⚡	**Warning! Internal Voltage**. Always turn off and disconnect the reader from the power supply for all cleaning operations.
⚠	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 eader. If this happens, contact the BioTek Service Department.
	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.
	Do not apply lubricants to the microplate carrier or carrier track. Lubrication on the carrier mechanism or components in the carrier compartment will attract dust and other particles, which may obstruct the carrier path and cause the reader to produce an error.

Routine Cleaning Procedure

You will need a mild detergent, deionized or distilled water, and clean, lint-free cotton cloths.

1. Turn off and disconnect the instrument from the power supply.
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 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.

Decontamination Procedure

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 into 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.

ⓘ	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.
⚠	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. 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. The bleach solution is caustic; wear gloves and eye protection when handling the solution.

Tools and Supplies

- Sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as an alternative to bleach)
- Deionized or distilled water
- Safety glasses

- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags
- 125 mL beakers
- Clean, lint-free cotton cloths

Procedure

1. Turn off and unplug the instrument.

2. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.

   - Check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10.0% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; if this is the case, prepare a 1:10 dilution.

3. Moisten a cloth with the bleach solution or alcohol. Do not soak the cloth.

4. 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.

5. Wipe the plate carrier and all exposed surfaces of the instrument.

6. 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.

7. Use a clean, dry cloth to dry all wet surfaces.

8. Discard the used gloves and cloths using a Biohazard trash bag and an approved Biohazard container.

Filter Storage and Handling

To properly store interference filters during extended periods of non-use, package the filters in a light-tight envelope or container, away from high humidity. 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.

Replacing and Aligning the Bulb

The lamp is rated for an average life of 600 hours. The intensity of the lamp will slowly drop over time until the run-time self check detects a low signal level and the instrument displays an error code. The bulb should be replaced and aligned at this time.

- For standard model readers, order replacement bulb kit PN 7330513.
- For UV and NB model readers, order replacement bulb kit PN 7330516.

The kits contain complete instructions for replacing and aligning the bulb. See page 92 for a detailed view of the lamp assembly.

Warning! The lamp will be hot immediately after the reader has been shut down. Allow the lamp to cool down for at least 10 minutes before attempting to replace the bulb.

Warning! The alignment procedure requires you to observe the light path while the bulb is turned on. To prevent possible vision impairment, avoid looking directly at the bulb while it is on.

Replace and Align the Bulb

Follow the instructions provided with the bulb replacement kit.
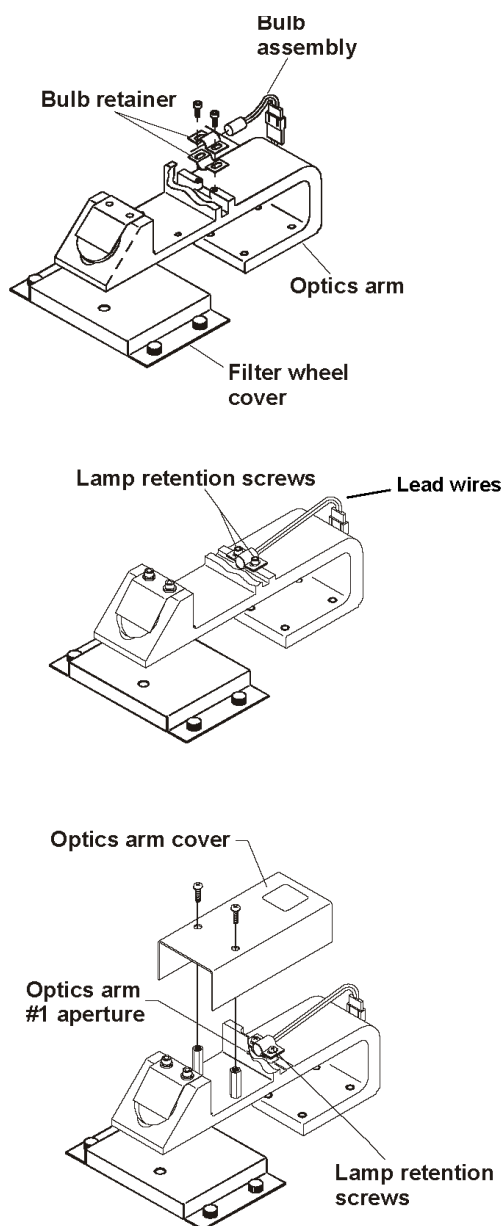
Figure 12: Lamp assembly

> ❖ An optics arm cover is only used in UV/NB models. The standard model of the reader does not include the cover.

Chapter 6

Troubleshooting and Error Codes

This appendix describes error codes that may be displayed in Gen5, KC4, or KCjunior, or on the ELx800, and suggests possible solutions for these problems.

Overview

An error code is displayed as a four-digit identifier. The first character will be 0, 1, 2, or A.

- "0", "1", or "2" indicates a noncritical or General Error. When this occurs, the instrument should still respond to input. Look up the error code on the following pages and try to resolve the error based upon the description/instructions offered for the code. If you cannot resolve the problem, contact BioTek's Technical Assistance Center.

- "A" indicates a Fatal Error. If this occurs, turn off the instrument, look up the error code on page 99, and contact BioTek's Technical Assistance Center.

The fourth digit of an error code typically signifies a particular motor sensor, optical sensor, or channel. This table lists the sensor/channel represented by the fourth digit:

Motor or Optical Sensor	Channel
0 = Carrier X-Axis	0 = Reference
1 = Carrier Y-Axis	1 = Measurement

When contacting BioTek (see Chapter 1), please provide the following information:

- Error code(s) displayed

- Reader serial number (on the back of the instrument)

- Reader software information: Part number, version number, checksum, and if applicable, Gen5, KC4, or KCjunior software information. See Chapter 4 for instructions for obtaining software versions.

General Error Codes (0100-1300)

General errors indicate nonfatal conditions that require attention.

Code	General Error Codes - Description and Probable Causes
0100	Abort The read or task has been aborted.
0101	Abort Error The read or task has been aborted. The 0101 indicates a software abort. Probable Causes: • User aborted read from KC4. • User aborted from another serial interface.
0200	X-axis (carrier in/out) motor did not find the opto (home) sensor A motor was unable to move to its "home" position as registered by feedback from an optical sensor. Probable Causes: • Defective or broken optical sensor. • Flex cable is disconnected or damaged. • X-axis movement is limited so that the optical sensor cannot be interrupted. Note: In cases where a sensor is not functioning, the motor will drive the axis to its mechanical stop and generate substantial noise.
0201	Y-axis (carrier left/right) motor did not find the home sensor A motor was not able to move to its "home" position as registered by feedback from an optical sensor. Probable Causes: • Defective or broken optical sensor. • Sensor not connected. • Y-axis movement is limited so that the optical sensor cannot be interrupted.
0202	Filter wheel did not find opto (home) sensor Probable Cause: • Failed lamp.
0300-0301	Light beam not found During the X-axis movement, the light beam (saturation) transition (max light to no light) was never found during autocalibration. Probable Causes: • Loose belt, loose motor pulley, or defective motor drive causing the carrier to ignore movement instructions. • Failed lamp. • Loose filter wheel drive gear or defective motor controller PCB.
0302	Filter wheel failed positional verify; lamp failed • A failed lamp generates a 0302 error on power-up.

Code	General Error Codes - Description and Probable Causes
0400	Carrier X-axis movement failed positional verify Motor X-axis failed to get to the same position when moved a known number of steps from the home position and back. Probable Causes: • The belt has slipped because of incorrect tension, a loose motor pulley, or a loose belt clamp. • Defective motor drive.
0401	Carrier Y-axis movement failed positional verify Motor X-axis failed to get to the same position when moved a known number of steps from the home position and back. Probable Causes: • The belt has slipped because of incorrect tension, a loose motor pulley, or a loose belt clamp. • Defective motor drive.
0402	Filter wheel failed positional verify; lamp failed Probable Causes: • Optical trigger flag has moved or is loose. • Filter wheel is binding against the motor gear. • Defective motor drive. • Bearings caused motors to fail.
0500	Filter not installed Probable Causes: • Empty filter location on the filter wheel (all filter locations must have either a filter or a filter blank, BioTek PN 2872086). • Entire filter wheel is not installed.
0601 to 0605	Filters #1 through 5 Gain out of range Errors 0601 through 0605 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. Probable Causes: • A missing, defective, or misplaced filter or filter blank will cause this error. Check to ensure that the filters in the wheel match the filters programmed in the software. Look for obvious physical defects in the filters. • Defective lamp providing reduced signal, causing the error. Check that the lamp is lit when the reader is ON. Replace lamp if necessary. • Misaligned optics.

Code	General Error Codes - Description and Probable Causes
0700	Reader failed noise test Significant variations in background electronic noise were detected when blocking the light and increasing the gain to maximum. Probable Causes: • External signals may be penetrating the measurement chamber. Ensure that the bottom and top shrouds, as well as the filter wheel cover, are correctly installed. • A faulty motherboard or daughter board. • Photodiode or power supply may be too close to the instrument. • Failure indicates excessive variation in the dark current (background) noise levels of the measurement circuit.
0800	Reader failed offset test During self-test, the background electronic signal detected is outside of acceptable limits at maximum gain when blocking the light. Probable Causes: If noise Max is > 20000. • Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened. • There may be an ambient light leak. Ensure that the plate carrier door is properly closed. • Faulty motherboard or daughter board. • Photodiode or power supply may be too close to the instrument. • A missing or loose filter may be allowing unintended lamp light into the measurement chamber. Check the filter wheel for empty positions.
0900	Read time dark value out of range The dark current value taken during the current read is significantly different than the same reading taken during the power-up self-check. Probable Cause: • The measurement electronics background noise has changed since the last power-up self-check. Electrical noise may be 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 may have increased since power-up. • Faulty motherboard or daughter board.
0A01- 0A05	Read time Air Blank out of range The blank (full signal) reading taken during the current read has changed significantly from the same reading taken during the power-up self-check. Probable Cause: • Lamp is unstable. Reboot system and/or install new lamp. Turn unit OFF when not in use to conserve lamp life.
0B00	Invalid assay An assay number that is not programmed was selected.

Code	General Error Codes - Description and Probable Causes
0C00	Printer timed out The time allotted for the instrument to make a valid connection to a printer has expired. Note: This error only appears in readers shipped before April 2004, and that have not had a basecode update. Probable Causes: • Printer not connected, on line, or powered up. • Printer parallel port may not be correctly selected.
Errors 0D00 through 1300 indicate that the reader has failed one or more of a number of self-tests. Contact BioTek's Technical Assistance Center for instructions. Refer to Chapter 1 for contact information.
0D00	Calibration checksum error Reader failed calibration checksum test. Probable Cause: • The stored checksum value for the calibration data does not match the actual checksum.
0E01- 0E05	Wavelength not found in table The specified wavelength is not detected in the instrument's filter table. The last number is the filter set number in the assay protocol. Probable Causes: • Assay wavelength does not match the filter table. Run system test. Verify that wavelengths in the filter wheel match settings in the filter table (see Chapter 2).
0F00- 0F05	Filter signal out of range The filter (1-5) has a signal that is out of range. Probable Causes: • This indicates a light level problem with one or more filters. • A "UV" filter may have been installed in a non-UV instrument. • A blank filter may be incorrectly installed in a filter position. • Verify that filters in the filter wheel match settings in the filter table (see Chapter 2).
1000	Configuration data missing Required reader configuration data was missing at the beginning of the self-test. Possible Causes: • Necessary configuration data was not downloaded or was downloaded incorrectly.
1100	Failed configuration checksum test The stored checksum value from the configuration data does not match the actual checksum of the current configuration data. Probable Cause: • The configuration data has changed and the checksum stored is no longer valid. The error is produced when outdated versions of assay definition software are used to create an assay configuration file. • File is incompatible with the operation code within the reader's memory. Recreate the assay definition using the correct version of assay definition software and re-download the software.

Code	General Error Codes - Description and Probable Causes
1200	Autocalibration data missing No Autocal data exists for the read location. Probable Cause: • Autocalibration was not performed following assay definition download.
1300	Motor not homed successfully If the motor is not correctly homed, the instrument sends an error and exits the function. Probable Causes: • Errors 0200 or 0300 have been ignored. Refer to the Probable Causes for these errors.

Fatal Error Codes (A100-A900)

Fatal Errors indicate conditions that require immediate attention. If a fatal error code is displayed, contact BioTek's Technical Assistance Center for further instructions. Refer to Chapter 1 for contact information.

Code	Description
A100	Task control block not available
A200	Read already in progress
A300	Device not available
A304	Printer device not available
A400	Failed code checksum on power-up
A500	Code flash readback timed out
A600	Quick flash configuration timed out
A900	Memory Manager corruption

Appendix A

Computer Control

This Appendix provides basic instructions for installing and configuring Gen5, KC4, or KCjunior software for control of the ELx800 reader.

Controlling the ELx800 with Gen5

Before installing Gen5 software, verify that your computer meets the minimum system requirements specified in the Gen5 Getting Started Guide.

Setting Up Gen5

These instructions briefly describe how to set up Gen5 for operation of the reader. Refer to the Gen5 Getting Started Guide or Help system for more detailed instructions.

1. Turn off the computer and the reader. Connect the appropriate serial cable between the two machines.

2. Turn on both machines.

3. Install Gen5 on the computer and register the software with BioTek.

4. Start Gen5. Login if prompted. The default System Administrator password is **admin**.

5. If a "Welcome to Gen5" screen appears, select **System Menu**.

6. From the main screen, select **System > Reader Configuration** to open the Reader Configuration dialog.

7. Click the **Add** button to open the Reader Settings dialog.

8. Use the Reader Type drop-down list to select **ELx800**.

9. Enter the appropriate **Com Port**. Gen5 requires a baud rate of 9600 for the ELx800.

10. Click **Test Comm.** Gen5 will attempt to communicate with the reader.

    - If the test is successful, click **OK** and **OK** again to save the settings. Click **Close** at the Reader Configuration dialog to return to the main screen.

    - If the test is not successful, refer to the *Problems* section below, or to the *Troubleshooting* section of the Gen5 Help system for assistance.

Problems

If Gen5 cannot communicate with the reader and displays an error, try the following:

- Confirm that the correct Reader Type was selected in step 8.

- Try a different COM port.

- Check the serial cable connections. Ensure that the cable is properly attached to the port defined in step 9.

- Confirm that the reader has passed its System Test and is at the main menu.

Refer to the Troubleshooting section in the Gen5 Help system for further assistance.

Getting Started with Gen5

These instructions describe how to create and run an Experiment in Gen5.

1. If the "Welcome to Gen5" screen appears, select **Read a Plate** and skip to step 4. Otherwise, select **File > New Experiment** from the main view.

2. Select **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.

   Gen5 and Gen5 Secure: If two readers are configured in Gen5, the Instrument Selection dialog appears. Select **ELx800** and click **OK**. The Procedure dialog will appear.

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 the **Help** button for assistance.

9. When complete, click **OK** to return to the Procedure dialog.

10. Click **Validate** to check the defined parameters. If any parameters are invalid, Gen5 will provide information for correcting the problem.

11. Click **OK** to save and close the Procedure dialog. The Plate Reading dialog will open.

12. Enter any desired information, place the plate on the carrier, and then click **READ** to begin the plate read. If the Save As dialog opens, enter a File name, choose a file location, and click **Save**.

13. 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.

    ❖ Gen5 Reader Control does not support data reduction.

Controlling the ELx800 with KC4

Before installing KC4 software, verify that your computer meets the minimum system requirements specified in the KC4 User's Guide or Help system.

Setting Up KC4

These instructions briefly describe how to set up KC4 for operation of the reader. Refer to the KC4 User's Guide or Help system for more detailed instructions.

1. Turn off the computer and the reader. Connect the appropriate serial cable between the two machines.

2. Turn on both machines.

3. Install KC4 on the computer and register the software with BioTek.

4. Start KC4. Login if prompted.

5. Select **System > Readers**. Scroll through the list of Available Readers and select the appropriate reader model. Click the **Port** button (and subsequent **Setup** button), to define the following communications parameters:

Port:	Select the COM port used for the RS-232 serial cable connection
Transmission Speed:	9600 (default), 1200, or 2400 (must match the baud rate on the reader)
Data Bits:	8
Parity:	No
Stop Bits:	2

6. Click the **Current Reader** button to attempt to establish communication with the reader, using the currently defined communication parameters.

7. If successful, click **OK** to save the settings and close the dialog box. If not successful, KC4 will provide appropriate instructions for resolving any problems.

Problems

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

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

Getting Started with KC4

These instructions describe how to read a plate using KC4.

1. Select **Data > New Plate.**

2. If prompted to select a protocol, select **Empty Protocol** and click **OK**. If not prompted, select **Protocol > New,** or use the Protocol Wizard to step through protocol creation.

3. Select **Protocol > Reading.** The Reading parameters dialog will appear.

4. Select a **Reading Type.**

5. Define the **Filters** (wavelengths) at which the plate will be read.

6. Select a **Plate Type** from the drop-down list.

7. Define other reading parameters as necessary. Click the **Help** button for assistance.

8. When complete, click **OK.**

9. Select **Data > Read Plate.** The Plate Reading dialog will appear.

10. Enter any comments, place the plate on the carrier, then click **START READING** to begin the plate read.

    *   The plate will be read and then the raw data results will display in KC4.

    *   To analyze, manipulate, or print results, Protocol parameters should be defined. Refer to the KC4 Help system or User's Guide for instructions.

Controlling the ELx800 with KCjunior

Before installing KCjunior software, verify that your computer meets the minimum system requirements specified in the KCjunior User's Guide or Help system.

Setting Up KCjunior

These instructions briefly describe how to set up KCjunior for operation of the reader. Refer to the KCjunior User's Guide or Help system for more detailed instructions.

1. Turn off the computer and the reader. Connect the serial cable between the two machines.

2. Turn on both machines.

3. Install KCjunior on the computer and register the software with BioTek.

4. Once installed, start KCjunior.

5. Select **Setup**, then **Reader1** or **Reader 2**. To select the reader and define the communications parameters, choose the following setup parameters:

Reader:	ELx800, EL800, or ELx800UV, EL800UV, or ELx800NB, EL800NB
Com Port:	COM1 or COM2 (the serial port used for the RS-232 cable connection)
Baud Rate:	9600 is the recommended rate
Data Bits:	8
Parity:	None
Stop Bits:	2
EOT Character:	Keep the default number.

6. Click the **Test Communications** button to attempt to establish communications with the reader, using the currently defined communication parameters. If a Serial Write Error dialog box is displayed, an incorrect Com Port may have been selected. Select a different port and then repeat this step.

7. If the test passes, click **OK** to save the settings and close the dialog box. If the test fails, follow the directions provided by KCjunior, then click **Test Communications** again.

Problems

If KCjunior cannot communicate with the reader, check the cable plug-in location to make sure it matches the setup choices and is not a Null cable. If this is suspected, add another Null and try again.

Getting Started with KCjunior

These instructions describe how to read a plate using KCjunior.

1. Click **Read Plate** from KCjunior's main screen. The Read Plate Dialog will appear.

2. If desired, enter a Results ID and a Plate Description, and then click **Read Plate.** The Protocol Definition dialog will appear.

3. Select a **Read Method Type.**

4. Define the wavelength(s) at which the plate will be read.

5. Select a **Plate Geometry** from the drop-down list.

6. Define other reading parameters as necessary. Click the **Help** button for assistance.

7. When complete, click **OK** to return to the Read Plate Dialog. If desired, enter a **Plate ID.**

8. Place the plate on the carrier, then click **OK** to start the plate read.

   - The plate will be read and then the raw data results will display in KCjunior.

   - To analyze, manipulate, or print results, protocol parameters should be defined. Refer to the KCjunior Help system or User's Guide for instructions.

Appendix B

Using 384-Well Geometry

When operated through the keypad, the ELx800NB does not have access to data reduction options for 384-well plate geometry. To capture and manipulate the raw data, you must use Gen5, KC4, or KCjunior software. This Appendix provides instructions for setting up and performing a 384-well plate read for data reduction in Gen5, KC4, and KCjunior.

Perform a 384–Well Plate Read in Gen5

These instructions only apply to the ELx800NB model.

1. If you have not already done so, perform the instructions in *Appendix A* under *Setting Up Gen5*.

2. If the "Welcome to Gen5" screen appears, select **Read a Plate** and skip to step 4. Otherwise, select **File > New Experiment** from the main view.

3. Select **Default Protocol** and click **OK**. Gen5 will open the Experiment workspace, which includes the Protocol menu tree and Plate screen.

4. Select **Plate > Read** or click the Read Plate icon. The Procedure dialog will open.

   Gen5 and Gen5 Secure: If two readers are configured in Gen5, the Instrument Selection dialog appears. Select **ELx800** and click **OK**. The Procedure dialog will appear.

5. Set the Plate Type to **384 WELL PLATE**.

6. Click **Read** to open the Read Step dialog.

7. Select a **Read Type**.

8. Select or enter the wavelength(s) at which the plate will be read.

9. Define other reading parameters as desired. Click the **Help** button for assistance.

10. When complete, click **OK** to return to the Procedure dialog.

11. Click **OK** to save and close the Procedure dialog. The Plate Reading dialog will open.

12. Enter any desired information, place the plate on the carrier, and 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**.

13. 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.

Perform a 384-Well Plate Read in KC4

These instructions only apply to the ELx800NB model.

1. If you have not already done so, perform the instructions in *Appendix A* under *Setting Up KC4*.

2. Select **Data > New Plate.**

3. If prompted to select a protocol, select **Empty Protocol** and click **OK**. If not prompted, select **Protocol > New,** or use the Protocol Wizard to step through protocol creation.

4. Select **Protocol > Reading.** The Reading parameters dialog will appear.

5. Select a **Reading Type.**

6. Define the **Filters** (wavelengths) at which the plate will be read.

7. Set the **Plate Type** to **<u>384 WELL PLATE</u>**.

8. Define other reading parameters as necessary. When complete, click **OK.**

9. Select **Data > Read Plate.** The Plate Reading dialog will appear.

10. Enter any comments, place the plate on the carrier, then click **START READING** to begin the plate read.

    - The plate will be read and then the raw data results will display in KC4.

    - To analyze, manipulate, or print results, Protocol parameters should be defined. Refer to the KC4 Help system or User's Guide for instructions.

Perform a 384-Well Plate Read in KCjunior

These instructions only apply to the ELx800NB model.

1. If you have not already done so, perform the instructions in *Appendix A* under *Setting Up KCjunior*.

2. Click **Read Plate** from KCjunior's main screen. The Read Plate Dialog will appear.

3. If desired, enter a Results ID and a Plate Description, and then click **Read Plate.** The Protocol Definition dialog will appear.

4. Select a **Read Method Type.**

5. Define the wavelength(s) at which the plate will be read.

6. Set the **Plate Geometry** to **16x24**.

7. Define other reading parameters as necessary. Click the **Help** button for assistance.

8. When complete, click **OK** to return to the Read Plate Dialog. If desired, enter a **Plate ID.**

9. Place the plate on the carrier, then click **OK** to start the plate read.

   - The plate will be read and then the raw data results will display in KCjunior.

   - To analyze, manipulate, or print results, protocol parameters should be defined. Refer to the KCjunior Help system or User's Guide for instructions.

Appendix C

Report Format

This appendix contains examples of reports that can be generated and/or printed from the ELx800. See *Printing Reports* in *Chapter 3* for details on how to print these reports.

Overview

> (i) **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 ELx800, this range is -3.0 OD to +3.0 OD. For assays defined onboard by a user (Assay 02 – Assay 55), it is -4.0 OD to +4.0 OD.

The following pages contain examples of reports that can be generated and/or printed directly from the reader. For details on how to print these reports, refer to *Specifying Data Output and Reporting Options* in Chapter 3.

In addition, an Assay List, Assay Definition, Map, and Result can be printed by choosing **REPORT** from the Main Menu.

Bio—Tek Instruments

Assay:Open Assay 04   Date:03/16/04   Lot: _____
                             Time:01:05:34PM   Operator:
Wavelength:405   Temp: Plate ID:

COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
A CALL	VAL	POS	POS	POS	NEG	POS	EQUIV	NEG	NEG	POS	POS	POS
CalcOD	0.109	0.085	0.090	0.610	1.501	0.112	0.846	1.465	2.524	0.216	0.187	0.279
Well	PC	SMP7	SMP15	SMP23	SMP31	SMP39	SMP47	SMP55	SMP63	SMP71	SMP79	SMP87
RSLT												
B CALL	VAL	EQUIV	POS	POS	NEG	EQUIV	NEG	EQUIV	POS	POS	NEG	NEG
CalcOD	0.119	1.054	0.091	0.105	1.672	1.007	1.939	0.828	0.715	0.372	1.675	1.625
Well	PC	SMP8	SMP16	SMP24	SMP32	SMP40	SMP48	SMP56	SMP64	SMP72	SMP80	SMP88
RSLT												
C CALL	POS	EQUIV	POS	POS	NEG	EQUIV	POS	POS	NEG	POS	NEG	POS
CalcOD	0.290	1.092	0.243	0.109	2.291	1.000	0.127	0.470	2.129	0.218	1.636	0.372
Well	SMP1	SMP9	SMP17	SMP25	SMP33	SMP41	SMP49	SMP57	SMP65	SMP73	SMP81	SMP89
RSLT												
D CALL	POS	NEG	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	POS
CalcOD	0.301	2.075	0.237	0.133	2.643	2.032	0.635	0.663	0.198	1.656	1.626	0.523
Well	SMP2	SMP10	SMP18	SMP26	SMP34	SMP42	SMP50	SMP58	SMP66	SMP74	SMP82	SMP90
RSLT												
E CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	POS	POS	EQUIV	POS
CalcOD	2.205	2.064	2.473	0.092	0.134	2.023	0.149	0.254	0.658	0.244	0.829	0.488
Well	SMP3	SMP11	SMP19	SMP27	SMP35	SMP43	SMP51	SMP59	SMP67	SMP75	SMP83	SMP91
RSLT												
F CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	NEG	POS	EQUIV	POS
CalcOD	2.811	3.214	2.381	0.651	0.589	3.856	0.283	0.753	3.803	0.355	0.880	0.328
Well	SMP4	SMP12	SMP20	SMP28	SMP36	SMP44	SMP52	SMP60	SMP68	SMP76	SMP84	SMP92
RSLT												
G CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	POS	EQUIV	POS	POS
CalcOD	3.499	3.663	2.857	0.088	0.183	3.776	0.150	0.207	0.213	1.151	0.260	0.215
Well	SMP5	SMP13	SMP21	SMP29	SMP37	SMP45	SMP53	SMP61	SMP69	SMP77	SMP85	SMP93
RSLT												
H CALL	NEG	EQUIV	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	3.225	1.045	2.889	0.090	0.149	0.100	0.532	0.158	0.712	0.225	0.721	0.238
Well	SMP6	SMP14	SMP22	SMP30	SMP38	SMP46	SMP54	SMP62	SMP70	SMP78	SMP86	SMP94
RSLT												

Samples with calls on a Matrix Report

Bio-Tek Instruments

Assay:ASSAY 01

Wavelength:450

Date:03/16/04
Time:02:27:57PM
Temp:

Lot: _____
Operator:
Plate ID:

COMMENTS

Standard Curve
(Linear Fit)

```
        0.310-                                                      +
             -
             -
             -
             -
        0.266-
             -
  A          -
  B          -
  S          -
  O   0.221-
  R          -
  B          -
  A          -
  N          -
  C   0.177-
  E          -
             -
             -
             -
             -
        0.133-
             -
             -
             -
             -
        0.089-+-------+-------+-------+-------+
            6.250                              12.500
```

CONCENTRATION   (   )

Curve: y = Ax + B
Coefficients: A=0.035 B=-.132
R-Sqr: 1.0000

Curve Fit Report

```
                    Bio-Tek Instruments
Assay:Open Assay 04              Date:03/16/04      Lot: _____
                                 Time:01:05:34PM    Operator:
Wavelength:405                   Temp:              Plate ID:
```

COMMENTS

Interpretation of Results

```
    GREYZONE=0.20              =0.200
    CUTOFF=1                   =1.000


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

    PC : PC > .01
```

Well	ID	BlkdOD	CalcOD	Call	Pred Conc	RSLT	Std Dev	CV%	Notes:
A01	PC	0.109	0.109	VAL					
B01	PC	0.119	0.119	VAL					
AVE		0.114	0.114				0.007	6.163	
C01	SMP0001	0.290	0.290	POS					
D01	SMP0002	0.301	0.301	POS					
E01	SMP0003	2.205	2.205	NEG					
F01	SMP0004	2.811	2.811	NEG					
G01	SMP0005	3.499	3.499	NEG					
H01	SMP0006	3.225	3.225	NEG					
A02	SMP0007	0.085	0.085	POS					
B02	SMP0008	1.054	1.054	EQUIV					
C02	SMP0009	1.092	1.092	EQUIV					
D02	SMP0010	2.075	2.075	NEG					
E02	SMP0011	2.064	2.064	NEG					
F02	SMP0012	3.214	3.214	NEG					
G02	SMP0013	3.663	3.663	NEG					
H02	SMP0014	1.045	1.045	EQUIV					
A03	SMP0015	0.090	0.090	POS					

Page 1

Samples with calls on a Column Report

```
                  Bio-Tek Instruments

Assay:Open Assay 04              Date:03/16/04      Lot: _____
                                 Time:01:05:34PM    Operator:
Wavelength:405                   Temp:              Plate ID:
                                                    _____

COMMENTS


Interpretation of Results

    GREYZONE=0.20              =0.200
    CUTOFF=1                  =1.000


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

    PC : PC > .01

 _____
 Well ID          BlkdOD   CalcOD   Call    Pred Conc  RSLT    Std Dev  CV%      Notes:
 _____
 A01  PC          0.109    0.109    VAL
 B01  PC          0.119    0.119    VAL
 AVE               0.114    0.114                              0.007    6.163

End of Report
```

A Column Report, without samples

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					

Page 1

Panel Report

```
01:47PM       03/16/04
_____

NAME:                    Open Assay 04
READ TYPE:               ENDPOINT
PLATE TYPE:               96
WAVELENGTH:              405
BLANK MAP:               AIR
CURVE-FIT TYPE:          LINEAR
EDIT STD OUTLIERS:       NONE
X/Y AXIS TYPE:           LIN
EXTRAPOLATE UNKNOWNS?    NO
_____


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

Assay Detail Report (Sheet 1 of 2)

```
                    Assay List
                    Version: 3.7
    _____

          1       ASSAY    01
          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
         31       ASSAY    31
         32       ASSAY    32

         33       ASSAY    33
         34       ASSAY    34
         35       ASSAY    35
         36       ASSAY    36

         37       ASSAY    37
         38       ASSAY    38
         39       ASSAY    39
         40       ASSAY    40
         41       ASSAY    41

         42       ASSAY    42
         43       ASSAY    43
         44       ASSAY    44
         45       ASSAY    45

         46       ASSAY    46
         47       ASSAY    47
         48       ASSAY    48
         49       ASSAY    49

         50       ASSAY    50
         51       ASSAY    51
         52       ASSAY    52
         53       ASSAY    53
         54       ASSAY    54
         55       ASSAY    55
```

Assay Detail Report (Sheet 2 of 2)

Appendix D

Comparison of the ELx800 and the EL800

Feature	ELx800	EL800	ELx800UV	EL800UV
340-750 nm			x	x
405-750 nm	x	x		
Single- and dual-wavelength reading	x	x	x	x
6-well plates	x			
12-well plates	x			
24-well plates	x		x	
48-well plates	x		x	
96-well plates	x	x	x	x
Edit Assay Name	x			
Absorbance Values	x	x	x	x
Formulas	x		x	
Curve fits	x		x	
# Assays available	55	10	55	10
# Results stored	8	1	8	1
Blanks and samples only in MAP		x		x
Blanks, standards, controls and blanks in MAP	x		x	
Panel Assay	x		x	
Computer Control capability*****	x	x	x	x
Serial and Parallel output	x	x	x	x
Customizable Assays	x		x	

*All **EL800** versions can be computer controlled and will function as ELx800's under computer control via Gen5 Software.

Appendix E

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 ELx800 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

This sample demonstrates the use of transformations and cutoffs.

Intended Use

This assay is designed for the in vitro screening of human 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 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 human 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 human serum. Ready to use.

- ANA Cutoff Control: 1 bottle containing 1.8 mL of diluted stabilized human serum. Ready to use.

- ANA Negative Control: 1 bottle containing 1.8 mL of diluted stabilized human serum. Ready to use.

- ANA Conjugate: 1 bottle containing 12 mL of peroxidase labeled antibody to human 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 µL.

- Multichannel Pipette: Recommended for dispensing 100 µL 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 ELx800

From the Main Menu, select **DEFINE**. Select the assay name and edit the name if desired. At the DEFINE menu:

STEP	COMMENTS
To program the reading method, press:	
METHOD	
WAVELENGTH (Dual or Single): Single	
MEASURE (Wavelength): 450	
PLATE TYPE: 96	
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

STEP	COMMENTS
NUMBER STDS: 00	
NUMBER CTLS: 03	
CONTROL 1: PC	
CONTROL 2: CTL1	Suggested choice for the cutoff control
CONTROL 3: NC	
NUMBER OF REPLICATES	
PC: 01	
CTL1: 03	
SAMPLES: 91	User-defined; recommendation is to fill the entire plate
SAMPLE REPLICATES: 01	
Use the MAP and MATH keys to create the control validation formula:	
FORMULA	
VAL	
CONTROL:	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.
PC;x > 1.200	
NO. OF REPLICATES: 01	
NC;x < 0.300	
NO. OF REPLICATES: 01	
To create the plate transformation for CALC, divide the ANA result by a cutoff standard.	
FORMULA	
*MORE	
TRANS-VAR	
SCOPE VARIABLE (SMP or OD)	Select OD to advance to the formula definition screen and define the transformation variable (Tvar)
FORMULA	
CTL1;X	Defining TRANS VAR = CTL1;X isolates the OD value for CTL1;X for use in transformation
TRANS:	
FORMULA: (OD/TVAR)*10	Converts all OD values on plate to "ANA Result" per kit insert instructions
To define a cutoff formula for Positive and Negative calls:	
FORMULA	
CUTOFF: 10.0	
GREYZONE: 00%	
SAMPLE > CUTOFF + 00%: POS	

Sample Human Anticardiolipin IgG Enzyme Immunoassay Kit

This sample demonstrates the use of a standard curve and cutoffs.

Intended Use

This assay is intended for the in vitro measurement of IgG anticardiolipin antibodies in human 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 precoated 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 human 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 human 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.

- Cardiolipin IgG Positive Control: 1 bottle containing 1.2 mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.

- Cardiolipin Negative Control: 1 bottle containing 1.2 mL of diluted human 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.

- Plate 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 µL.

- Multichannel Pipette: Recommended for dispensing 100 µL 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 GPLU/mL	Negative result
> 11 GPLU/mL	Positive result

Programming the Assay on the ELx800

From the Main Menu, select **DEFINE**. Select the assay name and edit the name if desired. At the define menu, follow steps below:

STEP	COMMENTS
To program the reading method, press:	
METHOD	
WAVELENGTH (Dual or Single): Single	
MEASURE (Wavelength[s] to use): 450	
PLATE TYPE: 96	
To program the plate map, press:	
MAP	
AUTO	
DOWN	
DOWN	
A01	

STEP	COMMENTS

BLANK MAP: AIR
 	
NUMBER STDS: 05
 	
NUMBER STD REPLICATES: 01
 	
CONCENTRATIONS:
      STD1: 6.25
      STD2: 12.5
      STD3: 25
      STD4: 50
      STD5: 100
 	
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:	Kit instructions specify that samples with concentration values greater than 11 should appear as positive. The ELx800 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	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.