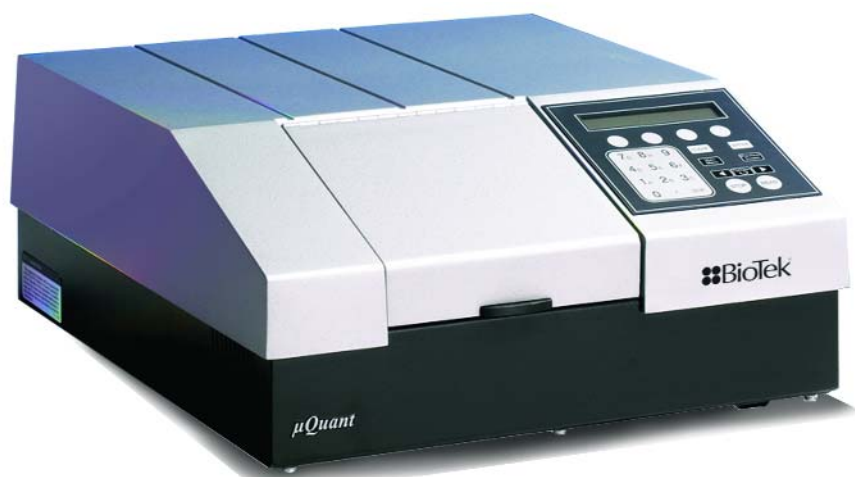


μ Quant™

Operator's Manual



μQuant™

**Microplate Spectrophotometer
Operator's Manual**

May 2006

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Part Number 7271000

Revision I

BioTek® Instruments, Inc.

Notices

BioTek® Instruments, Inc.

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Document Conventions

This manual uses the following typographic conventions:

Example	Description
	These icons call 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

Revision	Date	Changes
A	7/98	Release to Production
B	7/98	Changed λ accuracy from ± 3 nm to ± 2 nm (pages 1-6 and 4-16).
C	9/98	Added reference to 384-well microplate in Specifications (page 1-6).
D	2/99	Revised Appendix B, Computer Control. Added Appendix E, Error Codes. Revised compatible printer in Chapter 2.
E	7/02	Added references to new microplate geometries (6- and 12-plate) throughout manual. Deleted section on Reuse of Standard Curves, Chapter 3. Revised Preface, Chapters 2 and 4, and Appendices B and E. Edited format and text as necessary.
F	10/03	Updated Notices with current contact information. Updated Intended Use to distinguish between European Union and all other jurisdictions. Updated Warranty to include Bio-Tek's current warranty statement. Clarified bleach dilution solutions for Decontamination.
G	5/04	<p>General:</p> <ul style="list-style-type: none"> - Restructured layout of manual. Edited and reformatted text according to new template. - Modified appearance of display screens. - Changed "Abs" to "OD" throughout. <p>Preface:</p> <ul style="list-style-type: none"> - Updated information in Notices (page ii). - Updated contact information (page ix). - Expanded the Intended Use Statement (page xv). - Updated all user safety sections (pages xvi-xxi). <p>Chapter 1, Introduction:</p> <ul style="list-style-type: none"> - Updated descriptions of features and package contents (pages 3 and 4). - Clarified Specifications (pages 5 and 6). - Updated Technical Support (page 7). <p>Chapter 2, Instrument Description:</p> <ul style="list-style-type: none"> - Enhanced component description (page 10).

Revision History (Continued)

Revision	Date	Changes
G	5/04	<p>Chapter 3, Installation:</p> <ul style="list-style-type: none"> - Updated all illustrations. - Clarified installation instructions. <p>Chapter 4, Operation:</p> <ul style="list-style-type: none"> - Revised display screens throughout. - Clarified operation instructions. <p>Chapter 5, Performance Verification and IQ-OQ-PQ Procedures:</p> <ul style="list-style-type: none"> - Updated entire chapter. - Clarified liquid test procedures. <p>Added new Chapter 6, Maintenance and Decontamination.</p> <p>Added new Chapter 7, Troubleshooting and Error Codes.</p> <p>Added instructions for controlling the reader with KC4™ to Appendix A, Computer Control (formerly Appendix B).</p> <p>Added instructions for using KC4™ to manipulate data to Appendix B, Using 384-Well Geometry (formerly Appendix D).</p> <p>Updated sample reports in Appendix C, Report Format.</p> <p>Removed previous Appendix A, Decontamination.</p> <p>Removed Appendix E, Error Codes.</p>
H	11/04	<p>Removed references to “General Formula” (pages 41, 62, 63, 73, and 89).</p> <p>Preface: Updated Safety Symbol text.</p> <p>Chapter 1, Introduction:</p> <ul style="list-style-type: none"> - Corrected Service Manual part number listed under Optional Accessories (page 4). - Changed “VAC” to “V~” (pages 3 and 6). - Updated Hardware and Environmental Specifications (page 6). <p>Chapter 3, Installation:</p> <ul style="list-style-type: none"> - Changed “VAC” to “V~” (page 20).

Revision History (Continued)

Revision	Date	Changes
I	05/06	<p><i>General:</i></p> <ul style="list-style-type: none"> - Add Gen5 instructions/references wherever KC4 or KCjunior instructions/references were included throughout the manual. - Changed “Universal” to “Absorbance” in “Universal Test Plate” or “Universal Plate Test.” - Removed the hyphen from Bio-Tek (BioTek). - Removed “Universal” from “Universal Microplate Spectrophotometer.” <p><i>Cover:</i> Replaced cover with new design from Marketing (includes updated photo and new BioTek logo).</p> <p><i>Preface:</i> Updated Notices, Warnings, Hazards, and safety information. Removed Warranty and Registration Card.</p> <p><i>Chapter 1:</i> Replaced 2-page Technical Support section with 1-page Product Support & Service section.</p> <p><i>Chapter 5:</i></p> <ul style="list-style-type: none"> - Changed chapter title from “Performance Verification and IQ-OQ-PQ Procedures” to “Instrument Qualification.” - In Recommended Qualification Schedule, changed PQ frequency from monthly and semiannually to monthly and quarterly, and clarified criteria for running Liquid Tests 1, 2, or 3. - Added Gen5 instructions for performing the System Test and Absorbance Plate Test. - Changed “Sigma® P 3563 packets” to “Sigma® P4417 tablets (or equivalent)” in required materials for Liquid Test 3. - For Liquid Tests 1, 2, and 3, incorporated recommendation from Manual Update H1 to shake the plate or wait between pipetting and read steps. <p><i>Appendix A:</i> Added new section, “Controlling the Reader with Gen5.”</p> <p><i>Appendix B:</i> Added new section, “Reading a 384-Well Plate in Gen5.”</p>

Intended Use Statement

The μ Quant is a single-channel, automated, bench-top, general-purpose, Enzyme Immunoassay Analyzer that performs analyses and DNA quantification of a variety of enzyme immunoassays. Assay protocol variations are addressed by the developer of the ELISA test kit, in accordance with the test kit's procedure.

A versatile curve-fitting and statistical software program is preloaded on every μ Quant. The operator can use BioTek's Extensions™ Define Protocol software to define up to 57 assay protocols and blanking patterns that can be stored in memory and instantly accessed. Plate templates and formulas are automatically combined with the protocol assay setup. Data results may be printed out, or sent to a computer running a BioTek software package, such as Gen5™, KCjunior™ or KC4™ for Microsoft® Windows®.

- In the European Union: This product may **only** be used for Research and Development and other nonclinical purposes.
- In all other jurisdictions: This product may be used for Research and Development and in vitro Diagnostic purposes.



The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. The operator must evaluate this software in conjunction with the specific laboratory diagnostic assay. This reevaluation must include the establishment of new performance characteristics for the specific assay.

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.

Specimen Preparation

Samples should be obtained, treated and stored following instructions and recommendations contained in the package kit.

Repackaging and Shipping



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

Warnings



Operate the instrument on a 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 μ Quant™. However, the operator should be aware of certain situations that could result in serious injury; these may vary depending on the instrument model.

Hazards and Precautions

Hazards



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 cord directly to a three-prong receptacle with a functional ground.

Warning! Internal Voltage. Always turn off the power switch and unplug the power cord before cleaning the outer surface of the instrument.

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

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

Warning! Software Quality Control. The operator must follow the manufacturer's assay package insert when using the instrument's onboard software to modify software parameters and establish reading 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.

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

Precautions

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



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

Caution: Environmental Conditions. Do not expose the system to temperature extremes. For proper operation, ambient temperatures should remain between 18°-40°C. 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. A carrier shipping block must be removed before operating the reader. The shipping block must be reinstalled before repackaging the reader for shipment. See **Chapter 3, Installation**.

Caution: Warranty. Failure to follow preventive maintenance protocols may **void the warranty**. See **Chapter 6, Maintenance and Decontamination**.

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)."



Based on the testing described below and information contained herein, this instrument bears the CE mark.

Directive 89/336/EEC Electromagnetic Compatibility

Emissions – Class A

EN 50081-1:1992 and IEC 61326-1:1997

EN 55022: 1995 Class A

Immunity

EN 50082-1:1997 and IEC 61326-1:1997

EN 61000-4-2:1995 Electrostatic Discharge

EN 61000-4-3:1996 Radiated EM Fields

EN 61000-4-4:1995 Electrical Fast Transient/Burst

EN 61000-4-5:1995 Surge Immunity

EN 61000-4-6:1996 Conducted Disturbances

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

Directive 73/23/EEC Low Voltage

EN 61010-1:1993, Including Amendment 2:1995: “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).”

Electromagnetic Interference and Susceptibility

USA FCC Class A

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

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

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

Canadian Department of Communications Class A

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

Le present appareil numerique n'emet 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. 1010.1-92**
“Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements”

International

- **EN 61010-1:1993 and Amendment 2:1995**
“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)



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



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*



Caution (refer to accompanying documents)

Attention (voir documents d'accompagnement)
Achtung siehe Begleitpapiere
Atención (vease los documentos incluidos)
Attenzione, consultare la doc annessa



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 μ Quant™ and describes its hardware and software features. Also included are information about this manual and instructions for obtaining technical assistance, if needed.

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Introducing the μ Quant™

The μ Quant is a single-channel microplate spectrophotometer for research and development and in vitro diagnostic use, designed to automatically perform endpoint analysis.



In the European Union: This product may **only** be used for Research and Development and other nonclinical purposes.

In all other jurisdictions: This product may be used for Research and Development and in vitro Diagnostic purposes.

The reader can measure the optical density of solutions in 6-, 12-, 24-, 48-, 96- or 384-well microplates. The data files created can be printed in report form or stored as data files for a variety of ELISA-based applications. The reader features superior optical specifications, with an extended dynamic range of up to 4.000 absorbance units in some read modes. The wavelength range is from 200 nm to 999 nm. Kinetic analysis can be performed using computer control.

Assay definitions (consisting of protocols, templates and formulas) and the data they produce are managed by an onboard processor, via a 2-line x 24-character LCD screen and keypad. The μ Quant is 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. Data can be stored onboard, printed, and/or uploaded to controlling software on a host PC.

The μ Quant is backed by a superior support staff. If the instrument ever fails to work perfectly, please contact BioTek's Technical Assistance Center (refer to page 7 for contact information).

Quality Control

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

Hardware Features

- Xenon-flash light source
- Single-channel measurement system with reference channel
- Monochromator with wavelength range of 200-999 nm
- 2-line x 24-character LCD display
- Membrane keypad with 25 alphanumeric keys
- X-Y carrier movement
- Capability of reading 6-, 12-, 24-, 48-, 96- and 384-well microplates
- External 24-volt power supply compatible with 100-240 V~ @ 50-60 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
- Assays that can be programmed into memory and recalled instantly
- Up to 8 results that can be stored in memory
- Onboard spectral scanning
- Support for the following languages: English, French, German, Italian, and Spanish.

❖ Contact BioTek for information on changing to a different language.

Package Contents

- μ Quant™ Microplate Spectrophotometer
- Power cord (part number varies according to country of use)
- 24 VDC power supply (PN 61062)
- Operator's manual (PN 7271000)
- Printer cable (PN 71072)
- Dust cover (PN 7332040)
- RS-232 serial cable (PN 75053)
- Assay Reference Guide (PN 7271006)
- Unpacking instructions (PN 7271003); packing instructions (PN 7271012)
- Shipping document (PN 94075) that includes a Warranty Statement and Certificate of Compliance and Calibration
- Set of shipping materials (PN 7273000)
- Declaration of Conformity (PN 7271004)

Optional Accessories

- Product Qualification Package (PN 7270514)
- BioTek QC Check Solution #1 for liquid testing (PN 7120779 for 25 ml; PN 7120782 for 125 ml)
- BioTek wetting agent solution for liquid testing (PN 7773002)
- HP DeskJet printer: 110 V only (PN 97152)
- Adapter for 60-, 72-, and 96-well Terasaki plates (PN 7330531)
- Gen5™, KC4™ or KCjunior™ software (PNs and versions listed on Biotek.com or contact your local dealer)
- Gen5 Reader Diagnostics Utility (PN 5320201)
- Service Manual (PN 7331005)
- Absorbance Test Plate (PN 7260522 or 9000547)
- Bio-Cell™ for 1 cm wavelength readings (PN 7272051)
- Bio-Cell adapter plate for containing up to 8 Bio-Cells (PN 7270512)

Specifications

Microplates

Accommodates standard 6-, 12-, 24-, 48-, 96- and 384-well microplates, and 60*-, 72*- and 96-well* Terasaki trays.

*Adapter (PN 7330531) is required but not included.

Speed of Reading

The actual plate read time and accuracy are dependent on the method of reading:

- **Normal** Mode is the slower of the two available modes. After positioning the well over the beam, the instrument waits 100 ms before taking the measurement.

❖ **Note:** The 100 ms delay is to allow for more complete fluid settling.

- **Rapid** Mode is faster than Normal Mode because the instrument does not wait before taking the measurement.

The following read times are based on a 96-well endpoint read for single or dual wavelength measurements.

Normal Read Mode: 60 sec.

Rapid Read Mode: 45 sec.

Optical Specifications

λ range:	200 to 999 nm
λ accuracy:	± 2 nm
λ repeatability:	± 0.2 nm
λ bandpass:	2.4 nm

Optical Performance (96-well, flat-bottom and round-bottom plates, single-wavelength measurements):

Absorbance Measurement Range:	0.000 to 4.000 OD
Accuracy:	0.000 to 2.000 OD $\pm 1\% \pm 0.010$ OD Normal Read Mode 2.000 to 3.000 OD $\pm 3\% \pm 0.010$ OD Normal Read Mode 0.000 to 2.000 OD $\pm 1\% \pm 0.010$ OD Rapid Mode or Spectral Scanning 2.000 to 2.5000 OD $\pm 3\% \pm 0.010$ OD Rapid Mode or Spectral Scanning (Measured with neutral density filters calibration plate @ 405, 450, 490, 550, 630, 690 nm)
Linearity:	0.000 to 2.000 OD $\pm 1\%$ Normal Read Mode 2.000 to 3.000 OD $\pm 3\%$ Normal Read Mode 0.000 to 2.000 OD $\pm 1\%$ Rapid Mode or Spectral Scanning 2.000 to 2.500 OD $\pm 3\%$ Rapid Mode or Spectral Scanning (Measured @ 405 nm using yellow food dye)
Repeatability (STD):	0.000 to 2.000 OD $\pm 1\% \pm 0.005$ OD Normal Read Mode 2.000 to 3.000 OD $\pm 3\% \pm 0.005$ OD Normal Read Mode 0.000 to 2.000 OD $\pm 1\% \pm 0.005$ OD Rapid Mode or Spectral Scanning 2.000 to 2.500 OD $\pm 3\% \pm 0.005$ OD Rapid Mode or Spectral Scanning (Measured @ 405 nm using yellow food dye)

Hardware and Environmental Specifications

Display:	2-line x 24-character LCD display
Light Source:	Xenon flash
Dimensions:	42 cm x 38 cm x 18 cm (16" deep x 15" wide x 7" high)
Weight:	11.34 kg (25 lb. maximum)
Environment:	Operational temperature 18°-40°C (64.4°-104°F)
Humidity:	10% to 85%, noncondensing
Power Supply:	External 24 V power supply compatible with 100-240 V $\sim \pm 10\%$ @ 50-60 Hz

Product Support & Service

A superior support staff backs all of BioTek's products. If your instrument(s) or software ever fails to function perfectly, if you have questions about how to use or maintain them, or if you need to send an instrument to BioTek for service or repair, please contact our Technical Assistance Center.

Contacting the Technical Assistance Center (TAC)

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

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

Fax: 802-655-3399

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 keyboard 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 7, Troubleshooting and Error Codes**)

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 3, Installation**), write the RMA number on the shipping box, and ship to this address:

BioTek Instruments, Inc.
Technical Assistance Center
100 Tigan Street
Highland Park
Winooski, Vermont 05404 USA

Chapter 2

Instrument Description

This chapter includes principles of operation and descriptions of important components of the multi-detection reader.

Principles of Operation.....	10
External Components	10

Principles of Operation

The μ Quant™ is a single-channel microplate spectrophotometer. The instrument has a long-life xenon flash light source, a holographic grating-based monochromator, and UV grade fiber optics and lenses. This combination allows for absorbance measurements in a spectral range from 200 nm to 999 nm in 1-nm increments. The instrument bandpass is 2.4 nm.

Assay definitions, along with the data they produce, are managed by an onboard processor, via a 2-line x 24-character LCD screen and membrane switch. Data can be stored onboard, printed, and/or uploaded to controlling software on a host PC. The μ Quant is 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.

External Components

- The **power switch** is located on the right side of the instrument. The switch is labeled with “I/O”, indicating on and off, respectively.
- The front panel features a 2-line x 24-character Liquid Crystal Display (LCD), and a **25-key keypad**. Through this interface you can create and modify absorbance assays, read plates, store and print results, send results to an external computer, and more. See **Chapter 4, Operation** for more information.
- The **microplate carrier** supports the microplates and adapter plates described in **Chapter 1**, under **Specifications**. A spring clip holds the plate securely in place. The microplate carrier access door with its magnetic closure helps to ensure a light-impermeable measurement chamber.
- When a plate read is initiated, the plate carrier is drawn into the measurement chamber, and then moves in the X and Y axes to align each microwell with the probe, as specified in the assay. When the read is complete, the plate carrier is returned to its full-out position.
- The **back panel** contains the power supply jack, parallel (printer) port, and serial port. See **Setting Up the μ Quant** in **Chapter 3** for more information.

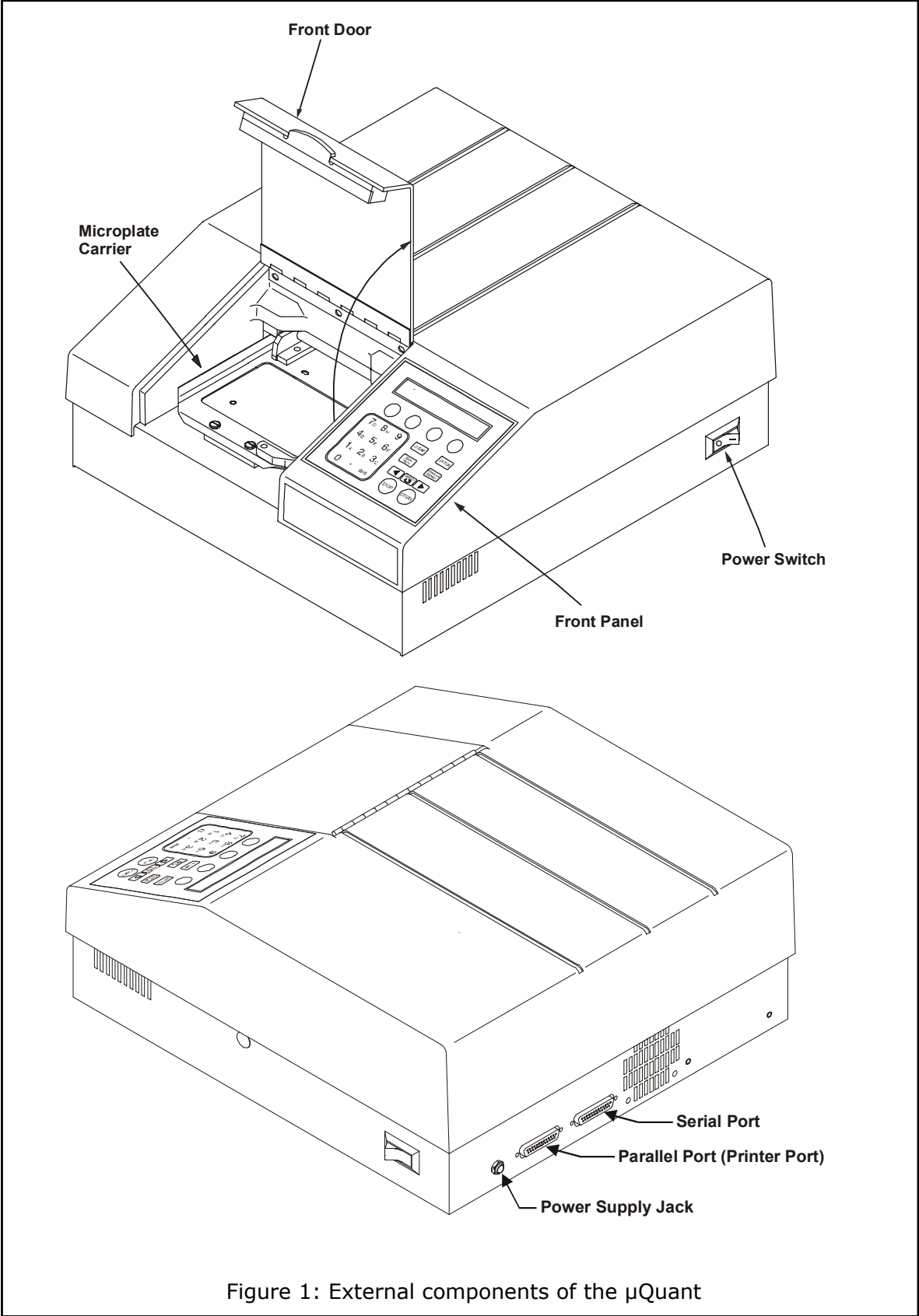


Figure 1: External components of the μ Quant

Chapter 3

Installation

This chapter includes instructions for unpacking and setting up the μ Quant™ and instructions for connecting printers and/or serial devices.

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Repackaging and Shipping the μ Quant.....	28

Unpacking and Inspecting the μ Quant™



Important! Save all packaging materials. If the μ Quant™ is shipped to the factory for repair or replacement, it must be carefully repackaged, according to the instructions on pages 28 through 33, using the original packing materials (PN 7273000). The individual packaging materials contained in PN 7273000 are listed in the table below. Using other forms of commercially available packing materials, or failure to follow the repackaging instructions may **void your warranty**. If the original packing materials have been damaged, replacements are available from BioTek.

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

If the 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 instrument immediately, before the shipping-related claim is settled.

Refer to the unpacking instructions and **Figures 2** through **6** on the following pages when removing the instrument and its accessories from the shipping container.

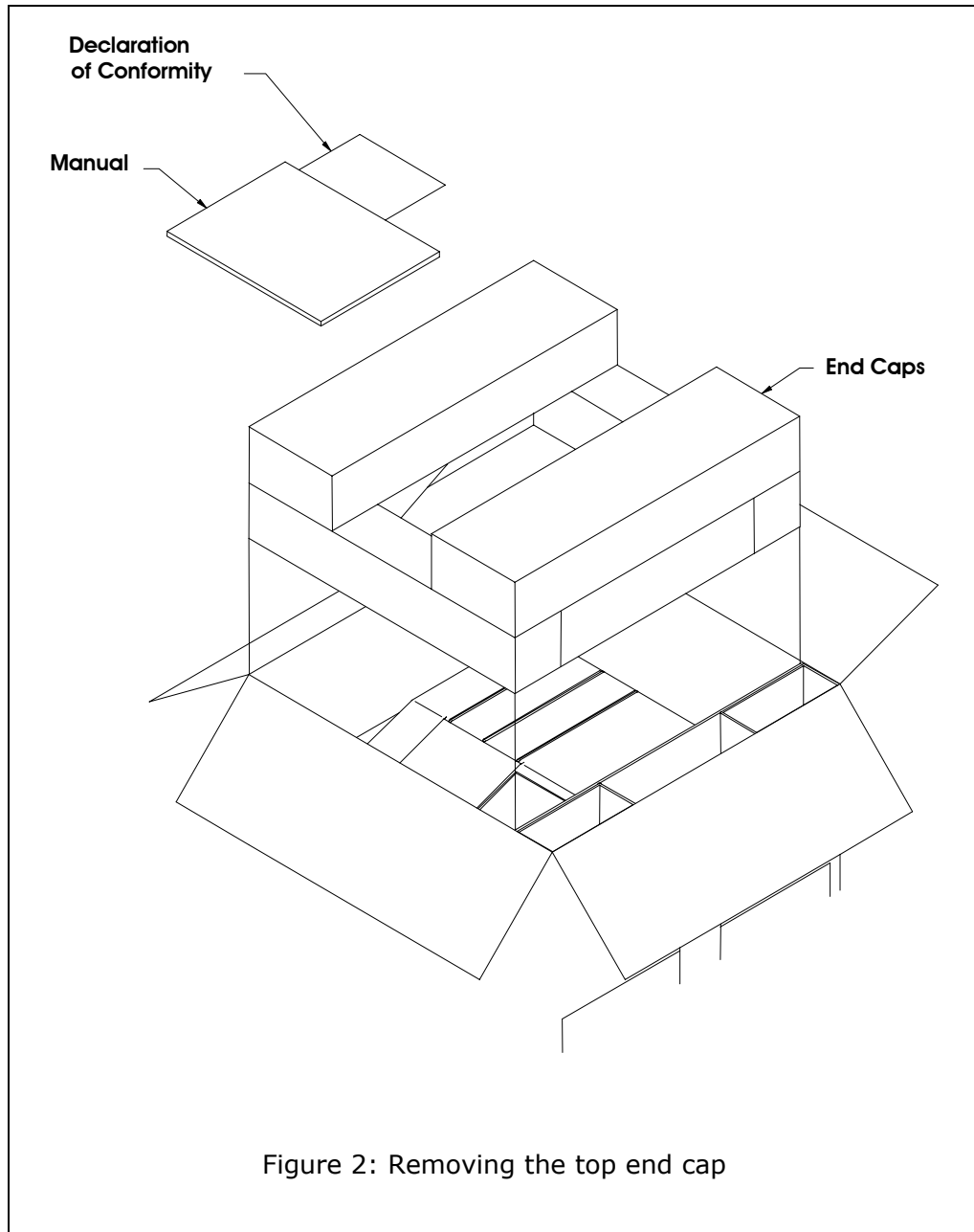
See **Repackaging and Shipping the μ Quant** at the end of this chapter for complete shipping instructions.

Packing Materials	PN
Outer shipping container	7272054
Inner shipping container (accessories)	7272055
Top foam end caps	7272057
Bottom foam end caps	7272056
2-Mil poly bag (μ Quant)	98085
8 ½" x 11" bubble bag (power supply)	91083

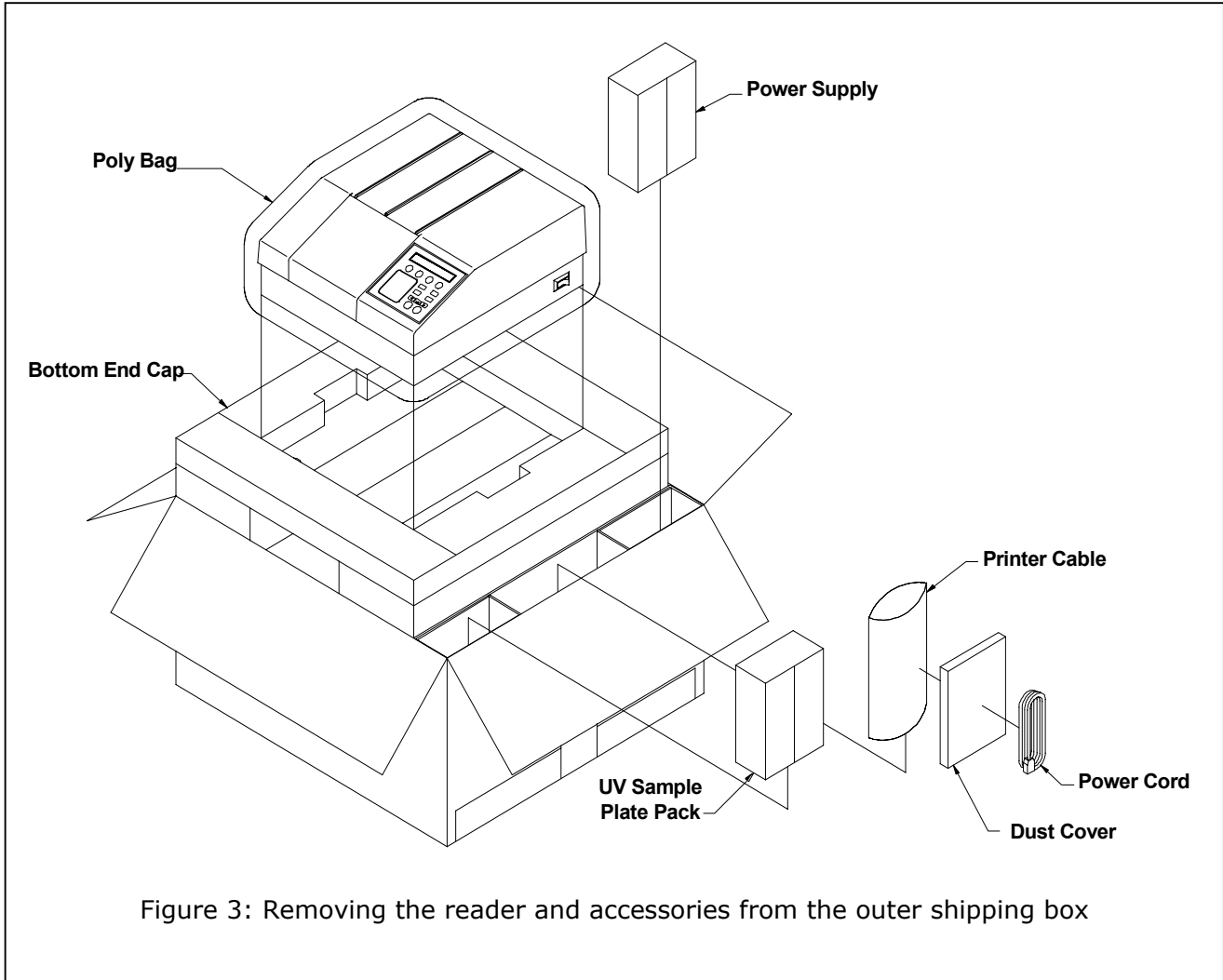
Required Tools	PN
Combination slotted/Phillips-head screwdriver	98145

Unpacking the Instrument and Its Accessories

1. Carefully open the top of the box.
2. Remove the top foam end caps, manual, and Declaration of Conformity (**Figure 2**).



3. Lift the reader out of the box (**Figure 3**) and place it on a level surface. Remove the reader from the poly bag. Remove the accessories and set them aside.



4. Place the instrument on a stable surface.
5. Use the slotted screwdriver to remove the two black dress screws from the rear panel of the instrument (**Figure 4**). Set aside these screws.

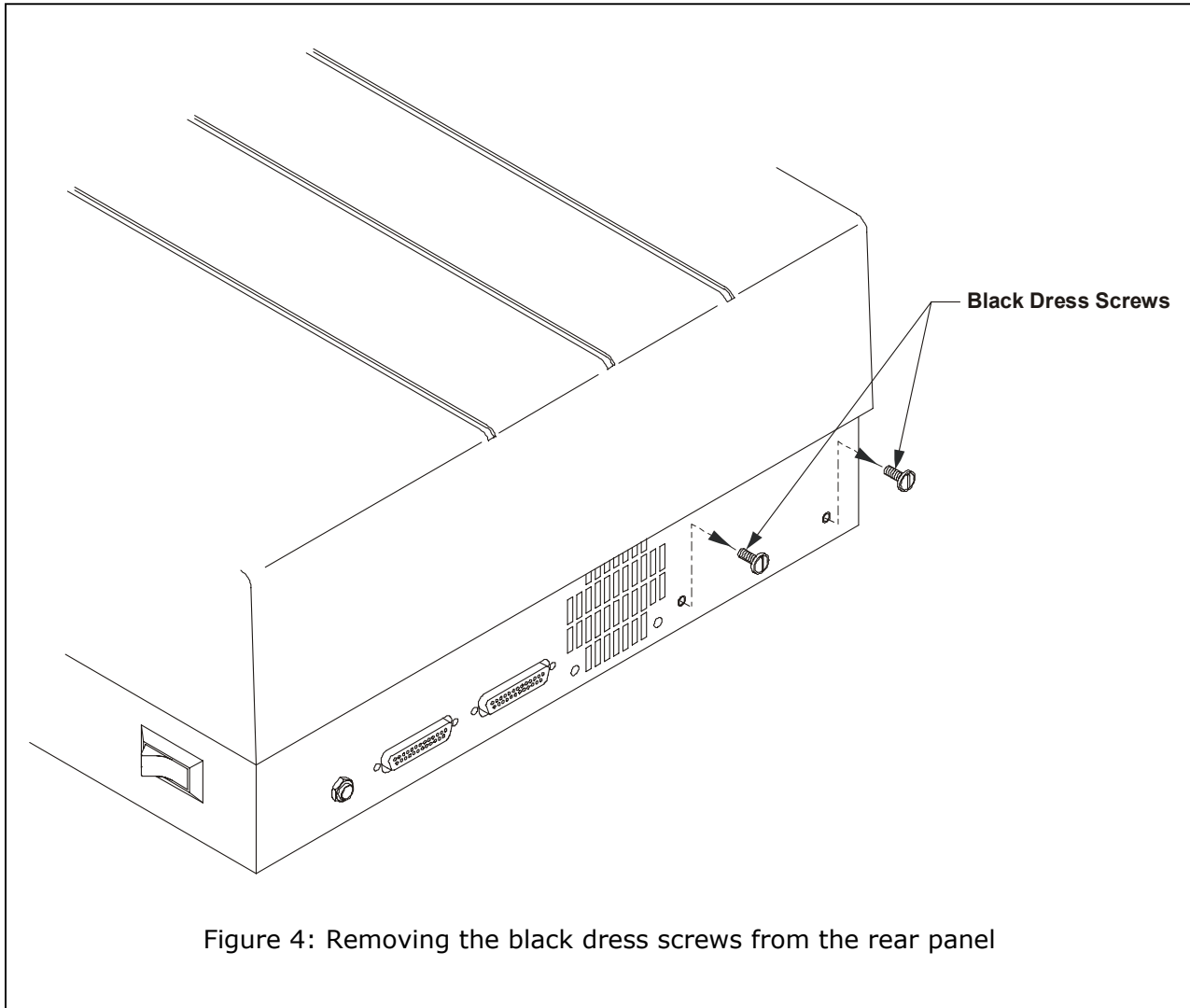


Figure 4: Removing the black dress screws from the rear panel

6. Turn the instrument around and open the front door. While holding the carrier in place, use a Phillips-head screwdriver to remove the shipping block and mounting hardware (**Figure 5**) and set them aside.



IMPORTANT! Do **NOT** remove the two black dress screws that secure the carrier-sliding block (**Figure 5**) to the carrier.

7. Insert the two black dress screws that were removed from the rear panel into the shipping block mounting holes.

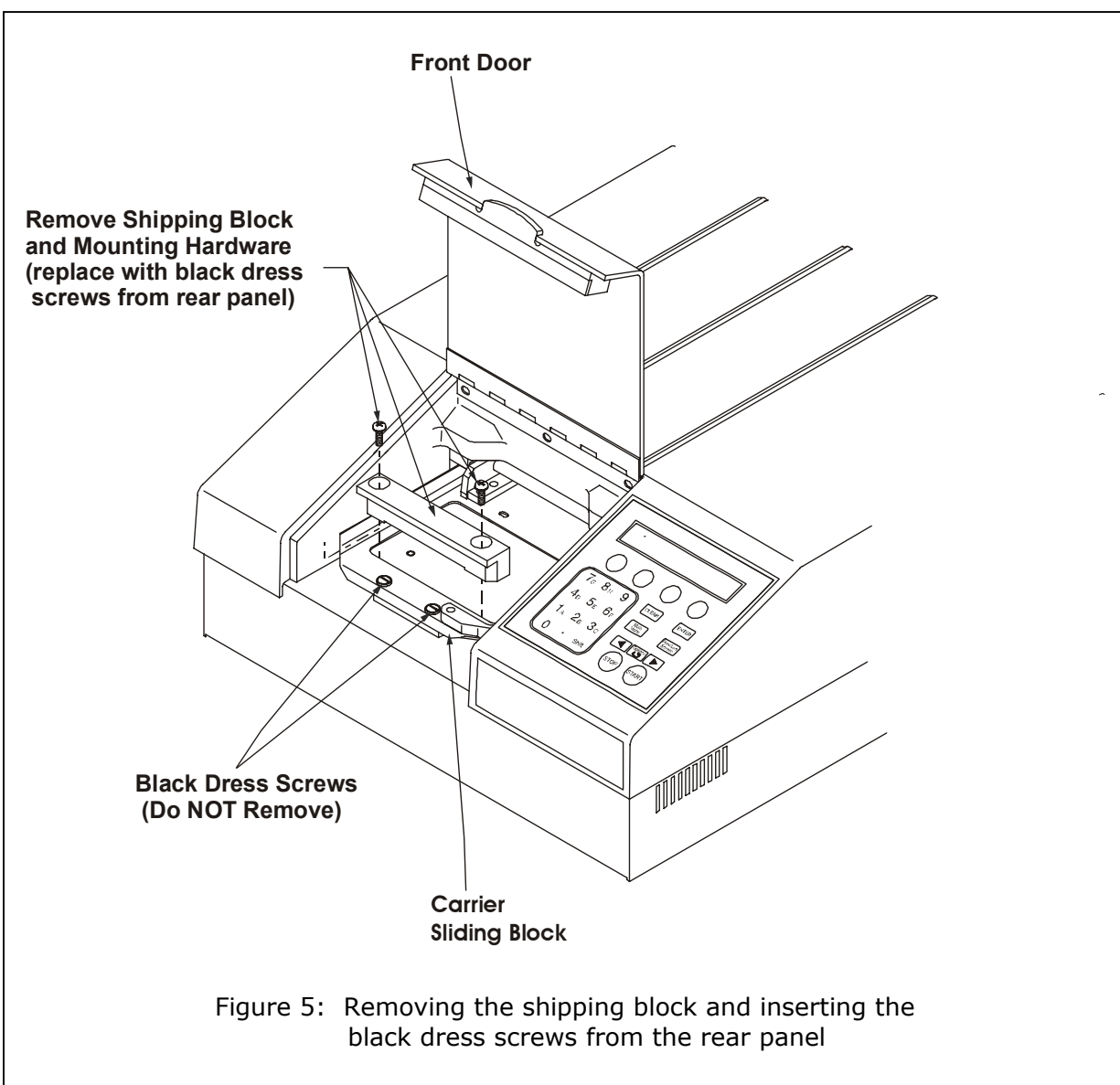
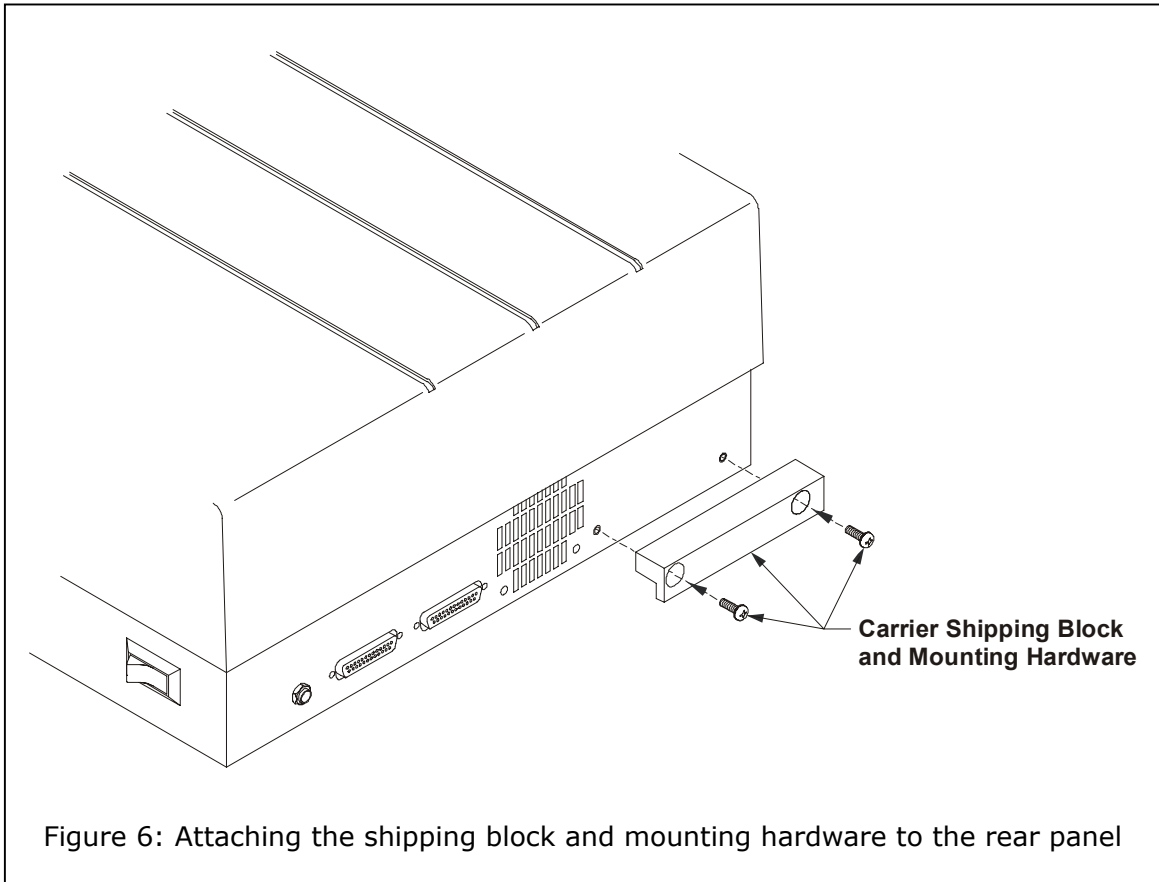


Figure 5: Removing the shipping block and inserting the black dress screws from the rear panel

8. Attach the shipping block and mounting hardware to the rear of the unit as shown in **Figure 6**.



Setting Up the μ Quant

Operating Environment

The μ Quant is designed to operate optimally when installed on a level surface in an area where ambient temperatures remain between 18°C (64.4°F) and 40°C (104°F). The reader is sensitive to extreme environmental conditions, and these conditions should be avoided:

- **Excessive humidity:** Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks. The specified humidity range for this instrument is from 10% to 85%, noncondensing.
- **Excessive ambient light:** Bright sunlight or strong incandescent light may affect the instrument's optics and readings, reducing its linear performance range.
- **Dust:** Optical density readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is essential to ensure accurate readings.

Electrical Connections



Caution: Power Supply. Only use the specified power supply to ensure proper operation of the unit. The μ Quant has a universal 24-VDC, 2 A power supply that functions from 100 to 240 V $\sim \pm 10\%$ @ 50 to 60 Hz without external switching.

1. Connect the power cord to the external power supply.
2. Locate the power supply jack on the rear of the μ Quant.
3. Plug the rounded end of the power supply line cord into the power supply jack.
4. Plug the 3-prong end of the power cord into an appropriate power receptacle.



Warning! Power Rating. The μ Quant 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 System Test

After you have installed the μ Quant and connected the power supply, turn on the instrument to run a system test. The on/off switch is located on the lower right side of the base.

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

If an error is detected, the reader will "chirp" and display an error code. See **Chapter 7** for a list of error codes. If no errors are detected, the reader will briefly display **SYSTEM TEST PASS**.

The power-up system test does not produce a printed results report. To run the test manually and obtain a printout of the system test values, start at the Main Menu and press **UTIL \rightarrow TESTS \rightarrow SYSTEM**. See **System Test** and **Checksum Test** in **Chapter 5**, or **Connecting a Printer to the μ Quant** on page 24 for more information.

μ Quant Main Menu

Following successful power-up of the μ Quant, the Main Menu appears:

```

R E A D Y      0 1 : 2 2 P M      0 1 / 2 3 / 0 4
R E A D      D E F I N E   R E P O R T   U T I L

```

- The Main Menu permits access to all onboard functions. See **Main Menu** in **Chapter 4** for more information, including a diagram showing the flow of functionality.
- The μ Quant front panel contains four circular buttons, referred to in this manual as "**SOFT KEYS**." One **SOFT KEY** is positioned directly below each selectable option in the display. To select a menu option, simply press its corresponding **SOFT KEY**. See **μ Quant Front Panel** in **Chapter 4** for additional instructions.

Adjusting the Wavelength Table

The μ Quant has six default wavelength settings installed at the factory. Before operating the instrument, configure the wavelength table with values that will confirm that the instrument is operating near its limits. (The recommended wavelengths are 200, 405, 490, 550, 630, and 999 nm.) You may choose values other than the upper or lower limits of the instrument if the μ Quant will never be used in these outer ranges. Note that you may also choose wavelength values that coincide with six of the wavelengths (405 to 750 nm) given in the BioTek Absorbance Test Plate (PN 7260522) with its accompanying Standards Certificate.

❖ The six wavelengths can be set at any time. The default values can be changed via Gen5™, KC4™, or KCjunior™; refer to the Gen5 Getting Started Guide or Help system, or to the User's Guides to KC4 or KCjunior.

To check or change the software wavelength from the instrument keypad:

1. Power up the μ Quant.
2. From the Main Menu, press **UTIL**. The **UTILITIES** menu appears.

```

R E A D Y      1 2 : 4 5 P M      0 1 / 2 3 / 0 4
R E A D      D E F I N E   R E P O R T   U T I L
  
```

```

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

3. From the **SELECT UTILITY OPTION** menu, press **SETUP**. The **EDIT SETUP INFORMATION** screen is displayed.

```

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

4. From this menu, press **LAMBDA**.
5. The wavelength will be displayed. Press **ENTER** to continue.

6. To change the wavelength number, use the **numeric** keypad to enter a number at the cursor location. The cursor will automatically advance to the next editable field. Press **ENTER** to save the entry and move to the next wavelength on the table.

```

E N T E R
L A M B D A # 1   W A V E L E N G T H : 4 0 5

```

❖ **Note:** Any wavelength value between 200 and 999 nm may be entered in 1-nm increments.

7. When the last wavelength has been entered, the software exits the routine, and displays the following screen:

```

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

```

8. Press the Main Menu key.
9. Perform a System Test to calibrate the system for the new lambda values. "SYSTEM TEST PASS" will be displayed.

Connecting a Printer to the μ Quant



Connect the printer to the μ Quant *only* if you are running the instrument in standalone mode. If you are using Gen5™, KC4™, or KCjunior™ software, skip this step, and go to page 26, **Setting Up the Serial Port for Communications**.

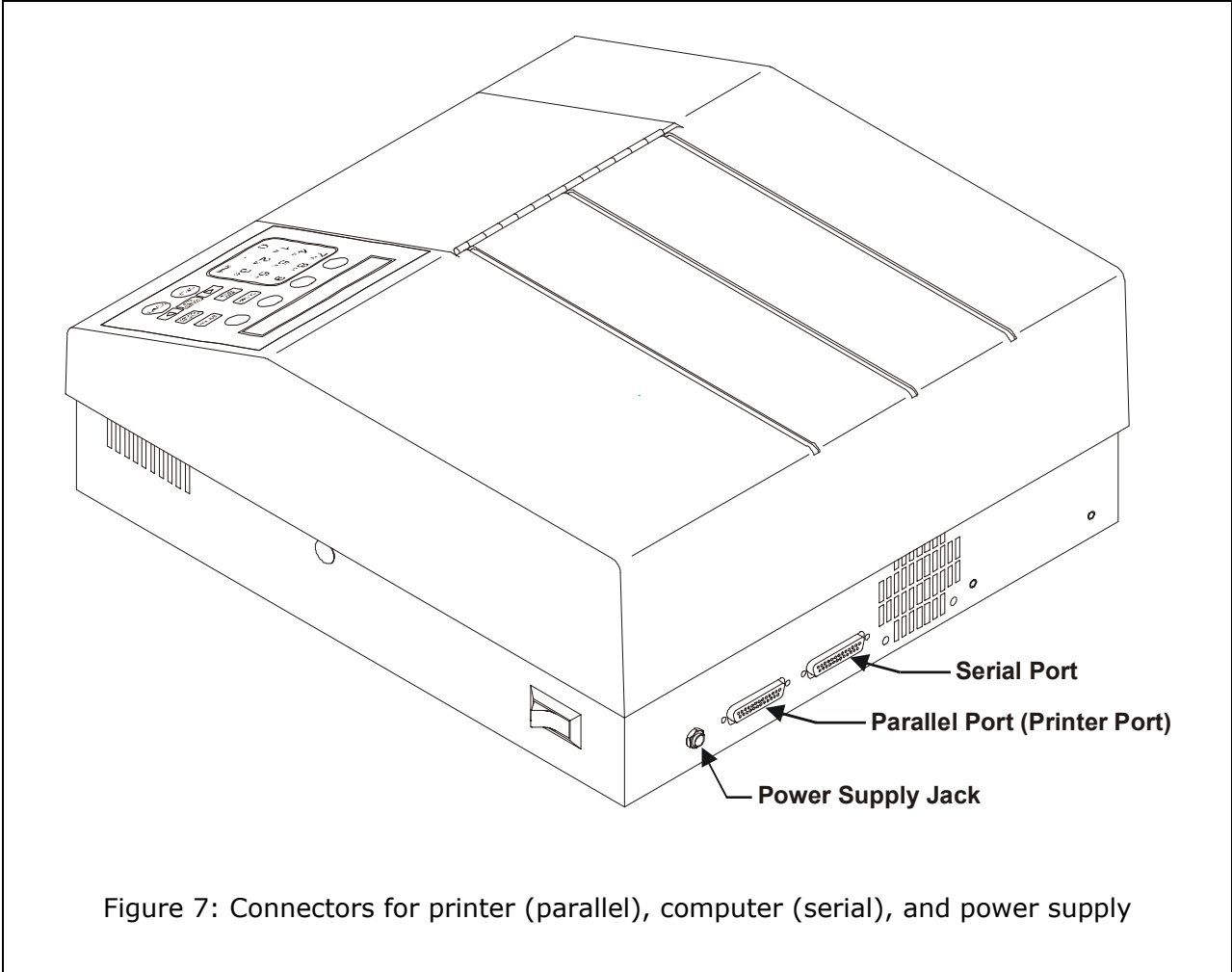
The μ Quant has a parallel port (LPT1) to allow connection to Epson-compatible printers or HP Deskjet™ printers. (See page 93, under **Specifying Data Output and Reporting Options**, for more information.) The port is illustrated in **Figure 7**. The parallel port requires a 25-pin D-sub connector. A parallel cable (PN 71072) designed to connect the reader to a printer is provided with the μ Quant.

To attach a printer to the μ Quant:

1. Turn the reader off.
2. Place the printer in a location adjacent to the μ Quant.
3. Attach one end of the cable to the parallel port on the printer.
4. Attach the other end of the cable to the parallel port on the μ Quant.
5. Tighten the securing screws on both ends of the cable.
6. Turn on the reader, and then turn on the printer.



To avoid system instability, be sure to connect the printer to the reader *before* powering up the reader.



(Optional) Setting Up the Serial Port for Communications

Before serial communication can be initiated between the μ Quant and another device (such as a host PC running BioTek's Gen5™, KC4™, or KCjunior™ software), the communication parameters must match between the devices.

The μ Quant has a 25-pin serial (RS-232) port located on the rear panel of the instrument. The serial port allows the reader to communicate with a computer, using standard communications software and/or RS-232 protocols.

The serial port also allows field upgrades of the μ Quant software.

❖ **Appendix A** contains information on required protocols for computer control of the reader.

Attaching the Cable

1. Power down the computer and the μ Quant.
2. Connect the appropriate serial cable to both machines. The serial port on the reader is a DTE configuration with a 25-pin (pin-male) D-sub connector.
3. Power up the reader and the computer.
4. Ensure that the μ Quant and the computer are operating with the same communications settings.

Installing the PC Software

Install Gen5, KC4, or KCjunior software on the computer's hard drive. Refer to the Gen5 Getting Started Guide or Help system, or to the User's Guides for KC4 or KCjunior for installation instructions.

Setting 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 bps, if necessary. The Data Bits, Stop Bits, and Parity settings **cannot** be changed.

To change the μ Quant's baud rate from the instrument keypad:

1. At the Main Menu, press **UTIL**.

```
R E A D Y      1 2 : 4 5 P M      0 1 / 2 3 / 0 4
R E A D      D E F I N E   R E P O R T   U T I L
```

2. At the **SELECT UTILITY OPTION** screen, press **SETUP**.

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

3. At the **EDIT SETUP INFORMATION** screen, press ***MORE** to continue.

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

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

4. 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** 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   D A T A - B I T S
```

To change the μ Quant's baud rate (or other communications settings) in Gen5™, KC4™ or KCjunior™, refer to their respective user guides, or to **Appendix A, Computer Control**.

Repackaging and Shipping the μ Quant



IMPORTANT! Failure to properly repack the reader increases the likelihood of damage to the instrument during shipping. The shipping system was designed to stabilize the reader's mechanical mechanisms, which would otherwise be free to move around during shipping.

If you need to ship the μ Quant Reader to BioTek for service or repair, be sure to use the original packing. Other forms of commercially available packing are not recommended and can **void the warranty**.

If the original packing materials have been damaged or lost, contact BioTek for replacement packing (see **Product Support & Service** in **Chapter 1** for contact information).



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.

Before Repackaging the Instrument

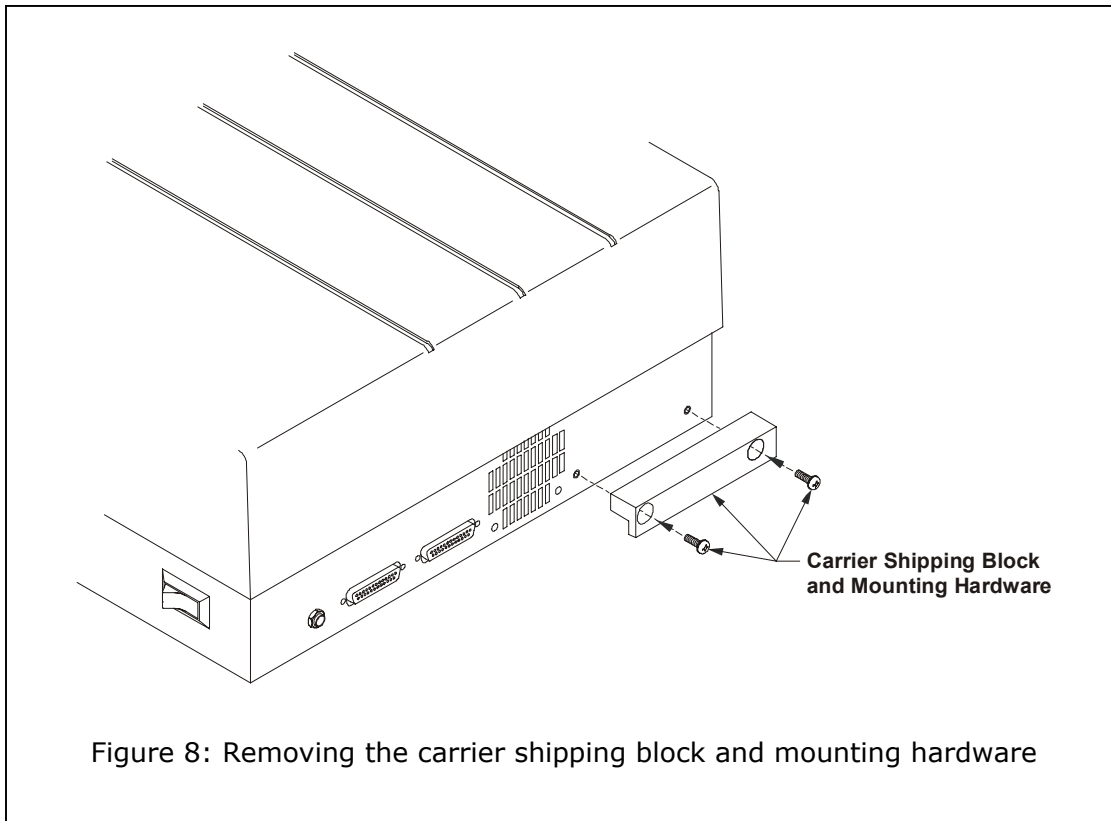
1. **Decontaminate the reader before repackaging it.** (See **Chapter 6, Maintenance and Decontamination**, for the Decontamination procedure.)
2. Once the instrument is clean, reattach the shipping block.

Repackaging the μ Quant and Its Accessories

Refer to **Figures 8** through **12** when repackaging the μ Quant.

Mounting the Shipping Block

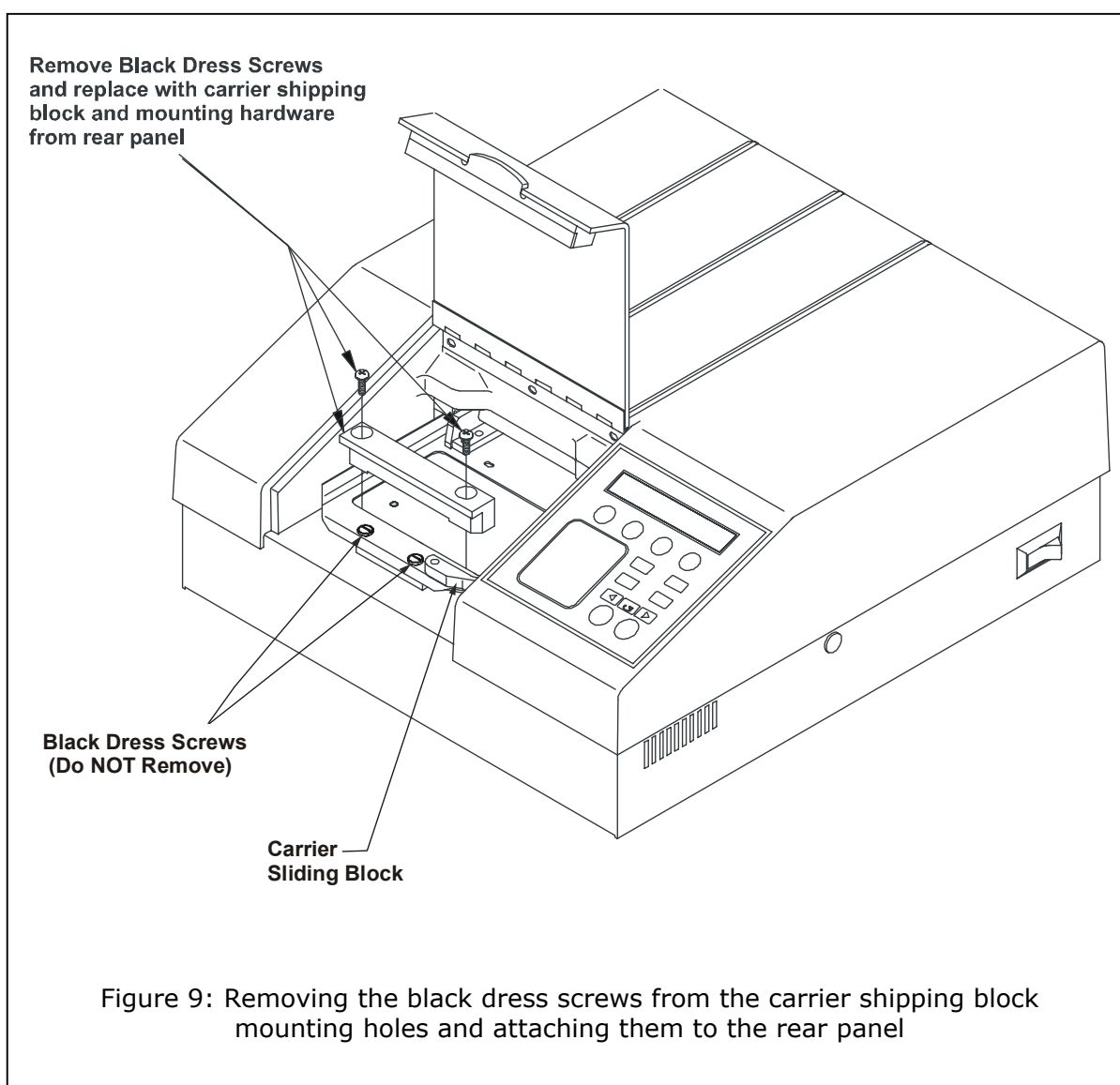
1. Turn off the unit and unplug the power supply.
2. Use the Phillips-head screwdriver to remove the shipping block and mounting hardware from the rear of the unit (**Figure 8**) and set them aside.



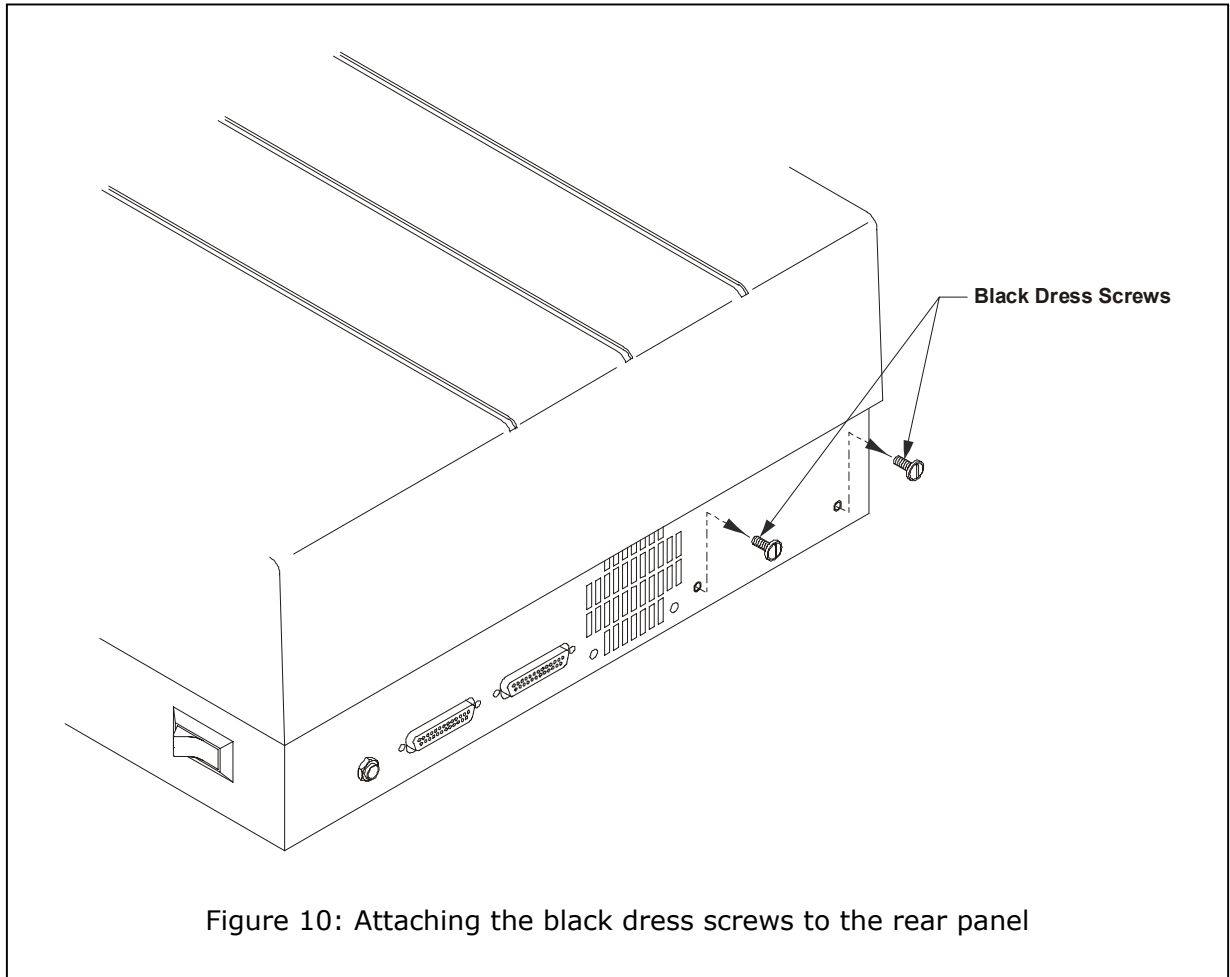
3. Turn the instrument around and open the front door. While holding the carrier, remove the two black dress screws from the carrier shipping block mounting holes **inside** the carrier and set them aside.
4. Install the carrier shipping block and mounting hardware that were removed from the rear panel (**Figure 9**).



IMPORTANT! Do **NOT** remove the two black dress screws that secure the carrier-sliding block to the carrier.

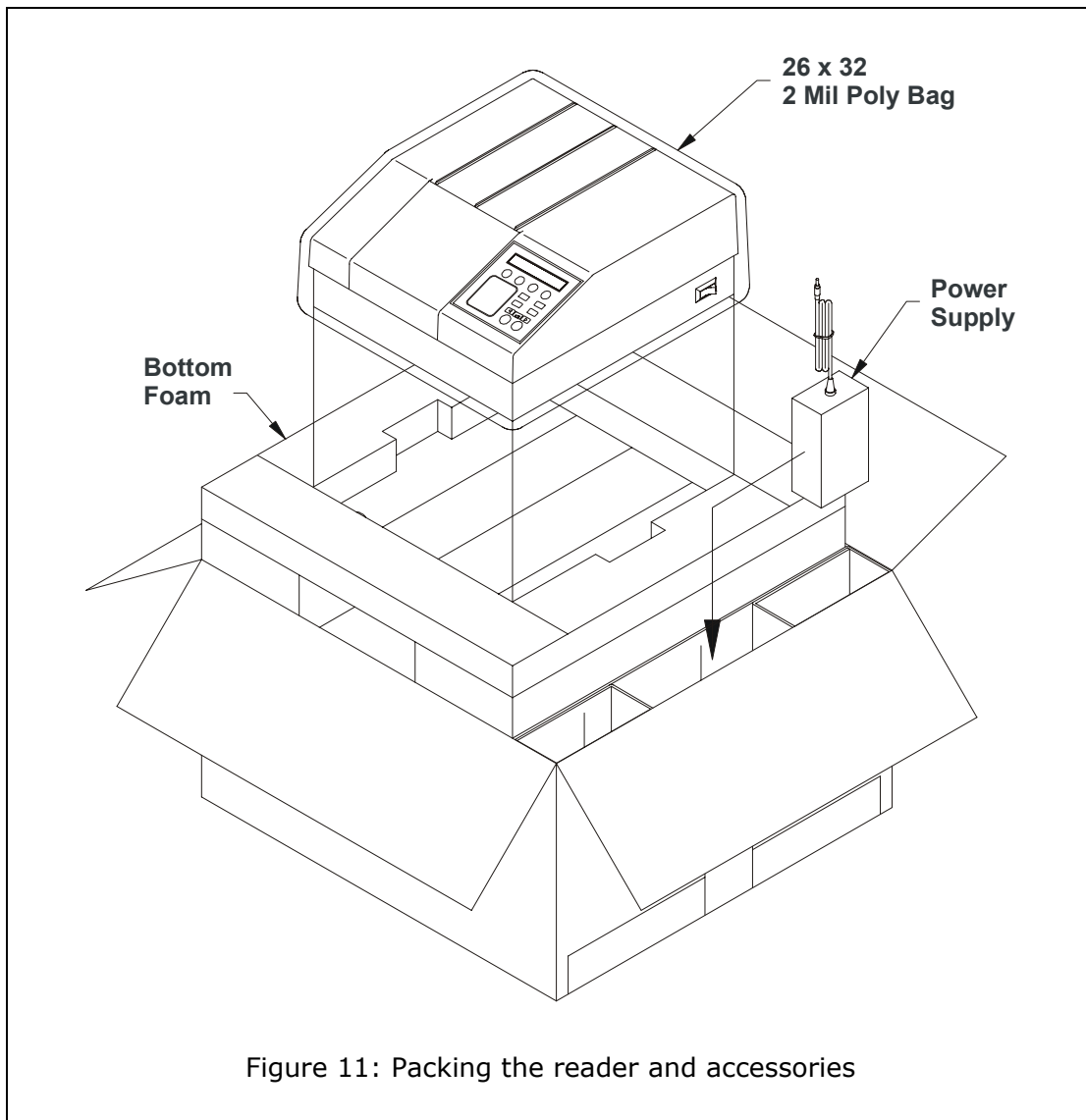


5. Attach the two black dress screws that were removed from the carrier shipping block mounting holes to the rear panel (**Figure 10**).



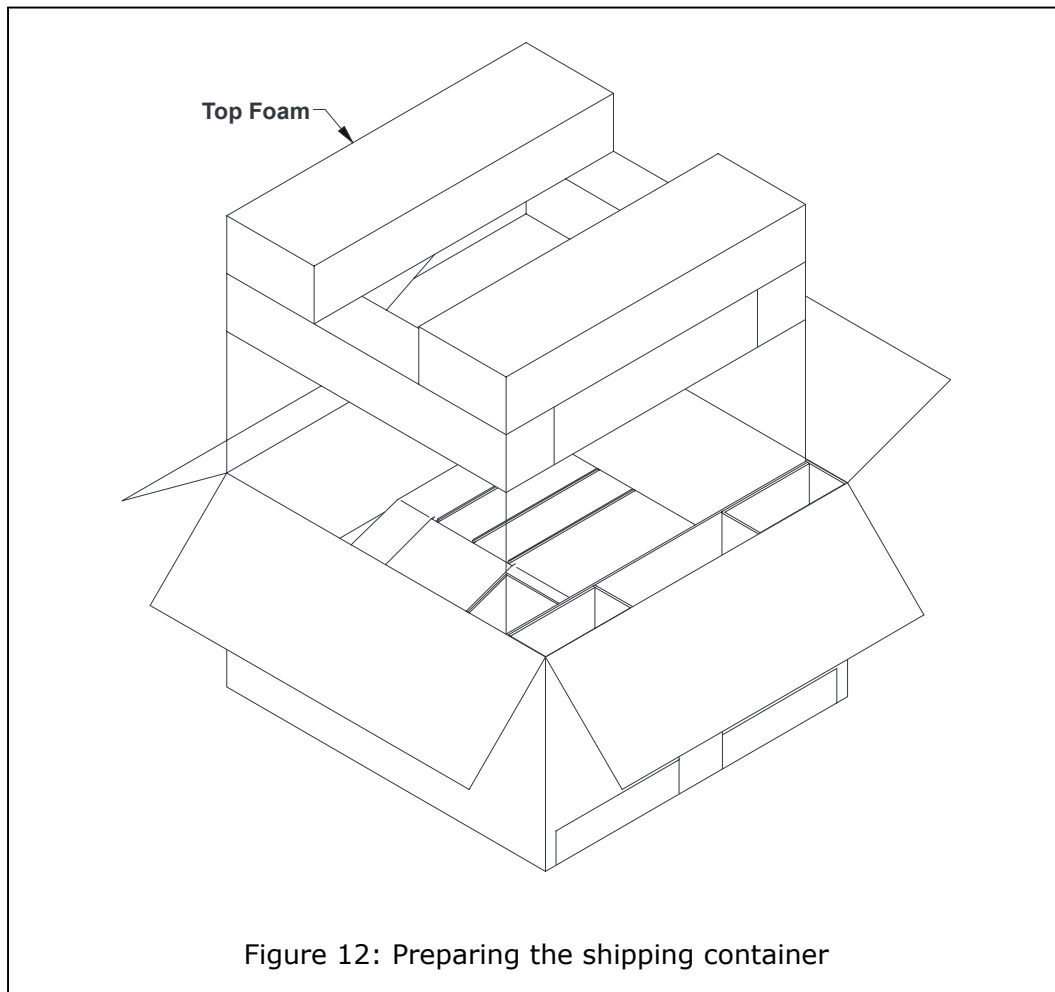
Packing the Reader and Accessories

1. Insert bottom foam end cap all the way into the bottom of the box. Note the orientation of the foam to the box (**Figure 11**).
2. Put the instrument inside the 2-mil poly bag and place it inside the bottom end cap.
3. Put the power supply inside the 8 ½" x 11" bubble bag. Place the power supply inside the pocket in the box as shown.



Preparing the Shipping Container

1. Place top foam on the instrument (Figure 12).
2. Obtain a Return Materials Authorization (RMA) number from BioTek's Technical Assistance Center through BioTek's Web site, fax, or e-mail address listed in Chapter 1.
3. When obtaining the RMA, explain whether the reader requires calibration, cleaning, periodic maintenance, warranty work, and/or repair. Make a note of any error messages displayed and their frequency.
4. Provide BioTek with the name and contact information of a person who may be contacted if questions arise.
5. Close the box and tape it shut.
6. Write "RMA" and the RMA number in large, clear letters on the outside of the shipping container, and ship the instrument to the BioTek address provided in the **Product Support & Service** section of **Chapter 1**.



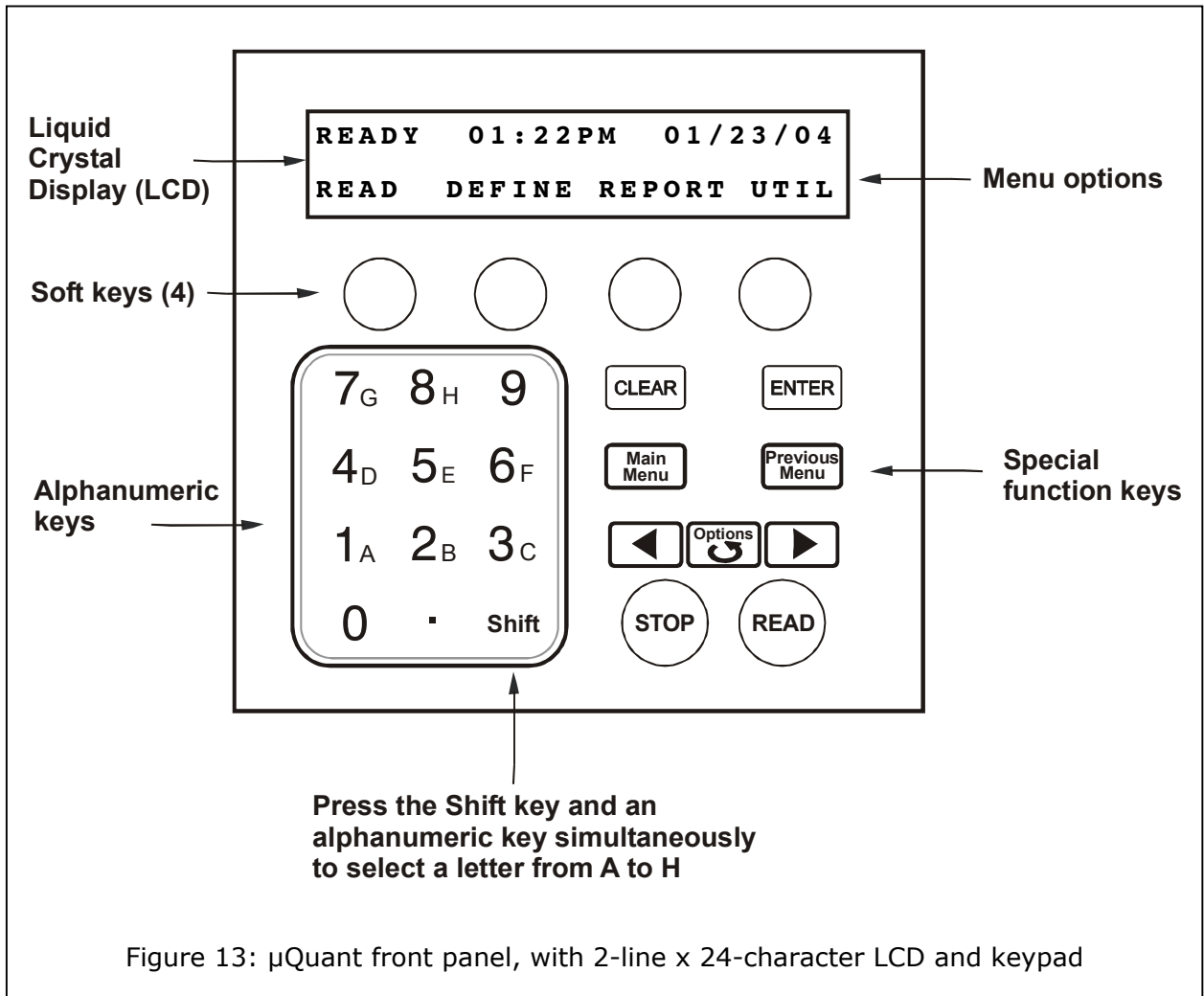
Chapter 4

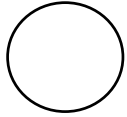
Operation

This chapter includes instructions for operating the μ Quant and its onboard software.

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μQuant Front Panel





The keypad has four **SOFT KEYS**, one below each selectable menu option. Press a **SOFT KEY** to make a selection. For example, from the Main Menu, press the leftmost **SOFT KEY** to select **READ**, the rightmost to select **UTIL**.



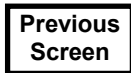
Exit the current screen and return to the **Main Menu**. Pressing Main Menu while defining or modifying an assay automatically saves the current settings.



Cycle through available options within a screen. For example, press **Options** within the **Select Assay Number** screen to cycle through the names of the onboard assays.



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



Pressing **Previous Screen** generally saves the current screen settings and returns control to the screen most previously viewed.



Press **CLEAR** to reset a numeric value to 0, or to clear all characters when editing an assay name. **Tip:** Press **Shift + Clear** at the **Map Generation** screen to “clear” a previously defined manual map.



Move the cursor to the left in data-entry screens.



Move the cursor to the right in data-entry screens.



Initiate a plate read.



Halt the read currently in progress.

Overview



IMPORTANT! Do not turn on the instrument until the carrier shipping block has been removed.

The μ Quant features a 25-pad keypad and a 2-line x 24-character LCD display, allowing you to access the reader's program menus and print test results. The reader's bidirectional serial port allows computer control of the instrument, and provides the means for downloading additional assay definition files to the instrument. This chapter describes the operation of the **open** (configurable) assays onboard the μ Quant.

- ❖ The μ Quant reader may come with preprogrammed assays already installed. Refer to **Table 1** under **Define** on page 40 for a list of preprogrammed assays.

System Startup

To turn on the μ Quant, press the on/off switch on the right side of the reader's base. The μ Quant will perform a System Test, displaying the screens shown below until initialization is complete. During this period, all keys are inactive.

If the instrument fails the System Test, a chirp will sound, and an error code will display.

- Refer to **System Self-Test** and **Checksum Test** in **Chapter 5** for more information.
- Refer to **Chapter 7, Troubleshooting and Error Codes** to interpret error codes, and **Chapter 1, Introduction** for information on contacting BioTek Instruments' Technical Assistance Center (TAC).

```
P o w e r u p   S e q u e n c e   V x . x x  
I n i t i a l i z i n g . . .
```

```
B i o - T e k   I n s t r u m e n t s  
S y s t e m   S e l f - T e s t
```

Main Menu

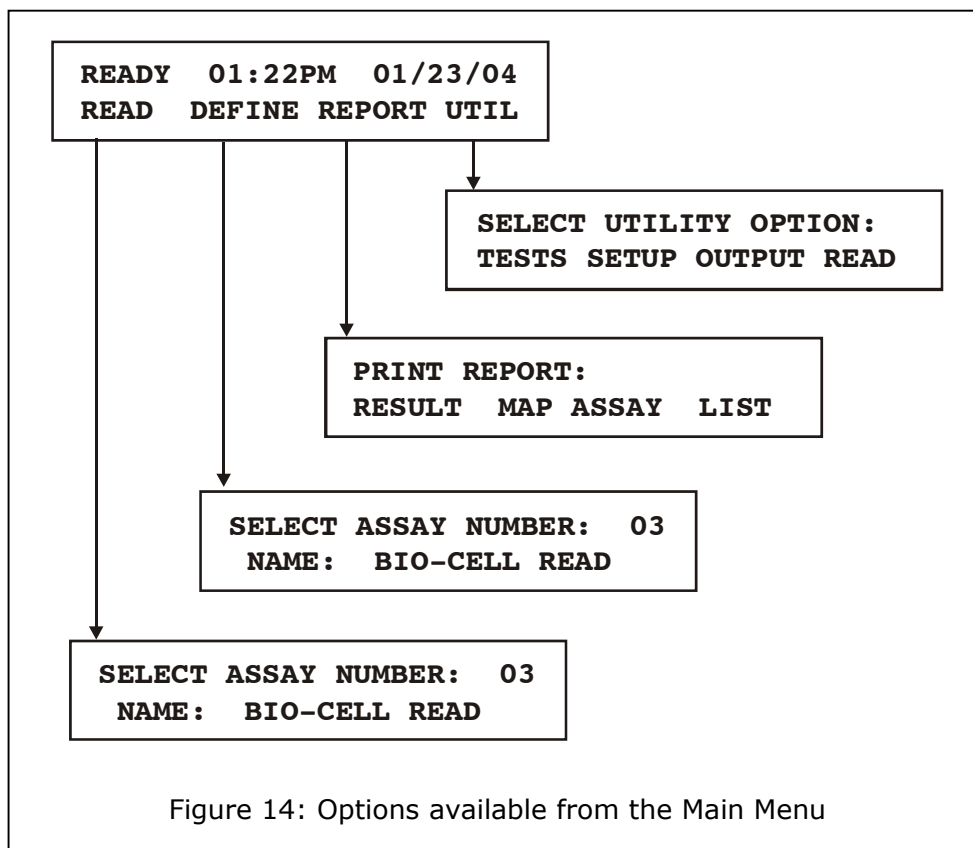
Following successful power-up of the μ Quant, the Main Menu appears:

```

R E A D Y      0 1 : 2 2 P M      0 1 / 2 3 / 0 4
R E A D      D E F I N E      R E P O R T      U T I L
  
```

The Main Menu permits access to all reader functions (**Figure 14**):

- **READ:** Choose a predefined assay for plate reading. Alternatively, press the key labeled **READ** on the keypad (page 83).
- **DEFINE:** Create a new assay or modify an existing one. Definable parameters include assay type, wavelengths, well identifiers, plate mapping options, formulas, and curve-fitting parameters (page 40).
- **REPORT:** Print a Result, Map, Assay, or List Report (page 88).
- **UTIL:** Run a **System Test** (page 101) or **Checksum Test** (page 107). Set up various global configuration options such as date and time, report output, and plate reading preferences (page 86).



Define

The Main Menu option **DEFINE** allows you to define the data acquisition and reduction parameters for a new assay, or modify preprogrammed assays stored in memory (Table 1).

Table 1
Preprogrammed Assay List

00	Spectral Scan assay	11	EDTA test assay	22	HRP TMB-S assay
01	Raw_OD assay	12	A320 Scattering assay	23	HRP-TMB assay
02	Raw_OD w/blk assay	13	Lowry Protein assay	24	Alk Phosphatase assay
03	Bio-Cell Read	14	Bradford Protein assay	25	B-galact ONPG assay
04	KFactor Bio-Cell	15	BCA Protein assay	26	Urease assay
05	DNA Quant assay	16	B-galactosidase assay	27	MTT assay
06	260/280 Ratio assay	17	NADH assay	28	XTT assay
07	RNA Quant assay	18	HRP 5AS assay	29	WST-1 assay
08	Oligo Quant assay	19	HRP ABTS assay	30	Methylene Blue assay
09	Protein Quant assay	20	HRP OPD-S assay		
10	Phenol assay	21	HRP OPD assay		

1. Start at the Main Menu and select **DEFINE** to display the **SELECT ASSAY NUMBER** screen.

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

2. Select an assay to define or modify, then press **ENTER**. See **Selecting an Assay to Define** on the next page for detailed instructions. The **EDIT ASSAY NAME** screen will appear.

❖ If you are modifying/selecting a PANEL assay (#99), see page 78 for instructions.

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


3. (Optional) Edit the assay name, then press **ENTER**. See **Editing the Assay Name** on the next page for detailed instructions. The **DEFINE** menu will appear:

```

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

```

The following options are available within the **DEFINE** menu:

- **METHOD:** Define the wavelength type (single or dual), wavelength(s), and plate geometry (page 43).
- **MAP:** Specify the plate layout, using blanks, controls, standards, and/or samples. Choose to map the plate manually, or let the software map it automatically (page 47).
- **FORMULA:** Define cutoff, transformation, and/or validation formulas. Create variables to be used within formulas (page 62).
- **CURVE:** Specify a curve fit type and x/y axis types (lin/log). Specify whether or not standard outliers can be edited, and then the method by which they will be edited. Enable or disable the extrapolation feature (page 74).

Selecting an Assay to Define

To select an assay to define or modify, start at the Main Menu and select **DEFINE** to display the **SELECT ASSAY NUMBER** screen.

```

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

```

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

Editing the Assay Name

Use the **EDIT NAME** screen to edit the name currently assigned to the assay. The assay name can contain up to 16 alphanumeric characters.

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

- The cursor is positioned at the first editable field (e.g., under “H”). Use the **alpha** and **numeric** keys to change the assay name.
- Use the **Options** key to sequentially advance the character positioned above the cursor. The characters will cycle through the alphabet (A-Z), with a space following Z.
- Use the **left** and **right arrow** keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Use the **CLEAR** key to remove the assay name from the display.
- Use **SOFT KEYS 1, 2, 3,** and **4** to select a dash, forward slash, colon, or space for inclusion in the assay name.

Define (Method, Map, Formula and Curve)

The **DEFINE** screen allows you to edit the **Method, Map, Formula,** or **Curve Fit** parameters for the currently selected assay.

```

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

Press the **SOFT KEY** beneath the displayed option to access the following functions:

- **METHOD**: Specify the wavelength type, wavelength(s), and plate geometry.
- **MAP**: Specify mapping formation.
- **FORMULA**: Access the formula entry screens.
- **CURVE FIT**: Specify curve-fit options.

Defining METHOD

The definition of a method includes selecting:

- Single or dual wavelength
- Wavelength(s)
- Plate geometry



The options appear on the display in the order that they were programmed in the assay. If the assay contains a closed variable (i.e., an element of the assay definition that you cannot access or modify), the entry screen is skipped.

Single or Dual Wavelength



Note: Only the Endpoint read method is supported here. There is one spectral scanning onboard assay available (see page 81 for more information).

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

- Select **SINGLE** or **DUAL** wavelength.
- Press **ENTER** to continue.

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

If **SINGLE** wavelength is chosen, the reader measures the optical density of each well at a single wavelength.

If **DUAL** wavelength is chosen, each well is read twice, each time at a different wavelength. The microplate is not removed from the reading chamber between the two measurements. The final reported optical density is the difference between the two readings (the delta OD). Dual-wavelength readings can significantly reduce optical interference caused by scratched or fingerprinted microplates, since the scratches or fingerprints reduce the amount of light on both wavelengths.

Meas Selection

The **MEAS** selection screen allows you to select the wavelength(s) for the assay. Press ***MORE** to cycle through the available options.

❖ The six wavelength values presented here for selection come from the μ Quant's "wavelength table." You can change the contents of the table by selecting **UTIL** → **SETUP** → **LAMBDA** from the Main Menu. See **Editing the Wavelength Table** on page 93 for more information.

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

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

- Select the **MEAS** (primary) wavelength.
- Use the right arrow key to move the cursor to **REF** and then select the Reference Wavelength.
- Press **ENTER** to continue.

Plate Type

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.

The μ Quant *does* allow you to select a Plate Type of **384**; however, it does *not* print results reports for assays with this plate type. After reading a 384-well plate, the μ Quant sends the raw data to a host PC where it can then be printed and/or further analyzed using data analysis software.

The most effective and versatile method for reading 384-well plates is to use BioTek's Gen5™, KC4™, or KCjunior™ for microplate data analysis and control software. See **Appendix B, Using 384-Well Geometry**, for more information.

P	L	A	T	E	T	Y	P	E	:	9	6	W	E	L	L	S
6	1	2	2	4	* M O R E											

P	L	A	T	E	T	Y	P	E	:	9	6	W	E	L	L	S
4	8	9	6	3	8	4	* M O R E									

P	L	A	T	E	T	Y	P	E	:	9	6	W	E	L	L	S
6	0	T	7	2	T	9	6	T	* M O R E							

P	L	A	T	E	T	Y	P	E	:	9	6	W	E	L	L	S
9	6	H	9	6	M	* M O R E										

- Press ***MORE** to cycle through the available options.

6: 6-well (2 x 3)	60T: 60-well Terasaki (6 x 10)
12: 12-well (3 x 4)	72T: 72-well Terasaki (6 x 12)
24: 24-well (4 x 6)	96T: 96-well Terasaki (8 x 12)
48: 48-well (6 x 8)	96H: 96-well Hellma Quartz (8 x 12)
96: 96-well (8 x 12)	96M: 96-well Metric (8 x 12, 9 mm well spacing)
384: 384-well (16 x 24)	

Defining MAP

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

- Automatic or manual map generation
- Mapping direction
- Replication direction
- Blank Map selection
- Blanking constant
- Number of blanks
- Location of blanks
- Number of standards
- Number of standard replicates
- Averaging of standards
- Concentration and location of standards
- Number of controls
- Control Type definition
- Number of control replicates
- Control location
- Number of samples
- Number of sample replicates
- Sample location



IMPORTANT! When 384-well plate type is selected, access to map, formula, or curve options is denied. The 384-well reads are preset for the map to have 384 samples. This does not include any blanking, controls, or standards.

MAP screens appear in the order that they were defined in the assay. If the assay has a closed variable, the screen for this variable is omitted.

```

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

- At the **DEFINE** options screen, select **MAP** to begin the plate map process.

Map Generation

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

```

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



Use the **SHIFT-CLEAR** keys to clear any previously defined map.

Mapping Direction

This option allows you to 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). The currently selected Mapping Direction appears on the top line of the display, and the available options appear on the bottom.

```

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.

Replication Direction

This option allows you to 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.
- Examples of mapping directions are shown on the next page.

Examples of Mapping Directions

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

Start Mapping at Well Location

The **START MAPPING AT WELL LOCATION** screen is only shown if automatic mapping is selected. This option allows you to enter the location of the well that will be the starting point for automatic mapping.

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

- Use the **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

This option allows you to select which blanking method to apply to the assay.

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

```

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

```

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

```

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

- Select the **BLANK MAP** type (see the descriptions on the next page).
- Press ***MORE** to cycle through the available options: **ROW** or **COLUMN**, and **P-ACROSS** or **P-DOWN**.
- Press **ENTER** to save the well location and continue.

Blank Map Definitions

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

❖ **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 entry screen only appears when a **CONSTANT BLANK** map is selected. Enter the value to be subtracted from each well on the plate.

E N T E R	
B L A N K I N G C O N S T A N T :	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

The **NUMBER OF BLANKS** field allows you to enter the number of blank wells on the plate. This entry screen is only displayed when Full, Column, or Row Blank maps are selected. Any previously defined value is displayed.

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

- Use the **numeric** keys to enter the number of blanks. The range is 0 to 48.
- Use the **CLEAR** key to clear the **NUMBER OF BLANKS** value from the display.

Blank Location

The **BLANK LOCATION** screen allows you to define where the blank well or wells occur on the microplate. This screen only appears if **manual mapping** was selected.

```

ENTER THE LOCATION OF
BLANK # 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

This option allows you to enter the number of standard **groups** that will be used in the assay. Any previously defined value will be displayed on the screen.

❖ **Note:** If the number of standards is altered, the number of replicates for the standard automatically reverts to 1.

```

ENTER NUMBER OF
STANDARDS : 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

This option allows you to enter the number of replicates per standard group in the assay. Any predefined value appears on the display.

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**. The range is 1 to 8 replicates. The software will verify that the number of replicates, multiplied by the number of standards, does not exceed the number of wells on the plate.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to continue.

Average Standards

The **AVERAGE STANDARDS** option allows you to select whether or not to average the replicates of each standard group. This average is used to calculate the standard curve instead of using the individual replicates of each standard.

❖ If the number of standard replicates is 1, this option is not available.

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

- 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

The Standard Concentration field allows you to 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 : C O 1

- Use the **numeric** and **alpha** keys and the **decimal point** key to enter standard concentration values. The range is .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. The following table lists 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
384-Well	A01-P24
60T	A01-F10
72T	A01-F12
96T	A01-H12
96H	A01-H12
96M	A01-H12

Number of Controls

The **NUMBER OF CONTROLS** screen allows you to enter the number of control **groups** that will be used in the assay. Any previously defined value will appear on the display.

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

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

Control Type

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

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

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

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

- Choose one control identifier for each type of control in your assay. The available options are: **Positive Control**, **Negative Control**, **High Positive Control**, **Low Positive Control**, **CTL1**, **CTL2**, **CTL3**, **CTL4**.
- After choosing an identifier for Control #1, press **ENTER** to choose the identifier for the next control.

Number of Control Replicates

The **NUMBER OF CONTROL REPLICATES** screen entry screen is presented if the number of control groups is greater than 0.

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 Number of [Control] Replicates.

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

Location of Controls

Use this option to 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 displayed location field can only be edited if **manual mapping** was selected (see page 49).

- 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

The number of sample groups on the plate can be defined here, and/or it can be defined at run-time if **UTIL** → **READ** → **PROMPT FOR SAMPLE COUNT?** is set to **YES**. See **Read Options** in **Chapter 3** for more information.

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

- 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

After the number of sample groups is specified, the **NUMBER OF SAMPLE REPLICATES** entry screen is presented.

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

- 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** is selected and samples are defined, the locations for each sample replicate must be specified.

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.

Defining FORMULA

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

To define formulas:

1. Start at the Main Menu and select **DEFINE**.
2. Select the assay then press **ENTER**. The **DEFINE** options screen will appear.

```

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
  
```

3. Select **FORMULA**. The **SELECT FORMULA TYPE** screen will appear.

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



To capture and manipulate the raw data using 384-well microplates with the μ Quant, you must use Gen5™, KC4™, or KCjunior™. Gen5, KC4 and KCjunior are PC-based software programs you can use to set up your assay, communicate with the μ Quant to run the assay, and then manipulate the raw data that is automatically retrieved from the reader. Refer to **Appendix B** for additional information.

Formula Type

The μ Quant 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, the **FORMULA ENTRY** screen appears. Each formula can contain a maximum of 24 characters. Spaces are not necessary.

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

F O R M U L A # 1 :

M A T H O T H E R M A P F U N C T N

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**, or **FUNCTION** options, continue to press the appropriate **SOFT KEY**. For example, press the **MATH SOFT KEY** several times to see +, -, *, /, %, =, etc. When the desired option appears, press the **right arrow** key to select it and advance to the next editable field.
 - Press the **left arrow** key to move the cursor to the left.
 - Press **CLEAR** to delete the item above the cursor.
 - When a formula is complete, press **ENTER** to continue.
- Select **MATH** to insert a mathematical symbol such as +, %, or <=.
- Select **OTHER** to insert an opening "(" or closing ")" parenthesis, or logical operators **AND** or **OR**.
- Select **MAP** to insert a well ID such as **BLK;x** or **NC;1**.
- Select **FUNCTN** to insert a mathematical function such as **LOG** or **SQRT**.



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

MATH

The following mathematical symbols can be used in formulas:

+	Addition
-	Subtraction
*	Multiplication
/	Division
%	Percent

==	Equal to
>	Greater than
>=	Greater than or equal to
<	Less than
<=	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
ALOG10	Anti Log Base 10
AB	Absolute Value
PWR	Power

ALOG	Anti Log
LOG	Log
SQRT	Square Root

EXAMPLES

LOG10- Log Base 10-
 $\text{Log}_{10} 2 = 0.301029995$

ALOG10- Anti Log Base 10-
 $\text{ALOG}_{10} (0.30102995) = 2$

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

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

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

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

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

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 63 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 REQUIRED CONTROLS**. The range is 1 through the number of defined replicates of a control or blank.
- ◆ Press the **CLEAR** key to clear the displayed value.
- ◆ Press **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 63 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 62 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.

Example:

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

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

The transformation formula is: **SMP-NC ; x**

◆ If **OD** is chosen:

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

Example:

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

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.

- At the **SCOPE VARIABLE** selection screen, choose **OD** and press **ENTER**.
- Enter the formula $(NC; x - CTL1; x)$ by using the **MATH**, **OTHER**, **MAP** and **FUNCTION** keys. Press **ENTER**.
- The formula definition screen is displayed. Choose **TRANS**.
- Enter the formula $(OD - (TVAR)) / (TVAR) * 100$ using the **MATH**, **OTHER**, **MAP**, and **FUNCTION** keys. ("TVAR" is available as MAP option.)

Example:

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

- At the **SCOPE VARIABLE** selection screen, choose **OD** and press **ENTER**.
- Enter **STD1** as the TVAR formula. Press **ENTER**.
- The formula selection screen is displayed. Choose **TRANS**.
- Enter the formula $(OD/TVAR) * 100$ using the **MATH**, **OTHER**, **MAP**, and **FUNCTION** keys. "TVAR" is available as a MAP option.

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

Defining CURVE

❖ **Note:** These screens are displayed on the μ Quant in the order in which they appear in the assay. If a closed variable is being used in the assay, the entry screen is omitted.

To define curve-fitting parameters for an assay:

1. Start at the Main Menu and select **DEFINE**.
2. Select the assay and then press **ENTER**. The **DEFINE** option screen will appear:

```

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
```

3. Select **CURVE**. The definable curve-fitting parameters include:
 - Curve-Fit Type
 - Editing of Outliers
 - Axis Identification
 - Extrapolation of Unknowns

❖ **Note:** If a transformation formula has been defined, then data used to generate a standard curve is based on the transformed data.

Curve-Fit Type

The μ Quant supports seven different curve-fitting methods: linear, quadratic, cubic, 4-P, 2-P, cubic-spline, and point to point.

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

```
C U R V E - F I T   T Y P E :   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 curve fit:** A simple best-fit straight line is plotted using the values of the standards.
- **Quadratic or “Quad” curve fit:** 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 curve fit:** A curve fit that uses the equation “ $ax^3 + bx^2 + cx + d = y$ ” to plot the standard’s values. This type of curve fit is affected even less than the quadratic fit when any particular standard has a poor value.
- **2-P (LOGIT/LOG):** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The logistic equation is algebraically transformed to a simpler form in which experimentally determined values are used for the responses at concentrations of zero and infinity.
- **Cubic Spline (C-Spline) curve fit:** A piecewise polynomial approximation consisting of joining a set of data points by a series of straight lines, which is then smoothed by using a cubic fit.

- **4-Parameter Logistic or “4-P”**: A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that becomes asymptotic to the upper and lower standard values. The 4 parameters are: Left asymptote, Right asymptote, Slope and Value at the Inflection point. This fit is most recommended for immunoassay data, and is more exact than Logit/Log.
- **Point to Point or “PT to PT”**: A plot that connects each standard point with a line, with no averaging of the values to “smooth” the curve at each standard.
- Press **SOFT KEYS 1, 2, 3, or 4** to select the curve-fit type that is displayed above the soft key. Select ***MORE** to display additional options.
- Press **ENTER** to save the selection and advance to the next screen.

Edit Standard Outliers

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

<p style="text-align: center;">E D I T S T D O U T L I E R S : M A N U A L</p> <p style="text-align: center;">N O N E M A N U A L</p>

- Select **NONE** to suppress the **EDIT STANDARD OUTLIERS** capability for this assay.
- Choose **MANUAL** to enable the capability.
 - If **AVERAGE STANDARDS** is set to **NO**, the individual standard replicates are available for editing. If set to **YES**, the standard **groups** are available for editing.
 - After the assay is run and reports are generated, press **REPORT** from the Main Menu. Press **RESULT**, select the assay, and then press **ENTER**. The **EDIT STD OUTLIERS? YES/NO** prompt will appear. See **Editing Standard Outliers** on page 89 for further instructions.

X/Y Axis Type

❖ **Note:** Linear only (LIN/LIN) axes are recommended when using the Cubic Spline fit.

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

```

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

```

- 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.

Extrapolation of Unknowns

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

```

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

Panel Assays

A Panel assay is a collection of up to 8 assays to be run on one plate.

- The most common reason to use a Panel assay is for confirmatory tests based on a screening test in clinical applications.
- 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.
- The assays specified within the Panel must all read at the same wavelength(s).
- Any curve-fit type, formulas, or standard concentrations previously defined for each assay will be used when the assay is selected for a Panel.
- The type and number of controls, blanks, standards, and replicates in the assays chosen for the Panel will be “copied” into the Panel definition. Map or assay parameters must first be changed in the predefined assay before they can change in the Panel.

❖ **Tip:** For use with mapping the Panel, consider printing a Map Report for each assay that will be included in the Panel.

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

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

- The default name is “PANEL”.
- Use the **alpha** and **numeric** keys to update the assay name, if desired.
- Press **ENTER** to continue. The **NUMBER OF ASSAYS** screen will appear.

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

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

```
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
```

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



The original mapping directions for the predefined assays are overwritten by the Panel's mapping directions. If the assay includes replicates, they will follow the Panel mapping direction.

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

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

- 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.
- After an assay is selected, its starting location must be defined.

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

- Use the **alpha** and **numeric** keys to choose the well location to begin the assay. Wells A01 through H01 are valid for **ACROSS** mapping; A01 through H12 are valid for **DOWN**.
- 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 “chirp” if you try to map into a well that is already assigned for use with the Panel.
 - The mapping for Assay 22 may begin at the next available well location after Assay 8 mapping is complete.
 - After all the assays have been entered into the Panel, consider printing the Panel’s Map Report to verify the map before reading the plate. Choose **REPORT** (from the Main Menu), **MAP, ASSAY 99**. The reader will print the map of each assay configured in the Panel.
 - The Panel Assay results are sorted by sample (unless a custom assay has been programmed by BioTek).

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

Spectral Scanning

Spectral scans measure peak absorbance without using filters.

- To run a Spectral Scan, start at the Main Menu, select **DEFINE**, and then choose assay number **00** to display the assay name.

```

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

- Press **ENTER** to bypass the **ASSAY NAME** and **DEFINE** options and display the **SELECT WAVELENGTH, START** screen. Press **ENTER** to continue.

```

S E L E C T   W A V E L E N G T H
S T A R T :                               2 0 0 n m
  
```

- Enter the wavelength to **START** the spectral scan. The valid range is 200 to 999 nm. Press **ENTER** to continue.
- Select the wavelength at which to **STOP** the scan. The valid range is the starting wavelength +1 (e.g., 201) to 999 nm. Press **ENTER** to continue.

```

S E L E C T   W A V E L E N G T H
S T O P   :                               9 9 9 n m
  
```

- Select the step increment between successive wavelengths for the spectral scan. The valid range is 1 to the difference between the **START** and **STOP** wavelengths. For example, if **START** | **STOP** | **STEP** are set to 340, 800, and 50, the μ Quant will read at 340, 390, 440, 490, and so on. Press **ENTER** to continue.

```

S E L E C T   W A V E L E N G T H
S T E P :                               _ 5 0 n m
  
```

- Choose the plate type. Press ***MORE** for further options. Press **ENTER** to continue.

```

P L A T E   T Y P E           9 6   W E L L S
      6           1 2           2 4           * M O R E
  
```

```

P L A T E   T Y P E :           9 6   W E L L S
      4 8           9 6           3 8 4           * M O R E
  
```

```

P L A T E   T Y P E           9 6   W E L L S
      6 0 T           7 2 T           9 6 T           * M O R E
  
```

```

P L A T E   T Y P E :           9 6   W E L L S
      9 6 H           9 6 M           * M O R E
  
```

- Select the well location of the scan. The valid range depends on the plate geometry. Press **ENTER** to continue.

```

S P E C T R A L   S C A N
A T   W E L L   L O C A T I O N :           C 0 3
  
```

- Choose **YES** to precalibrate the instrument before the scanning data is collected. This procedure will collect the new dark and air-blank values. Otherwise, choose **NO**, and press **ENTER** to return to the Main Menu.

```

P R E - C A L I B R A T E           Y E S
      Y E S           N O
  
```

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.

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

To read a plate:

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** on the next page for detailed instructions.
3. If required, enter the number of Samples, Plate ID, and/or Sample ID.

❖ **Note:** The options to present these screens are configurable by selecting **UTIL** → **READ**. See **Read Options** in **Chapter 3** for more information.

```

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

```

```

P L A T E   I D :   1 2 3 4 A
-           /           :           S P A C E

```

```

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

```

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

❖ Alternately press the **READ** key on the lower right of the keyboard.

Selecting an Assay to Run

To select an assay to run, start at the Main Menu and select **READ**. The **SELECT ASSAY NUMBER** screen will appear:

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

- 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.
- 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 the assay was created or downloaded from BioTek's Extensions™ Define Reader Protocol software.

- Prompts enable 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, such as one that was created using Extensions™ software, typical prompts might include:
 - The number of samples
 - Standard concentrations
 - Assay ID
 - Fill pattern
 - Blank method
 - First well location
 - Replicate count for each well type
 - Wavelength mode
 - Report preferences, etc.

Enter Number of Samples

If the **ENTER NUMBER OF SAMPLES** prompt is presented, indicate the number of sample **groups** on the plate. The number of sample replicates is typically 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 “chirp” and automatically change the value to the maximum permissible number of samples.

Enter Plate ID

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



Use caution when creating multiple Plate IDs. The reader does not warn you that you 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.



If the optional internal bar code scanner is installed, the reader will automatically scan the plate/bar code label and use this as the Plate ID.

```

P L A T E   I D :   1 2 3 4 A
-           /           :           S P A C E

```

- Use up to 10 alphanumeric characters. See pages 36 and 33 for instructions on using the keypad.
- Press **ENTER** to advance to the **ENTER SAMPLE ID** screen.

Enter Sample ID

If the **ENTER SAMPLE ID** prompt is presented, enter a **starting** sample identification number.

```

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

```

- The valid entry range is from 0001 to 9999.
- The software will automatically increment each subsequent sample identification number by 1.
- 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.

```

S A M P L E #   1           L O C A T I O N
                    R E P #   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 on the display, the reader is ready to read a plate:

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

- Before reading the plate, ensure that the printer is connected, turned on, and full of paper.
- Place the plate in the carrier and press the **READ** key to initiate the plate read.
- 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

Reports are automatically generated after a plate has been read (see **Output Options** in **Chapter 3** for information on selecting reports). Results reports also can be regenerated manually by using the **REPORT** option from the Main Menu. In addition, Map, Assay, and Assay List reports can be printed.

❖ **Note:** See **Appendix C** for sample reports.

```

R E A D Y      0 1 : 2 2 P M      0 1 / 2 3 / 0 4
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

```

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

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 4
  
```

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

Editing Standard Outliers

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

```

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

- 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.



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

Printing Results

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

```

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

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

Map 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.

Utility

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 will appear:

```

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

2. Select **DATE**. The **DATE** entry screen will appear:

```

D A T E :      0 1 / 2 3 / 0 4                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.

Editing the Wavelength Table

❖ The wavelength table contains six user-defined wavelength values that are presented for selection when defining an assay. See **Meas Selection** on page 45.

To edit the wavelength table:

1. From the Main Menu, select **UTIL** → **SETUP**. At the **EDIT SETUP INFORMATION** menu, select **LAMBDA**. The **EDIT WAVELENGTH** screen will appear:

```

E N T E R
L A M B D A # 1      W A V E L E N G T H : 6 5 0
  
```

- Enter the correct wavelength for each of the six positions, using the **numeric** keys. The range is from 200 to 999 nm.
- Continue to scroll through the six available wavelength positions, pressing **ENTER** between each.
- When all six wavelengths have been defined, or skipped, the software will automatically return to the **EDIT SETUP INFORMATION** menu.

Specifying Data Output and Reporting Options

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

- The onboard software provides several different 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.

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
  
```

- The current output option is displayed on the top line. Select **PRINT** to send reports directly to a printer, **COMPUTER** to send data out through the serial port, or **BOTH**.

❖ **Note:** These options have no effect on data output if the instrument is being controlled by software (such as Gen5™, KC4™, or KCjunior™) running on a host PC.

- 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 μ Quant supports printers using either HP's PCL3 language, such as the HP DeskJet series, or Epson's LQ language. For the latest list of compatible printers, consult the BioTek Web site (www.biotek.com), or call BioTek Instruments' Technical Assistance Center (refer to **Chapter 1** for contact information).
- Select **EPSON** or **HP** as appropriate.
- 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

```

- 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 examples of Reports.

- Press **ENTER** to continue. The **SAMPLES IN 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

```

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

```

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

```

- Select **YES** to print the standard curve (only applies to quantitative assays).
- Press **ENTER** to return to the **SELECT UTILITY OPTION** screen.

Selecting Read Options

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

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

- The **PROMPT FOR PLATE ID** screen will appear. Press **ENTER** to cycle through the prompt screens.

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

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

R	E	A	D	I	N	R	A	P	I	D	M	O	D	E	?	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.
 - RAPID MODE** prompts for the plate to be read in Fast Mode or Regular Mode.

❖ **RAPID MODE** reads a 96-well plate in approximately 30 seconds. If **NO** is selected, the plate will be read in **NORMAL MODE** (approximately 50 seconds). Refer to **Chapter 1** for specifications.

- Pressing **ENTER** after each selection advances the display.
- When selections are completed, the display returns to the **SELECT UTILITY OPTION** menu.

Chapter 5

Instrument Qualification

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

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Recommendations for Achieving Optimum Performance

Microplates should be perfectly clean and free of dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.

Although the μ Quant™ supports standard flat, U-bottom, and V-bottom microplates, optimum performance is achieved with optically clear, flat-bottomed wells.

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

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

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.

Recommended Qualification Schedule

The schedule shown in **Table 2** defines the factory-recommended intervals for performance testing for a microplate reader used for one shift seven days a week.

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

Table 2
Recommended Qualification Schedule

Tests	Installation Qualification	Operational Qualification	Performance Qualification	
	Initially	Initially/ Annually	Monthly	Quarterly
System Self-Test, p. 101	✓	✓	✓	
Checksum Test, p. 107	✓			
Absorbance Plate Test, p. 108		✓	✓	
Liquid Test 1, p. 121*		✓		✓
Liquid Test 2, p. 123*		✓		✓
Liquid Test 3, p. 127** (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; it is provided for sites requiring verification at wavelengths lower than those attainable with the Absorbance Test Plate.

Qualification Procedures

You may use the tests outlined in this section to confirm initial and ongoing performance of the μ Quant. Set up the μ Quant according to the instructions in **Chapter 3, Installation**. Confirm that the instrument powers up and communicates with peripherals for the Installation Qualification. After Installation Qualification, conduct the Operational Qualification Tests.

❖ **Note:** An Installation/Operational/Performance Qualification (IQ/OQ/PQ) package (PN 7270514) for the μ Quant is available for purchase. Contact your local dealer for more information.

Your μ Quant reader was fully tested at BioTek before shipment and should operate properly upon initial setup. If you suspect that problems may have occurred during shipment, if you reshipped the instrument, or if regulatory requirements dictate that Performance Qualification Testing is necessary, you should perform the following tests. After the initial confirmation of operation, you should perform the Absorbance Plate Test monthly and Liquid Testing semiannually.

- The **System Test** (described on the next page) verifies proper gains, bulb operation, and low electronic noise.
- The **Checksum Test** (page 107) compares the onboard software with internally recorded checksum values to ensure that no corruption has occurred.
- The **Absorbance Plate Test** (page 108) confirms the optical accuracy, linearity, mechanical alignment, repeatability, and wavelength accuracy of the instrument.
- **Liquid Testing** (page 117) quantifies the instrument using liquids, which verifies operation in a way that glass test filters cannot.



Set the μ Quant to **Normal/Standard Read Mode** when running all verification procedures (**Chapter 3**).

System Test



IMPORTANT! Do not turn on the instrument until the carrier shipping block has been removed.

The System Test is performed automatically whenever the instrument is turned on. It can also be performed manually through the μ Quant Main Menu.

The System Test feature conducts a series of tests, at each of six set wavelengths, which confirm adequate light levels, low electronic noise, adequate photodiode sensitivity, and overall system cleanliness. The testing is designed to ensure that the μ Quant will give in-specification performance for each set wavelength over the specified OD range.

The reader will “chirp” repeatedly if the power-on System Test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek. A system test should then be initiated to try to retrieve an error code from the reader.

To run the System Test from the instrument keypad:

1. If running a System Test, attach a printer to the instrument. See **Connecting a Printer to the μ Quant** in Chapter 4 for instructions.
2. From the Main Menu, select **UTIL**. The **SELECT UTILITY OPTION** screen will appear:

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

3. From the **SELECT UTILITY OPTION** screen, select **TESTS**. The **SELECT TEST** screen will appear:

```
S E L E C T   T E S T
S Y S T E M   C H K S U M           C A L P L A T E
```

❖ **Note:** The desired wavelengths must be entered into the wavelength table before running the test.

4. Select **SYSTEM** to run the System Test.

To run the System Test using Gen5, select **System|Diagnostics|Run System Test**. If the **Instrument Selection** dialog appears (Gen5 and Gen5 Secure only), select **uQuant**, then click **OK**.

To run the System Test using KC4™, open the **System** menu and select **Diagnostics| Run Optics Test**. Enter the **Reader Serial Number**, then click **Start**.

To run the System Test using KCjunior™, open the **Utilities** menu and select **Diagnostics| Reader System Test**. Enter the **Reader Serial Number**, then click **Run Test**.

The System Test report lists results in a Pass/Fail format (**Figures 15** and **16**) and can be viewed, saved, and printed. Print the report to document periodic testing or troubleshoot.

❖ **Note:** This report varies slightly depending upon how it was run (via the keypad, Gen5™, KC4™ or KCjunior™).

Gen5 System Test Report

Reader: uQuant (Serial Number: 128755)
 Basecode: P/N 7270201 (v2.02)
 Date and Time: 1/6/2006 10:37:03 AM
 User: Administrator
 Company:
 Comments: System Test performed during IQ

Test Results

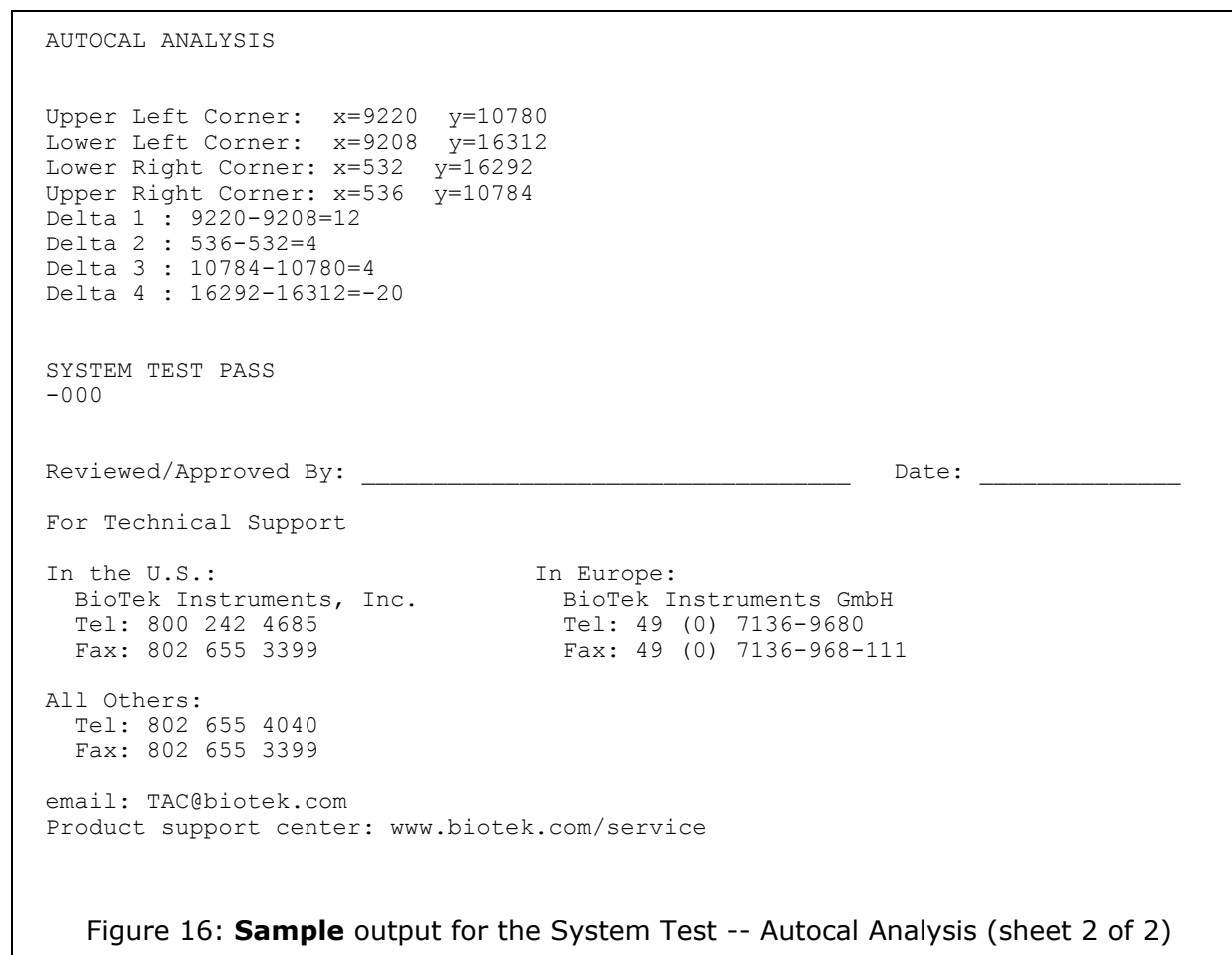
Operator ID: _____

Notes: _____

11:53AM 01/06/06 SYSTEM SELF TEST

Lambda: 405	Gain: 2.10	Resets: 4	
Channel:	Ref 1		
Air:	8656 39546		
Dark:	5021 5290		
Delta:	3635 34256		
Lambda: 450	Gain: 1.73	Resets: 4	
Channel:	Ref 1		
Air:	8497 39846		
Dark:	5019 5241		
Delta:	3478 34605		
Lambda: 490	Gain: 2.29	Resets: 8	
Channel:	Ref 1		
Air:	8338 39832		
Dark:	5018 5096		
Delta:	3320 34736		
Lambda: 550	Gain: 1.74	Resets: 2	
Channel:	Ref 1		
Air:	8194 39947		
Dark:	5025 5577		
Delta:	3169 34370		
Lambda: 630	Gain: 2.51	Resets: 1	
Channel:	Ref 1		
Air:	7899 39176		
Dark:	5047 6786		
Delta:	2852 32390		
Lambda: 730	Gain: 1.77	Resets: 2	
Channel:	Ref 1		
Air:	7552 39440		
Dark:	5025 5584		
Delta:	2527 33856		
Channel:	Ref 1		
Noise Max:	5031 5380		
Noise Min:	5028 5377		
Delta:	3 3		

Figure 15: Sample output for the System Test (sheet 1 of 2)



❖ If the reader fails the System Test, it will not perform a read until the error condition is cleared. See **Chapter 7, Troubleshooting and Error Codes**, for a list of possible error codes that may be displayed if the System Test indicates a failure.

Photodiodes

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

Light Bulb

The Optics test also indicates if the light bulb is within operational limits.

The System Test includes subtests for Gain Values and Resets, Air-Dark Value, Noise Test, and Autocal Analysis. The data and results for these tests can be reviewed on the System Test report.

❖ **Note:** The lamp life is rated at an average of 1 billion flashes. The lamp should last beyond the useful life of the μ Quant reader. If there is a problem with the lamp, the intensity may drop, and the runtime self-check would detect a low signal level that would appear on the instrument's display. Contact BioTek's Technical Assistance Center for assistance if the lamp needs to be replaced. Refer to **Chapter 1** for contact information.

Gain Values and Resets

The energy from the Xenon flash bulb is filtered by the monochromator and focused into microwells and the transmitted energy is then focused on the photodetector. Each flash of the bulb results in a burst of current from the photodetector. This current is collected by an electronics integrator. The output of the integrator is voltage. This voltage is multiplied by the Gain before being digitized by the ATD (analog to digital converter) and passed to the microprocessor.

The Resets are used to get to the full scale of the ATD to ensure the best readings possible. In Normal Mode, 8 flashes are used per well. The energy of the flashes can be accumulated on the integrator, but the integrator needs to be reset before the ATD reaches full scale and overflows.

If the energy of a single flash is high on a given wavelength, the integrator has to be reset after each flash (e.g., 8 resets will be used for 8 flashes). If the accumulated energy of two flashes does not saturate the ATD, then 2 flashes will be accumulated before resetting the integrator, and 4 resets will be used. Two resets will be used if the energy of 4 flashes is accumulated; 1 reset will be used if the energy of 8 accumulated flashes does not saturate the ATD.

- The Gain is used as a fine resolution multiplier to ensure the full range of the ATD is used for measurements. The Signal on a given wavelength is proportional to Resets/Gain. When this value drops below a certain threshold, the Self Test fails. To monitor instrument light levels, one can plot this value over time (e.g., monthly) for a given set of wavelengths.
- At low light levels (Resets = 1), if the Gain is over 20, you may want to contact BioTek Technical Support to schedule preemptive service on the instrument. The instrument will continue to operate at these signal levels and pass the internal system test, but preemptive service is advised.

Air - Dark Value

Just before each flash, the Dark reading is taken representing the background signal in the instrument. This dark is subtracted from a reading taken on Air (with no plate in the light path). The difference between the Air and Dark readings represents a true level of light available for measurements. If this level drops below a certain threshold on any channel, the Self-Test fails.

Noise Test

The noise test ensures that there is minimal variation in signal between “Dark” readings with no light present. The noise test values are given in the lower section of the System Test report.

The Noise test could fail if there is:

- An ambient light leak or electrical “noise” penetrating the measurement chamber. The bottom and top shrouds provide light and electrical shielding. Make sure that the plate chamber door is fully closed, and the top and bottom shrouds are properly installed and fastened without any gaps.
- Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding. This type of problem would require factory servicing.

Autocal Analysis

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 (see page 104). In the field, the alignment test of the Absorbance Plate Tests is used to verify that the carrier positioning is correct.

Checksum Test

This test also runs automatically when the reader is turned on. The test compares the software to the internally recorded checksum values to ensure that the programming has not been corrupted. It verifies the checksum, part numbers, and versions of software currently loaded onto your reader. If there are any errors during the power-on checksum test, they will be displayed.

1. Select **CHKSUM** at the **SELECT TEST** screen to run the Checksum Test. The information displayed will *resemble* the following:

```

7 2 7 0 2 0 1      V E R S I O N   2 . 0 2
C O D E   C H E C K S U M :           ( F F 2 A )

```

- 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 display:

```

7 2 7 0 2 0 3 - F W           V 2 . 0 0 . 1

```

- The second checksum test display will show the **assay configuration** software part number and version number. After a few moments, the Main Menu will reappear.

Absorbance Plate Test

Description

- ❖ Gen5's Reader Diagnostics Utility (PN 5320201) must be installed on the computer's hard drive, if you wish to run the Absorbance Plate Test using Gen5™.
- ❖ The Absorbance Test Plate is also referred to as "CALPLATE" on the display of the μ Quant and as "Universal Test Plate" in KC4 and KCjunior software.

This test uses BioTek's **7-Filter Absorbance Test Plate** (PN 7260522) to confirm the mechanical alignment; optical density accuracy, linearity, and repeatability; and wavelength accuracy of the μ Quant.

The Absorbance Plate Test compares the reader's optical density and wavelength measurements to NIST-traceable values.

- ❖ An alternate method that may be used to determine accuracy/linearity, repeatability, and alignment is **Liquid Test 2**, described on page 123.

The Absorbance Plate Test confirms the following:

- **Mechanical Alignment.** Mechanical alignment of the plate carrier and standard microplates is confirmed by the four-corner positional accuracy check.
- **Accuracy.** Accuracy of the Optical Density readings – the comparison of the optical density readings with those listed on the Absorbance Test Plate's Standards Certificate will confirm the accuracy of the optical density readings at specific wavelengths.
- **Linearity.** Linearity of the Optical Density readings is confirmed by default if the optical density readings are accurate.
- **Repeatability.** Ensures that the instrument meets its repeatability specification by reading each Test Plate neutral density filter twice with the filter in the same location.
- **Wavelength Accuracy.** To check the wavelength accuracy, use BioTek's 7-Filter Absorbance Test Plate (PN 7260522). This test plate provides a multiband test filter in location C6, for the "Peak Wavelength Test."
BioTek's 6-Filter Absorbance Test Plate (PN 9000547) does not have a filter in the C6 location. This test plate can be used on any BioTek reader; however, if this plate is used, the Peak Wavelength Test will not be performed.

Test Plate Certificates

To run the Absorbance Plate Test on the μ Quant, you will need BioTek's 7-Filter Absorbance Test Plate (Part Number 7260522), with its accompanying certificates.

- The Standards Certificate contains standard OD values for the filters at several different wavelengths. See the sample Standards Certificate in **Figure 17** below.
- The Peak Wavelength Certificate contains one or more “Peak Wavelength” values for the glass filter in position C6 on the plate. Each value has a valid test range associated with it. For example, a Peak Wavelength value may be 586 nm with a test range of 580 to 590 nm (or tolerance values of -6/ +4). See the sample Peak Wavelength Certificate in **Figure 19** on page 116.

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			0.568	0.485	0.434
G3	1.133	1.052				1.040	0.881	0.783
H6	1.701	1.578				1.560	1.323	1.179
F5	2.279	2.024	1.976	1.956	1.893	1.865	1.537	1.272
D4	2.945	2.604	2.545	2.513	2.437	2.400	1.972	1.632
Set # 2453					Serial # 161259			
Figure 17: Sample Standards Certificate for the Absorbance Test Plate								

Before the Absorbance Plate Test can be performed, the standard OD values and the peak wavelength value(s) must be entered via the keypad or via Gen5™, KC4™, or KCjunior™ software. This only has to be done once.

Instructions for entering the Absorbance Test Plate data and for running the test are provided on the following pages.

Entering the Absorbance Test Plate Data

Use the Standards Certificate and Peak Wavelength Certificate included with the test plate when performing the instructions below and on the following page.

To enter the test plate data into the reader from the instrument keypad:

1. Start at the Main Menu and select **UTIL** → **SELECT UTILITY OPTION** → **SETUP**. The **EDIT SETUP INFORMATION** menu will appear:

```

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

2. Press ***MORE** to display the second **EDIT SETUP** menu. Select **CALPLATE** to access the **CALIBRATION LAMBDA** menu.
3. Using the Standards Certificate packaged with the test plate, select the filter wavelength.

```

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

```

C A L I B R A T I O N   L A M B D A :   4 0 5
4 0 5           4 5 0           4 9 0           * M O R E
  
```

4. Press **ENTER**. The **WELL LOCATION/CALIBRATION VALUE** screen appears:

```

W A V E L E N G T H : 4 0 5   W E L L : C 0 1
C A L I B R A T I O N   V A L U E :   0 . 1 5 8
  
```

5. Enter the calibration values listed on the test plate's Standards Certificate. After each entry, press **ENTER** to advance to the next consecutive well location.
6. When all values have been entered, press the Main Menu key.
7. **For the Peak Wavelength Test:** Note the test range specified in the Peak Wavelength Certificate, then define a Spectral Scan assay (Assay 00) to scan the C6 filter across the specified range in 1-nm increments. Refer to **Selecting an Assay in Chapter 4** for detailed instructions on setting up an assay.

To enter the test plate data into Gen5™, select **System|Diagnostics|Test Plates|Add/Modify Plates**, then click **Add**. Click the **Help** button for guidance when setting the wavelengths and entering the OD and peak wavelength value(s).

To enter the test plate into KC4™, select **System|Diagnostics|Define Universal Plates**, then click **Add**. Click the **Help** button for guidance when setting the wavelengths and entering the OD and peak wavelength value(s).

To enter the test plate data into KCjunior™, select **Utilities|Diagnostics|Universal Plate Test**, then click **New Data Sheet**. Click the **Help** button for guidance when setting the wavelengths and entering the OD and peak wavelength value(s).

Running the Absorbance Plate Test



IMPORTANT: Before running the Absorbance Plate 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.

To run the Absorbance Plate Test from the instrument keypad:

1. Start at the Main Menu and select **UTIL → TESTS → CALPLATE**. The **SELECT TEST** menu will appear:

```
S E L E C T   T E S T   :
S Y S T E M   C H K S U M           C A L P L A T E
```

2. Select the appropriate wavelength at the **CALIBRATION LAMBDA** screen, and press **ENTER** to save the value and continue.

```
C A L I B R A T I O N   L A M B D A   :   4 0 5
4 0 5           4 5 0           4 9 0           * M O R E
```

3. When prompted, place the Absorbance Test Plate on the plate carrier, and press the **READ** key to begin the test.

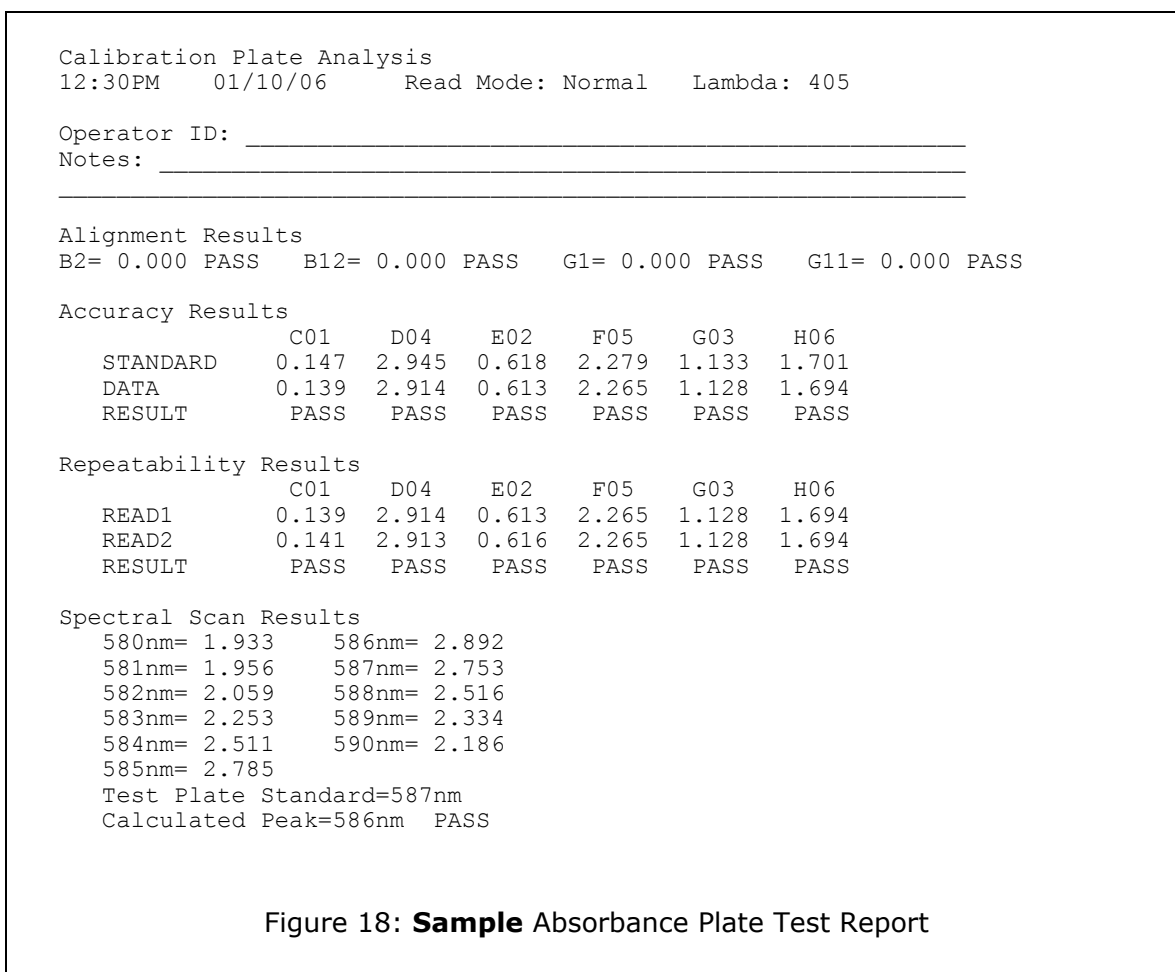
The Absorbance Test Plate report (“Calibration Plate Analysis”) will be sent to a printer when the test is run (see **Figure 18** for a sample report).

To run the test in Gen5™, select **System|Diagnostics|Test Plates|Run**. Select the appropriate reader, if the **Instrument Selection** dialog appears. Select the appropriate test plate, if the **Select Test Plate** dialog appears. Enter the reader **Serial Number** and **User** name (required information), then click **Start Test**.

To run the test in KC4™, select **System|Diagnostics|Run Universal Plate Test**. Select the appropriate **Universal plate**, enter the reader **Serial Number**, then click **Run Test**.

To run the test in KCjunior™, select **Utilities|Diagnostics|Universal Plate Test**. Select a **Universal Plate ID (Wavelength)**, enter the **Reader Serial Number**, then click **Run Test**.

For wavelength accuracy: While performing the steps above, click **Perform peak wavelength test** in Gen5 or KCjunior, or click **Peak absorbance search** in KC4.



❖ **Note:** The format varies depending on the software used to run the test.

Absorbance Plate Test Results and Troubleshooting Tips

If any of the Absorbance Plate Test parameters report as "FAIL," confirm that the standard values on the test plate Standards Certificate match the values on the printout. If not, correct and retest. If the test still fails, contact BioTek's Technical Assistance Center (refer to **Chapter 1** for contact information). Please have a copy of the test and the reader's serial number available when you call.

- **Mechanical Alignment:** The Test Plate has several groups of precisely machined holes to confirm the mechanical alignment of different microplate readers. The amount of light that shines through these holes 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 μ Quant may be out of alignment. If the reader fails the alignment test, review the following possible problems and solutions:
 - Ensure that the Test Plate is correctly seated in the microplate carrier. 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.

If these remedies are ineffective, call your BioTek representative or BioTek Technical Support.

- **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 has to be linear. Note that there may not be a Pass/Fail indication for filter values that are beyond the specified accuracy range of the instrument.

If the reader fails the accuracy test, review the following possible problems and solutions:

- Check the neutral density filters on the test plate to ensure they are clean. If necessary, clean them with lens paper. **Important!** 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 via the keypad, Gen5™, KC4™, or KCjunior™ are the same as those on the Test Plate Standards Certificate.
- Ensure that the Test Plate is within its calibration certification period.

If these remedies are ineffective, or if the Test Plate requires recalibration, call your BioTek representative or BioTek Technical Support.

- **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 that 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 reader fails the repeatability test, review the following possible problems and solutions:

- Check the neutral density 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.
 - If these remedies are ineffective, call your BioTek representative or BioTek Technical Support.
- **Wavelength Accuracy:** The 7260522 Test Plate has a glass filter in position C6 that is used to check wavelength accuracy of the monochromator.

The Test Plate Peak Wavelength Certificate lists the certified wavelength(s) at which the maximum absorbance peak occurs. Gen5, KC4, and KCjunior each have an option that can be enabled to scan the region of interest during the Absorbance Plate Test and confirm the accuracy of the glass reading.

❖ **Note:** You can use the instrument keypad to run a spectral scan assay (Assay 00) The resulting printout will indicate the wavelength at which the maximum absorbance peak occurs, thus confirming the wavelength accuracy.

The C6 filter is scanned across a specified wavelength range in 1-nm increments. The wavelength of the maximum absorbance is compared with the peak wavelength values entered via the keypad, Gen5, KC4, or KCjunior, which comes from the Peak Wavelength Certificate supplied with the test plate. The accuracy of the wavelength should be ± 3 nm (± 2 nm instrument, ± 1 nm filter allowance).

For example, if the test range is 580 to 590 nm, the Certificate value is 587 nm, and the reader reports a peak value of 590 nm, then the reader meets specifications. If the reader reports 591 nm, then the reader does not meet specifications.

If the reader fails the wavelength accuracy test, review the following possible problems and solutions:

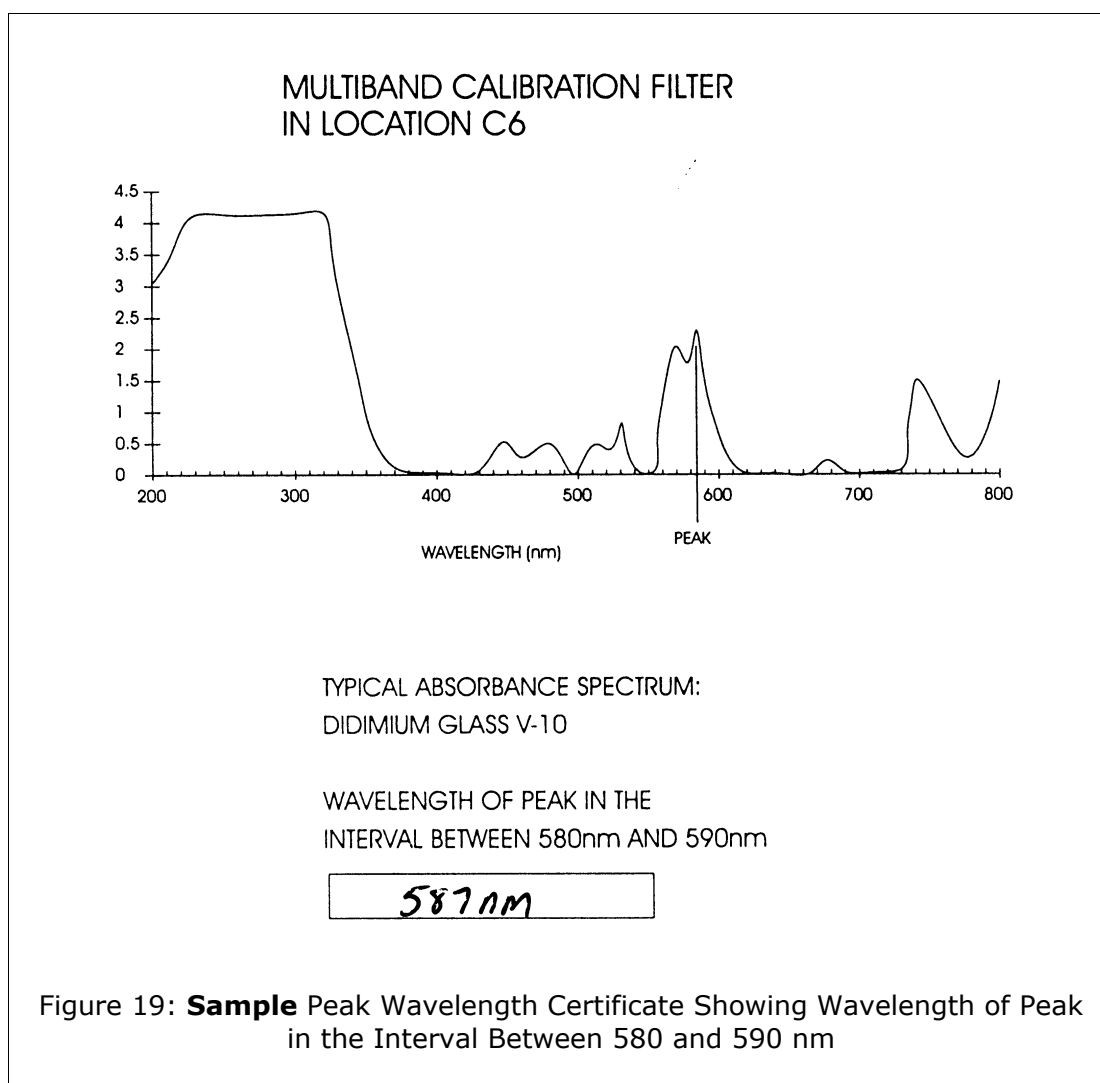
- Make sure that the test plate actually has a filter in location C6. BioTek's Absorbance 6-Filter Test Plate (PN 9000547) does not have this filter.
- Check the C6 filter to ensure that it is clean. If necessary, clean it with lens paper.



IMPORTANT! Do not remove the filter from the test plate, and do not use alcohol or other cleaning agents.

- Make sure that the information entered via the keypad, Gen5™, KC4™, or KCjunior™ matches the information on the Test Plate's Peak Wavelength Certificate.
- Make sure the Test Plate is within its calibration certification period.
- Check the microplate carrier to ensure that it is clear of debris.

If these remedies are ineffective, or if the test plate requires recalibration, call your BioTek representative or BioTek Technical Support.



Liquid Testing

Conducting Liquid Tests confirms the μ Quant'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** tests the alignment, accuracy, and repeatability of the reader and can be used for routine testing if you have the Absorbance Test Plate (see page 121).
- **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. Prepare the series of solutions of varying absorbances as described on page 123.
- **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 is optional because the reader has good "front end" linearity throughout its wavelength range (see page 127).
- BioTek offers a dye solution (PN 7120779, 25 ml; or 7120782, 125 ml) that may be used in the stock solution formulation for Liquid Tests 1 and 2, or, if you prefer, you may use the dye solution described in **Table 4** (page 119). The purpose of the formulation is to create a solution that absorbs light at ~ 2.000 OD full strength when dispensed at 200 μ l in a flat-bottom microplate well.
- Alternatively, any solution that gives a stable color will suffice. (This includes substrates incubated with an enzyme preparation and then stopped with an acidic or basic solution.) Some enzyme/substrate combinations that may be used as alternates to the described dye are shown in **Table 3** (page 118).

Repeatability

This test ensures that the instrument meets its repeatability specification by reading a color absorbing solution five times without removing the microplate. A dye solution that absorbs at 405 nm is acceptable, but other dyes that absorb at other wavelengths may also be used. Two columns of the microplate are filled with the test dye, and the well with the greatest variance between readings is reviewed for compliance with the specification.

If the reader fails the repeatability tests, review the following possible problems and solutions:

- Check the microwells to ensure that there is no debris that may have shifted and caused changes.
- Check the microplate carrier to ensure that all the holes are free of debris that may have shifted and caused changes.

If these remedies are ineffective, call your BioTek representative or BioTek Technical Support.

Alignment

This test confirms the mechanical alignment of the instrument by comparing the measurements read with a test solution in the microplate with those obtained when the plate is rotated so that A1 is now in the H12 position. The first dye OD readings from the repeatability testing listed above are compared to the same readings when the plate is turned 180°. This indicates if the same well will give similar values in the opposite position on the carrier. If the readings do not meet the specifications provided in **Chapter 1**, the reader may be out of alignment.

Table 3

Typical Enzyme-Substrate Combinations and Stopping Solutions

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

Stock Solution Formulation

The stock solution for Liquid Tests No. 1 and No. 2 may be formulated from the chemicals listed below (Solution A), or by diluting a dye solution available from BioTek (Solution B).

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

Table 4

Stock Solution Formulation for Liquid Test Nos. 1 and 2

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

Preparation of Stock Solution:

1. Weigh out 0.092 gram of FD&C No. 5 yellow dye powder into a weigh boat.
2. Rinse the contents into a 1-liter volumetric flask.
3. Add 0.5 ml of Tween 20, or 5 ml of BioTek's wetting agent.
4. Make up to 1 liter with DI water, cap, and shake well.

❖ This should create a solution with an absorbance of about 2.000 when using 200 μ l in a flat-bottom microwell. The OD value will be proportional to the volume in the well and the amount of FD&C No. 5 dye used. You can use a larger or smaller well volume, or add more dye or water to adjust the solution. Note that too small a well volume may result in increased pipetting-related errors.

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

Preparation of 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.

❖ This should create a solution with an absorbance of about 2.000 when using 200 μ l in a flat-bottom microwell. The OD value will be proportional to the volume in the well and the amount of QC Check Solution No. 1 used. You can use a larger or smaller well volume, or add more Check Solution or water to adjust the stock solution. Note that too small a well volume may result in increased pipetting-related errors.

Liquid Test 1



A 96-well, flat-bottom microplate is required for this test (Corning Costar® #3590 is recommended). Use a new microplate; any fingerprints or scratches may cause variations in readings.



IMPORTANT: Before running the liquid 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.

1. Using a freshly prepared stock solution (see Solution A on page 119 or Solution B on page 120), prepare a 1:2 dilution using DI water (one part stock, one part DI water; the resulting solution is a 1:2 dilution). The concentrated stock solution should have an optical density of 2.000 or lower.
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/Standard Read Mode (**Chapter 3**), single wavelength, no blanking (“Normal” plate position).
5. Rotate the microplate 180° so that well A1 is now in the H12 position. Read the plate five more times (“Turnaround” plate position).
6. Print the raw data or export it to an Excel spreadsheet using Gen5™, KC4™, or KCjunior™ software.

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

Repeatability:

7. Calculate the mean value for each physical well location in columns 1 and 2 for the five plates read in the Normal position, and then again for the five plates read in the Turnaround position. This will result in 32 mean values.
8. Perform a mathematical comparison of the mean values for each microwell in its Normal and Turnaround positions (A1/H12, A2/H11, B1/G12, B2/G11, and so on). To pass this test, the differences in the compared mean values must be within the accuracy specification for the instrument.

Example comparison calculation:

If the mean value for well A1 in the Normal position is 1.902, where the specified accuracy is $\pm 1\% \pm 0.010$ OD, then the expected range for the mean of the same well in its Turnaround (H12) position is 1.873 to 1.931 OD.

$$1.902 * 0.010 + 0.010 = 0.029; 1.902 - 0.029 = \mathbf{1.873}; 1.902 + 0.029 = \mathbf{1.931}$$

Accuracy Specification:

For comparison in this test, the following accuracy specifications are applied, using Normal/Standard Mode and a 96-well microplate:

$\pm 1\% \pm 0.010$ OD from 0.000 to 2.000 OD

$\pm 3\% \pm 0.010$ OD from 2.000 to 3.000 OD

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)

Preparation of Dilutions:

1. Set up a rack containing 10 tubes, numbered consecutively.
2. If you have not already done so, prepare concentrated stock Test Solution A or B. Refer to **Table 5** when executing steps 3 and 4.

Table 5

Test Tube Dilutions

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

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 **Table 5**.

Plate Preparation:

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

- ❖ **Note:** The choice of dilutions and the absorbance of the original solution can be varied. Use **Table 5** 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.

Read Plate

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

- ❖ **Note:** 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™ software.

- ❖ 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.

3. Calculate the mean absorbance for each well, and average the means for each concentration.
4. Perform a regression analysis on the data to evaluate linearity.

For example, using Microsoft® Excel:

In a spreadsheet, create two columns labeled X and Y. Enter the actual absorbance values in column X. Enter the expected absorbance values in column Y.

Select **Tools** → **Data Analysis** → **Regression**. Identify column X as the “Input X Range” and column Y as the “Input Y Range” and then click OK to perform the analysis.

❖ **Note:** If the Data Analysis command is not available on the Tools menu, you may need to install the Analysis ToolPak in Microsoft® Excel. Consult Excel’s help system for assistance.

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 $\pm 1.0\%$ ± 0.005 OD. If above 2.000 OD, apply the $\pm 3\% \pm 0.005$ specification.
3. The standard deviation for each set of readings should be less than the allowed deviation.

For example:

Absorbance readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a mean of 1.951, and a standard deviation of 0.0026. The mean (1.951) multiplied by 1% ($1.951 * 0.010$) = 0.0195, which, when added to the 0.005 ($0.0195 + 0.005$) = 0.0245 OD, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specifications:

$\pm 1\% \pm 0.005$ OD from 0.000 to 2.000 OD

$\pm 3\% \pm 0.005$ OD from 2.000 to 3.000 OD

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 $\pm 1\% \pm 0.010$ OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. ($1.902 * 1\% = 0.019 + 0.010 = 0.029$, 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 specifications are applied, using Normal/Standard Mode and a 96-well microplate:

$\pm 1\% \pm 0.010$ OD from 0.000 to 2.000 OD

$\pm 3\% \pm 0.010$ OD from 2.000 OD to 3.000 OD

Liquid Test 3

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 μ Quant™ 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

The stock solution for Liquid Test 3 may be formulated from the chemicals listed below (Solution A), or by diluting a dye solution available from BioTek (Solution B).

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

Required Materials:

- Deionized water
- Ingredients shown in **Table 5**
- β -NADH powder (β -Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma® bulk catalog number N 8129, or preweighed 10-mg vials, Sigma® number 340-110

❖ Store the β -NADH powder according to the guidelines on its packaging.

1. Prepare the stock buffer solution using the ingredients in **Table 6**:

Table 6**PBS 10X Concentrate Solution**

KH ₂ PO ₄ anhydrous	0.2 grams
NaCl	8.0 grams
Na ₂ HPO 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® P4417 tablets (or equivalent)
- β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma® bulk catalog number N 8129, or preweighed 10-mg vials, Sigma® number 340-110

❖ Store the β-NADH 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**.

Procedure

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 100% Test Solution:

- If using the Sigma PBS Solution, use 5 ml as the diluent.
- If using the 10x Concentrate PBS Solution, 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 100% Test Solution:

- 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 μ l of the 100% Test Solution into all wells of columns 1 and 2
- 150 μ l of the 75% Test Solution into all wells of columns 3 and 4
- 150 μ l of the 50% Test Solution into all wells of 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/Standard Mode, single wavelength at 340 nm, no blanking (or blank on air).

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

❖ If you are exporting the data to an Excel spreadsheet, perform the calculations described on the following page, 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 format of $\pm 1.0\% \pm 0.005$ OD (Mean * 0.010 + 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% (0.8004 * 0.010) equals 0.008, and when added to the 0.005 (0.008 + 0.005) equals 0.013, which is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.013, 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.

Example using Microsoft® Excel:

In a spreadsheet, enter the three mean values in ascending order and label the column as the Y values. Enter 0.50, 0.75, and 1.00 and label the column as the X values.

Select **Tools** → **Data Analysis** → **Regression**. Identify column Y as the “Input Y Range” and column X as the “Input X Range” and then click **OK** to perform the analysis.

❖ If the Data Analysis command is not available on the Tools menu, you may need to install the Analysis ToolPak in Microsoft® Excel. Consult Excel’s help system for assistance.

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 6

Maintenance and Decontamination

This chapter contains procedures for maintaining and decontaminating the μ Quant.

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Maintenance

A daily maintenance schedule 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.

Routine Cleaning Procedure



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 instrument. 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 instrument to produce an error.

Purpose

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

Tools and Supplies

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

Procedure

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.

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 cotton cloths

Procedure



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

Do not immerse the instrument, spray it with liquid, or use a “wet” cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department.

Do not soak the keypad – this will cause damage.



Important! Turn off and unplug the instrument for all decontamination operations.

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.
 - Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution.

3. Moisten a 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.

Chapter 7

Troubleshooting and Error Codes

This appendix introduces the test protocols necessary for IQ/OQ/PQ Qualification. This appendix also describes problems that you may experience with the μ Quant™ during testing, lists potential error codes, and suggests possible solutions for these problems.

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Overview

If an error code appears on the display (or in Gen5™, KC4™, or KCJunior™ software), consult the error code list to see if the error is easily correctable. If you cannot resolve the problem, run the instrument **System Test** and note the exact error code and serial number of the reader. Then call BioTek's Technical Assistance Center (refer to **Chapter 1** for contact information).

System Test Description

The System Test feature conducts a series of tests, at each of six set wavelengths, which confirm adequate light levels, low electronic noise, adequate photodiode sensitivity, and overall system cleanliness. The testing is designed to ensure that the μ Quant will give in-specification performance for each set wavelength over the specified OD range.

The reader automatically runs an internal System Test each time it is powered on. The reader will "chirp" repeatedly if the power-on System Test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek. A system test should then be initiated to try to retrieve an error code from the reader.

Glossary of Terms

- **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.

Error Codes

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

❖ **Note:** If an error code is displayed, run a System Self-Test for diagnostic purposes.

- “0”, “1”, or “2” indicates a noncritical error, and the instrument will respond to keypad input. See General Errors on the next few pages for more information.

Motor or Optical Sensor	Channel
0 = Carrier X-Axis	0 = Reference
1 = Carrier Y-Axis	1 = Measurement
2 = Monochromator Order-Sorting Filter Wheel	
3 = Monochromator	

- “A” indicates a more serious error. In this case, turn off the instrument. Upon restarting the instrument, you should be able to use the keypad. See **Fatal Errors** on page 163 for more information.

Contact BioTek's Technical Assistance Center for further assistance if any of error codes are displayed. Refer to **Chapter 1** for contact information.

General Errors

General errors indicate nonfatal conditions that require attention.

Code	Description and Probable Causes
0100	<p>Abort</p> <p>The read or task has been aborted.</p>
0101	<p>Abort Error</p> <p>The read or task has been aborted. The 0101 indicates a software abort.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • User aborted read from KC4 software. • User aborted from another serial interface.
0200	<p>X-axis (carrier in/out) motor did not find the opto (home) sensor</p> <p>A motor was unable to move to its “home” position as registered by feedback from an optical sensor.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Nylon slider bushings on the X-axis rail are dirty and worn, causing too much friction; dirt in roller bearings causing bearings to jam. • Support pin on the carrier has moved, preventing it from properly sitting between the two roller bearings on the bearing block. • Defective or broken optical sensor. • Defective motor controller PCB. • Carrier front-support screws are not adjusted or are worn, making the carrier uneven. The support pin is no longer inserting properly into the roller bearings. • X-axis PCB not adjusted far enough to the right. This will not allow the flag to enter the opto sensor enough to trip the sensor. Loosen the two screws and slide the PCB to the right and retighten. Run the carrier autocal. • Carrier not able to move into read chamber. An object may be obstructing the carrier’s path.

General Errors (Continued)

<p>0201</p>	<p>Y-axis (carrier left/right) motor did not find the home sensor</p> <p>A motor was not able to move to its “home” position as registered by feedback from an optical sensor.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Bearings on the Y-axis rails are dirty and worn and causing too much friction. • Defective or broken optical sensor. • Defective motor controller PCB or cable. • Carrier not able to move into read chamber. Check the carrier’s path for obstructions.
<p>0202</p>	<p>Order-sorting filter wheel did not home.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Filter wheel is not tight and can wobble. • Filter wheel is too close to the motor gear and is binding. • Defective or broken optical sensor. • Defective motor, motor controller PCB, or cable.
<p>0300</p>	<p>Saturation transition failed in the X-axis movement (light beam never found)</p> <p>During the X-axis movement, the light beam (saturation) transition (max light to no light) was never found during autocalibration.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Dirty rail and nylon bushings or bearings causing the carrier to jam. • Autocal jig is not in the carrier. • Order-sorting filter wheel is jammed and unable to turn to the open hole.

General Errors (Continued)

<p>0301</p>	<p>Saturation transition failed in the Y-axis movement (light beam never found)</p> <p>During the Y-axis movement, the light beam (saturation) transition (max light to no light) was never found.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Dirty rail and nylon bushings or bearings causing the carrier to jam. • Autocal jig is not in the carrier. • Order-sorting filter wheel is jammed and unable to turn to the open hole.
<p>0303</p>	<p>Saturation transition failed in the monochromator motor movement (light beam never found)</p> <p>During instrument initialization, the monochromator is homed by rotating the monochromator mirror until the white light (full light) is detected. This requires a fully functional flash lamp/detection system.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Defective flash lamp and or flash lamp power supply (inconsistent flashes). • Defective analog PCB. • Defective motor/power PCB. • Defective monochromator (low probability).
<p>0400</p>	<p>Carrier X-axis movement failed positional verify</p> <p>Motor X-axis failed to get to the same position when moved a known number of steps from the home position and back.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The optical trigger flag has moved or is loose. • Dirty rail and nylon bushings or bearings. • Carrier support pin is out of adjustment.

General Errors (Continued)

<p>0401</p>	<p>Carrier Y-axis movement failed position verify (not user serviceable)</p> <p>Motor Y-axis failed to get to the same position when moved a known number of steps from the home position and back.</p> <ul style="list-style-type: none"> • The optical trigger flag has moved or is loose. • Foreign object in the path of the carrier. • Dirty rail and bearings.
<p>0402</p>	<p>Order-sorting filter wheel failed positional verify</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The optical trigger flag has moved or is loose. • Filter wheel is binding against the motor gear.
<p>0403</p>	<p>Monochromator failed to find the zero order position (white Light or positional verify)</p> <p>The order-sorting filter wheel is homed and moved to the open hole position. The monochromator is moved until the optical system detects saturation. It is then moved to the point where there is no saturation and then moved back to the saturation point. This error is indicating the saturation did not clear or appear.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Flash lamp is missing flashes or is not flashing. • The optic system does not detect the saturation (analog PCB). • Order-sorting filter wheel is binding against the motor gear and not allowing the open hole to line up correctly. • Bearings inside the grating mirror are causing monochromator to jam.

General Errors (Continued)

<p>0500</p>	<p>Light beam saturated (too much light). Air reading reached 65535.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During an absorbance filter calibration or wavelength scan, the reference channel was saturated when storing to memory. • During a spectral scan read, the reference channel reached saturation at one or more of the selected wavelengths. <p>Probable Causes (Spectral Scan):</p> <ul style="list-style-type: none"> • Monochromator has a defect in the mirror gradients. • Analog PCB intermittently failed. • Order-sorting filter wheel is jammed, not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
<p>0501</p>	<p>Light beam saturated (too much light). Air measurement channel reading reached 65535.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During a spectral scan read, the measurement channel reached saturation at one or more of the selected wavelengths. • Filter one has saturated the measurement channel. <p>Probable Causes:</p> <p><i>Spectral Scan Read</i></p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Missed flashes or erratic flash lamp. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.

General Errors (Continued)

0501 (Cont.)	<p>Reference Channel</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
0502	<p>Light beam saturated (too much light). Air measurement channel reading reached 65535.</p> <p>This error indicates that filter two has saturated the reference channel.</p> <p>Probable Causes (Reference Channel):</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Missed flashes or erratic flash lamp. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
0503	<p>Measurement channel light beam saturated (too much light). Fails if > 3 steps of error.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The monochromator is unable to find the center of the white light (home position), or it found home in a different location than it had previously. • This error indicates that filter three has saturated the reference channel.

General Errors (Continued)

<p>0503 (Cont.)</p>	<p>Probable Causes: <i>Monochromator Not Found Home</i></p> <ul style="list-style-type: none"> • Flash bulb skipped a flash due to poor lamp connection or defective power supply. • Order-sorting filter wheel is jammed and is not aligning the through hole to the light path. • Monochromator is defective. • Analog PCB intermittently failed. <p><i>Reference Channel</i></p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
<p>0504</p>	<p>Light beam saturated (too much light). Air measurement channel reading reached 65535. Filter four has saturated the reference channel.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.

General Errors (Continued)

<p>0505</p>	<p>Light beam saturated (too much light). Air reading reached 65535. Filter five has saturated the reference channel.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
<p>0506</p>	<p>Light beam saturated (too much light). Air measurement channel reading reached 65535. Filter six has saturated the reference channel.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.

General Errors (Continued)

0510	<p>Reference channel light beam saturated (too much light). Air reading reached 65535.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During an absorbance filter calibration or wavelength scan, the measurement channel was saturated when storing to memory. • During a spectral scan read, the measurement channel was saturated for one of the wavelengths. <p>Probable Causes:</p> <p><i>Filter Calibration</i></p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Absorbance analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply. <p><i>Spectral Scan Read</i></p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
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General Errors (Continued)

<p>0511-0516</p>	<p>Measurement channel light beam saturated (too much light). Air reading reached 65535.</p> <p>Either during a read or system test, one of the filters from one to six has saturated the measurement channel. The last number is the lambda table position number.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The monochromator mirror/grating has a defect. • Analog PCB intermittently failed. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path, or the through hole is allowing white light to pass. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.
<p>0611-0616</p>	<p>Measurement channel gain out of range for the selected wavelength. Fails if Air reading is > 60000 with a gain of = 1.</p> <p>While calibrating a filter or testing the reader before a read, one of the filters saturated the measurement channel. The last number is the lambda table position number.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The monochromator mirror/grating is damaged. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light. • Analog PCB intermittently failed. • Missed flashes or erratic flash lamp. • Voltage to the lamp has increased because the lamp power supply has failed, or the motor power supply PCB sent the incorrect voltage request to the lamp power supply.

General Errors (Continued)

0700	<p>Absorbance reference channel noise test at max gain See noise "Delta" on self-test fail if < 20.</p> <p>Significant variations in background electronic noise were detected when blocking the light and increasing the gain to maximum.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Verify that the shrouds are installed and properly fastened. • The coaxial cable ground between the reference channel and the absorbance analog PCB may be floating or not connected. • There may be an ambient light leak. Ensure that the plate carrier door is properly closed. • Analog PCB failure; the photodetector is noisy. • A faulty analog PCB or faulty internal grounding may cause internal electronic noise.
0710	<p>Absorbance measurement channel noise test at max gain See noise "Delta" on self-test fail if < 20.</p> <p>Significant variations in background electronic noise were detected when blocking the light and increasing the gain to maximum.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check to see if the shrouds are installed and are properly fastened. • The coaxial cable ground between the reference channel and the absorbance analog PCB may be floating or not connected. • There may be an ambient light leak. Ensure that the plate carrier door is properly closed. • Analog PCB failure; the photodetector is noisy. • A faulty analog PCB or faulty internal grounding may cause internal electronic noise.

General Errors (Continued)

0800	<p>Absorbance reference channel failed offset range. See "noise Max" < 20000 and "noise Min" > 10 on the system test.</p> <p>During self-test, the background electronic signal detected is outside of acceptable limits at maximum gain when blocking the light.</p> <p>Probable Causes:</p> <ul style="list-style-type: none">• If noise Max is > 20000.<ul style="list-style-type: none">➤ The photodetector is too noisy and defective.➤ Absorbance channel analog PCB is defective.➤ A faulty analog PCB or faulty internal grounding may cause internal electronic noise.➤ There may be an ambient light leak. Ensure that the plate carrier door is properly closed.➤ Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Ensure that the shrouds are installed and properly fastened.• If noise Min is < 10.<ul style="list-style-type: none">➤ The photodetector is not connected or is defective, producing a noise reading of zero.➤ Analog PCB is defective.
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General Errors (Continued)

0810	<p>Measurement channel failed offset range. See "noise Max" < 20000 and "noise Min" > 10 on the system test.</p> <p>During self-test, the background electronic signal detected is outside of acceptable limits at maximum gain when blocking the light.</p> <p>Probable Causes:</p> <ul style="list-style-type: none">• If noise Max is > 20000:<ul style="list-style-type: none">➤ The photodetector is too noisy and defective.➤ Analog PCB is defective.➤ A faulty analog PCB or faulty internal grounding may cause internal electronic noise.➤ There may be an ambient light leak. Ensure that the plate carrier door is properly closed.➤ Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Verify that the shrouds are installed and properly fastened.• If noise Min is < 10.<ul style="list-style-type: none">➤ The photodetector is not connected, or the optic spray is damaged or defective, producing a noise reading of zero.➤ Analog PCB is defective.
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General Errors (Continued)

0900	<p>The absorbance reference channel Dark current value failed. See "Dark" on the system test. See criteria below.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The reference channel failed during a read or spectral scan for one of the following reasons: <ul style="list-style-type: none"> ➤ Dark value was < 100 during a spectral scan using 8 flashes and 8 resets during sweep mode. ➤ Dark value was < 100 during a spectral scan using 8 flashes and the number of resets saved for that wavelength during normal or rapid mode. • The reference channel failed < 100 or >20000 during filter calibration or spectral scan with the flash on. • The reference channel failed < 100 during filter calibration or spectral scan with the flash off. <p>Probable Causes:</p> <ul style="list-style-type: none"> • If failed < 100: <ul style="list-style-type: none"> ➤ Absorbance analog PCB or reference channel analog PCB is defective. ➤ Shielding of the cable between reference channel and analog PCB is defective or disconnected. ➤ Reference channel photodetector is defective. • If failed > 20000: <ul style="list-style-type: none"> ➤ Reference channel photodetector is defective. ➤ A faulty analog PCB or faulty internal grounding may cause internal electronic noise. ➤ There may be an ambient light leak. Ensure that the plate carrier door is properly closed. ➤ Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check to see if the shrouds are installed and are properly fastened. ➤ Order-sorting filter wheel is jammed and not aligning the filter wheel to block the light path, or the filter is degraded and not passing enough light energy.
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General Errors (Continued)

<p>0901-0906</p>	<p>The absorbance reference channel Dark current value failed See "Dark" on the system test. See criteria below.</p> <p>The last number is the lambda table position number. This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The reference channel failed < 100 during optic test with the flash on. • The reference channel failed < 100 during a read or blank read not in sweep mode with the flash off. • The reference channel failed < 100 or the Dark value has changed more than 10% from the last self-test data during a read or blank read with the flash on. <p>Probable Causes:</p> <ul style="list-style-type: none"> • Absorbance analog PCB or reference channel analog PCB is defective. • Cable between reference channel and analog PCB is defective or disconnected. • Reference channel photodetector is defective or the optic spray is damaged. • A faulty analog PCB or faulty internal grounding may cause internal electronic noise. • There may be an ambient light leak. Ensure that the plate carrier door is properly closed. • Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Verify that the shrouds are installed and properly fastened. • Order-sorting filter wheel is jammed and not aligning the filter wheel to block the light path, or the filter is degraded and not passing enough light energy.
<p>0910</p>	<p>The absorbance measurement channel Dark current value failed See "Dark" on the system test. See criteria in text below.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The measurement channel failed during a read or spectral scan for one of the following reasons: <ul style="list-style-type: none"> ➤ Dark value was < 100 during a spectral scan using 8 flashes and 8 resets during sweep mode. ➤ Dark value was < 100 during a spectral scan using 8 flashes and the number of resets saves for that wavelength during normal and rapid mode.

General Errors (Continued)

<p>0910 (Cont.)</p>	<ul style="list-style-type: none"> • The measurement channel failed < 100 or > 20000 during filter calibration or spectral scan with the flash on. • The measurement channel failed < 100 during filter calibration or spectral scan with the flash off. <p>Probable Causes:</p> <ul style="list-style-type: none"> • If failed < 100: <ul style="list-style-type: none"> ➤ Absorbance analog PCB or measurement channel analog PCB is defective. ➤ Shielding of the cable between measurement channel and analog PCB is defective or disconnected. ➤ Measurement channel photodetector is defective. • If failed > 20000: <ul style="list-style-type: none"> ➤ Measurement channel photodetector is defective. ➤ A faulty analog PCB or faulty internal grounding may cause internal electronic noise. ➤ There may be an ambient light leak. Ensure that the plate carrier door is properly closed. ➤ Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Verify that the shrouds are installed and properly fastened. ➤ Order-sorting filter wheel is jammed and not aligning the filter wheel to block the light path, or the filter is degraded and not passing enough light energy.
<p>0911- 0916</p>	<p>The absorbance measurement channel Dark current value failed. See "Dark" on the system test. See criteria below.</p> <p>The last number is the lambda table position number. This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The measurement channel failed < 100 during optic test with the flash on. • The measurement channel failed < 100 during a read or blank read not in sweep mode with the flash off. • The measurement channel failed < 100 or if the Dark value has changed more than 10% from the last self-test data during a read or blank read with the flash on.

General Errors (Continued)

<p>0911-0916 (Cont.)</p>	<p>Probable Causes:</p> <ul style="list-style-type: none"> • Absorbance analog PCB or measurement channel analog PCB is defective. • Shielding of the cable between measurement channel and analog PCB is defective or disconnected. • Measurement channel photodetector is defective or the optic spray is damaged. • A faulty analog PCB or faulty internal grounding may cause internal electronic noise. • There may be an ambient light leak. Ensure that the plate carrier door is properly closed. • Electrical noise may be penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Verify that the shrouds are installed and properly fastened. • Order-sorting filter wheel is jammed and not aligning the filter wheel to block the light path, or the filter is degraded and not passing enough light energy.
<p>0A01-0A06</p>	<p>Reference Channel Air/Blank out of range. See criteria below.</p> <p>This error is indicating the Air reading at the time of the plate read was < 50% of the air reading at the time of the optic test. The last number is the lambda table position number.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Flash lamp has missed flashes or erratic flash during the blank read. • Dirty optics or spilled substance on the optics. • Order-sorting filter wheel is jammed and not aligning the filter wheel to block the light path, or the filter is degraded and not passing enough light energy or is blocking the light.

General Errors (Continued)

0A11-0A16	<p>Measurement Channel Air/Blank out of range. See criteria below.</p> <p>The Air reading at the time of the plate read was < 50% of the air reading at the time of the optic test. The last number is the lambda table position number.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Flash lamp has missed flashes or erratic flash during the blank read. • Dirty optics or spilled substance on the optics. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light.
0C00	<p>Printer timed out</p> <p>The time allotted for the instrument to make a valid connection to a printer has expired.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Printer not connected, on line, or powered up. • Printer parallel port may not be correctly selected.
0D00	<p>Wavelength calibration data missing during a spectral scan</p> <p>Wavelength data is missing prior to a spectral scan, meaning the wavelength has not been calibrated. This includes the gain test data (looking for the resets to be < 1 or > 8 to fail).</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Memory corrupt. • The “calibrate before read” check box is not selected and this wavelength has not been used prior to this error. • The gain test was skipped or failed for this wavelength.
0D01-0D06	<p>Wavelength calibration data missing during an absorbance read. See criteria below.</p> <p>Wavelength data is missing prior to an absorbance read, meaning the wavelength has not been calibrated. This includes self-test and gain test. (looking for the resets to be < 1 or > 8 to fail). The last number is the lambda table position number.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The wavelength was not calibrated prior to the read. • The gain test skipped or failed for this wavelength.

General Errors (Continued)

<p>0E01-0E06</p>	<p>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.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Wavelength not entered correctly or missing in filter table. • Wavelength entered correctly in PC software but never sent to reader. • Verify the lambda table has the wavelengths loaded into the instrument from the controlling PC software. Compare the contents of the lambda table with the software's filter table.
<p>0F00</p>	<p>Reference channel correction outside limits. See criteria below. This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During a spectral scan, the flash-on value minus flash-off < 500 normal mode, or < 500 * calibrated resets / 8 for sweep mode. • During spectral scan the blanking on air uses minimal flashes to test the light performance. The following ratio is used to determine the performance of the lamp: Reference channel blank for wavelength / (flash-on reference data - corrected reference dark offset). The ratio will fail if < 0.5 or > 2.0. <p>Probable Causes:</p> <ul style="list-style-type: none"> • Lamp failure, alignment or lamp power supply. • Reference channel analog PCB or absorbance channel analog PCB or the cable in between. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light. • Damaged optic spray.

General Errors (Continued)

<p>0F01-0F06</p>	<p>Reference channel correction outside limits. See Delta (Air – Dark) on self-test. See criteria below.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During system test the Delta was < 500 for one of the wavelengths. • This error indicates that during sweep mode only the blanking on air uses one flash to test the light performance. The following ratio is used to determine the performance of the lamp: <p>Reference channel blank for wavelength / (flash-on reference data – corrected reference dark offset). The ratio will fail if < 0.5 or > 2.0.</p> <ul style="list-style-type: none"> • During a spectral scan or blank read, the flash-on value minus flash-off < 500 normal mode, or < 500 * calibrated resets / 8 for sweep mode. <p>Probable Causes:</p> <ul style="list-style-type: none"> • Lamp failure, alignment or lamp power supply. <p>Reference channel analog PCB or absorbance channel analog PCB or the cable in-between.</p> <ul style="list-style-type: none"> • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light. • Damaged optic spray.
<p>0F10</p>	<p>Measurement channel correction outside limits. See criteria below.</p> <p>This error indicates that during a spectral scan the flash-on value minus flash-off < 8000 normal mode, < 8000 * calibrated resets / 8 for sweep mode.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • Lamp failure, alignment or lamp power supply. • Reference channel analog PCB or absorbance channel analog PCB or the cable in between. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light. • Damaged optic spray. • See the next page for more situations.

General Errors (Continued)

0F11-0F16	<p>Measurement channel correction outside limits. See criteria below. See Delta (Air – Dark) on self-test.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • During system test the Delta was < 8000 for one of the wavelengths. • During a spectral scan or blank read the flash-on value minus flash-off < 8000 normal mode, < 8000 * calibrated resets / 8 for sweep mode. <p>Probable Causes:</p> <ul style="list-style-type: none"> • Lamp failure, alignment or lamp power supply. • Absorbance channel analog PCB. • The order-sorting filter wheel is jammed and not aligning the correct filter to line up with the light path; the filter is degraded and not passing enough light energy; or the filter is blocking the light. • Damaged optic spray.
1000	<p>Configuration data missing.</p> <p>Indicates the configuration data was missing at the beginning of the self-test.</p> <p>Possible Causes:</p> <ul style="list-style-type: none"> • Memory corruption • New main PCB installed • Assay code corrupted or not downloaded
1100	<p>Failed configuration checksum test.</p> <p>This error indicates that during self-test or at the end of a plate read, the checksum calculated for configuration flash memory page 0 doesn't match saved checksum.</p> <p>Probable Cause:</p> <ul style="list-style-type: none"> • The flash memory on the 7270400 PCB is defective or corrupt. The basecode software may need to be redownloaded.
1101	<p>Failed configuration checksum test.</p> <p>This error indicates that during self-test or at the end of a plate read the checksum calculated for configuration flash memory page 1 doesn't match saved checksum.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The flash memory on the 7270400 PCB is defective or corrupt. The basecode software may need to be redownloaded.

General Errors (Continued)

<p>1200</p>	<p>Autocalibration data missing for absorbance reads.</p> <p>No autocal data exists for the read location.</p> <p>Probable Cause:</p> <p>The 7270400 PCB was changed and the Flash memory does not have the calibration values loaded. Perform the Autocalibration procedure to correct this.</p>												
<p>130x</p>	<p><Motor> not homed successfully.</p> <p>This error indicates that the <motor> is not at home. At the beginning of the motor_position function, the basecode verifies that the motor is homed. If not, it sends an error and exits the function.</p> <p>Probable Causes:</p> <p>If an error 0200 is ignored. See the probable causes for 0200.</p> <table border="1" data-bbox="513 873 1328 1058"> <thead> <tr> <th>Error</th> <th>Motor</th> <th>See probable causes for</th> </tr> </thead> <tbody> <tr> <td>1300</td> <td>X-axis</td> <td>0200</td> </tr> <tr> <td>1301</td> <td>Y-axis</td> <td>0201</td> </tr> <tr> <td>1302</td> <td>Order-sorting filter wheel</td> <td>0202</td> </tr> </tbody> </table>	Error	Motor	See probable causes for	1300	X-axis	0200	1301	Y-axis	0201	1302	Order-sorting filter wheel	0202
Error	Motor	See probable causes for											
1300	X-axis	0200											
1301	Y-axis	0201											
1302	Order-sorting filter wheel	0202											
<p>1600</p>	<p>Computer control assay definition error.</p> <p>Used only for software development purposes.</p>												
<p>1700</p>	<p>Kinetic interval not correct for selected options.</p> <p>This error indicates one of the following situations:</p> <ul style="list-style-type: none"> • The kinetic interval in the current assay is too short. • The kinetic interval for an absorbance read, the total time or kinetic interval = 0. <p>Probable Causes:</p> <ul style="list-style-type: none"> • User programming error. Increase or decrease the kinetic interval. 												
<p>1900</p>	<p>Memory allocation failed.</p> <p>Indicates that the process failed while saving or moving data. If this occurs, turn off the unit, wait 30 seconds, and then turn on the unit.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> • The memory is corrupt. Replace the processor PCB. • If the error persists, contact BioTek TAC. 												

General Errors (Continued)

1C00	A/D calibration STBY line never went low.										
1C01	<p>A/D calibration STBY line went low but never transitioned to a high.</p> <p>The analog PCB circuit failed when trying to initialize the A/D, or the cable to the defective PCB has lost continuity.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> Problems with the absorbance analog PCB or the cable between the analog PCB and motor power supply PCB 										
2100	<p>Invalid parameter value selected</p> <p>This error can occur only during computer control, indicating that an invalid assay configuration was sent to the instrument.</p>										
280x	<p><Motor> currently in use</p> <p>This error indicates that the <motor> is not available for this model or already has a task assigned to it. At the beginning of the motor_setup function the basecode checks to see if the motor is currently in use or not available. This error can occur with any motor request. See table below for error.</p> <table border="1" data-bbox="626 926 1214 1110"> <thead> <tr> <th>Error</th> <th>Motor</th> </tr> </thead> <tbody> <tr> <td>2800</td> <td>X-axis</td> </tr> <tr> <td>2801</td> <td>Y-axis</td> </tr> <tr> <td>2802</td> <td>Order-sorting filter wheel</td> </tr> <tr> <td>2803</td> <td>Monochromator</td> </tr> </tbody> </table> <p>Probable Causes:</p> <ul style="list-style-type: none"> User selected the incorrect model in computer software. Downloaded incorrect basecode. 	Error	Motor	2800	X-axis	2801	Y-axis	2802	Order-sorting filter wheel	2803	Monochromator
Error	Motor										
2800	X-axis										
2801	Y-axis										
2802	Order-sorting filter wheel										
2803	Monochromator										
2F00	<p>Results data being sent not acknowledged by host PC.</p> <p>This error is indicating the handshaking between the host PC software and the reader did not complete. This is a lost data condition.</p> <ul style="list-style-type: none"> Software development only. 										
3100	<p>Plate read took longer than kinetic interval.</p> <p>Probable Causes:</p> <ul style="list-style-type: none"> User defined interval is incorrect. 										
3201	<p>No absorbance A/D ready transition.</p> <p>This error indicates that the A/D chip on the analog PCB is not ready for input from the photodiodes.</p>										

Fatal Errors

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

Code	Description and Probable Causes
A100	Task control block not available
A200	Read already in progress
A300	Motor not available This error indicates that a motor is not available, but it does not identify which motor was requested.
A301	Real-time clock not available
A302	Display device not available
A303	Flash lamp not available
A304	Printer device not available
A400	Failed code checksum on power-up
A600	Data flash write timed out
A700	Data flash readback did not match write
A800	Code flash write timed out
A900	Memory allocation heap corrupted

Appendix A

Controlling the μ Quant with Gen5™, KC4™, or KCjunior™ Software

The μ Quant can be controlled either from BioTek's Gen5™, KCjunior™ or KC4™ PC software. This Appendix provides instructions for programming the computer to control the instrument.

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Overview

BioTek's Gen5™, KC4™, or KCjunior™ software provide the user even more power and flexibility. For example, the μ Quant™ can run computer-controlled kinetic assays using up to six wavelengths on the same microplate. Spectral scan assays may be performed that use the entire wavelength range from 200 nm to 999 nm at 1-nm increments.

This Appendix provides instructions for controlling the μ Quant with BioTek's Gen5, KC4, or KCjunior software.

Controlling the Reader with Gen5™

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

Setting Up Gen5

The μ Quant™ can be operated using a computer running BioTek's Gen5 software. The following instructions *briefly* show you how to set up Gen5 for operation of the reader. Refer to Gen5's Getting Started Guide or Help system for more detailed instructions.

1. Turn off the computer and the reader. Connect the appropriate serial cable (PN 75053) between the two machines. See **Table 7** on page 175 for the cable pinouts.
2. Turn on both machines.
3. Install Gen5 on the computer's hard drive and register the software with BioTek.
 - If you purchased Gen5's Reader Diagnostics Utility (required for the Absorbance Test Plate), install this software on the computer's hard drive.
4. Open Gen5.
5. Login if prompted (Gen5 Secure). The default Administrator password is **admin**.
6. At the 'Welcome to Gen5' screen, select **System Menu**.
7. From Gen5's main screen, select **System|Reader Configuration** to open the 'Reader Configuration' dialog.
8. Click the **Add** button to open the 'Reader Settings' dialog.
 - Gen5 and Gen5 Secure: Up to two readers may be added in Gen5.
9. Use the drop-down list in **Reader Type** to select **uQuant**.
10. Enter the appropriate **Com Port**.
 - The **Baud Rate** is set at **9600** and cannot be changed.
11. Click **Test Comm**. Gen5™ will attempt to communicate with the reader.
 - If you receive "The reader is communicating!" message, click **OK**, and then **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 and you receive an error message, refer to the **Problems** section below, or to the Troubleshooting section of Gen5's Help system for assistance.
- Gen5 Secure only: An 'Audit Trail' dialog will appear after exiting Reader Configuration, whenever you add, modify, or delete a reader. Enter any comments, if desired, then click **Close**.

Problems

If Gen5 fails to communicate with the reader, try the following troubleshooting suggestions:

- Confirm that the correct Reader Type was selected in step 9.
- Try a different COM port.
- Check the serial cable connections. Ensure that the cable is properly attached to the port defined in step 10 and is not a Null cable. If this is suspected, add another Null and try again.
- Confirm that the reader has passed its Self Test. The reader will not communicate if it fails an internal system test.

If the test still fails, refer to the Troubleshooting section in Gen5's Help system for further assistance.

Getting Started with Gen5

The following instructions *briefly* show you how to define and perform a "Quick Read" in Gen5 (**File|New Experiment|Default Protocol**). It's called "Quick" because you can perform a reading without having to take the time to create a new protocol.

If the reading is part of an experiment or assay that you will perform numerous times, you will need to create a new protocol (**File|New Protocol**).

Refer to Gen5's Help system early and often to learn how to create protocols, assign well identifiers, read plates, print results, perform data reduction, and more.

To perform a "Quick Read":

1. At the 'Welcome to Gen5' screen, select **System Menu**, then at the main screen select **File|New Experiment**. (Alternative: select **Read a Plate** at the Welcome screen, then proceed to step 4 on the following page.)
2. Click **Default Protocol**, then 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 more than one reader was added in Gen5, the 'Instrument Selection' dialog will appear instead of the Procedure dialog. Select **uQuant**, then click **OK**. The Procedure dialog will then appear.
4. Select a **Plate Type**.
5. Click **Read** to open the 'Read Step' dialog.
6. Select a **Read Type**.
7. Define 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.
 - Click **Validate** if you would like Gen5 to verify the defined parameters. If all parameters are valid, you will receive confirmation. If any parameters are invalid, Gen5 will provide information for correcting the problem. Refer also to the Troubleshooting section of Gen5's Help system.
10. Click **OK** again to save and close the Procedure dialog. The 'Plate Reading' dialog will appear.
11. Enter any desired information, place the plate on the carrier, then click **READ** to begin the plate read. 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 Gen5's Help system for instructions.

❖ Gen5 Reader Control does not support data reduction.

Controlling the Reader with KC4™

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

Setting Up KC4

The μ Quant™ can be operated using a computer running BioTek's KC4 software. The following instructions *briefly* show you how to set up KC4 for operation of the reader. Refer to KC4's User's Guide or Help system for more detailed instructions.

1. Turn off the computer and the reader. Connect the appropriate serial cable (PN 75053) between the two machines. See **Table 7** on page 175 for the cable pinouts.
3. Turn on both machines.
4. Install KC4 on the computer's hard drive, and register the software with BioTek.
5. Once installed, start KC4. Log in, if prompted.
6. Select **System|Readers**. The 'Reader Selection' dialog will appear.
7. Scroll through the list of **Available Readers** and select the **μ Quant**. Click the **Port** button (and subsequent **Setup** button), to define the following communications parameters:
 - Port: **COMn** (select the COM (serial) port used for the RS-232 cable connection)
 - Transmission Speed: **2400, 4800, or 9600** (default); must match the baud rate on the reader
 - Data Bits: **8**
 - Parity: **No**
 - Stop Bits: **2**

❖ Although the transmission speed (baud rate) may be changed to 2400 or 4800, BioTek recommends keeping the default rate of 9600.

8. Click **OK** to close the 'Port Setup' dialog, then **OK** again at the 'Port Selection dialog'.

9. Click **Filters/Wavelengths**, then **Get Wavelengths** to align KC4's wavelengths table with the reader's internal table. Click **OK** to return to the Reader Selection dialog.
10. Click the **Current Reader** button to attempt to establish communications with the reader, using the currently defined communication parameters.
11. If the test passes, click **Close** to save the settings and return to the main screen. If the test fails, KC4 will provide appropriate instructions for resolving any problems. See also the **Problems** section below.

Problems

If KC4 fails to communicate with the reader and displays a serial communications error, check the cable plug-in location to ensure that it matches the setup choices in **Table 7** and is not a Null cable. If this is suspected, add another Null and try again.

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

Getting Started with KC4™

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

To read a plate using KC4:

1. Select **Data|New Plate**.
2. If prompted to select a protocol, select "Empty Protocol" and click **OK**. If not prompted, select **Protocol|New**, or use KC4's Protocol Wizard to step through protocol creation.
3. Select **Protocol|Reading**. The 'Reading parameters' dialog will appear.
4. Select a **Reading Type**.
5. Define the **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 desired information, place the plate on the carrier, then click **START READING** to begin the plate read.
 - The plate will be read and then the raw data results will display in KC4.
 - To analyze, manipulate, or print results, **protocol** parameters should be defined. Refer to KC4's Help system or User's Guide for instructions.

Controlling the Reader with KCjunior™

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

Setting Up KCjunior

The μ Quant™ can be operated using a computer running BioTek's KCjunior software. The following instructions *briefly* show you how to set up KCjunior for operation of the reader. Refer to KCjunior's User's Guide or Help system for more detailed instructions.

1. Turn off the computer and reader. Connect the appropriate serial cable (PN 75053) between the two machines. See **Table 7** on page 175 for the cable pinouts.
2. Turn on both machines.
3. Install KCjunior on the computer's hard drive and register the software with BioTek.
4. Once installed, start KCjunior.
5. Select **Setup**, then **Reader1** or **Reader 2** to open the 'Reader Setup' dialog.
6. Scroll through the **Reader Type** list and select the **uQuant**. Define the communication parameters as follows:
 - Com Port: **COMn** (select the COM (serial) port used for the RS-232 cable connection)
 - Baud Rate: **1200, 2400, or 9600** (default); must match the baud rate on the reader
 - Data Bits: **8**
 - Parity: **None**
 - Stop Bits: **2**
 - EOT Character: Keep the default number.

❖ Although the baud rate may be changed to 1200 or 2400, BioTek recommends keeping the default rate of **9600**.

7. 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 is displayed, an incorrect COM port may have been selected. Select a different port and then repeat this step.
 - If the test passes, at 'The communications test was successful' dialog, click **OK** to close the dialog, then click **OK** again to save the settings and close the Reader Setup dialog.
 - If the test fails, follow the directions provided by KCjunior, then click **Test Communications** again. See the **Problems** section below.

Problems

If KCjunior fails to communicate with the reader, and displays a serial communications error, check the cable plug-in location to make sure it matches the setup choices in **Table 7** and is not a Null cable. If this is suspected, add another Null and try again.

Getting Started with KCjunior™

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

To read a plate using KCjunior:

1. Click **Read Plate** from KCjunior's main screen. The 'Read Plate Dialog' will appear.
2. If desired, enter a **Results ID** and a **Plate Description**, and then click **Read Plate**. The 'Protocol Definition' dialog will appear.
3. Select a **Read Method Type**.
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 or manipulate results, a **Protocol** should be defined. Refer to KCjunior's Help system or User's Guide for instructions.

Table 7
Serial Cable Pinout Description

Serial Cable Pinout*	
PC (9-pin female)	Reader (25-pin female)
1+6	4
2	2
3	3
5	7
7	5+6+8
8	20
Shell	Shell (shielding)

* For a 25-pin PC connection using BioTek serial cable (PN 75053) plus 9pM-25pF adapter (PN 49755)

Appendix B

Using 384-Well Plate Geometry

When used in standalone mode, the μ Quant™ 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, or KCjunior.

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Reading a 384-Well Plate in Gen5™

1. Start Gen5. Log in, if prompted (Gen5 Secure users).
2. When the 'Welcome to Gen5' screen appears, select **System Menu**.
3. At Gen5's main screen, select **System | Reader Configuration** to open the 'Reader Configuration' dialog:

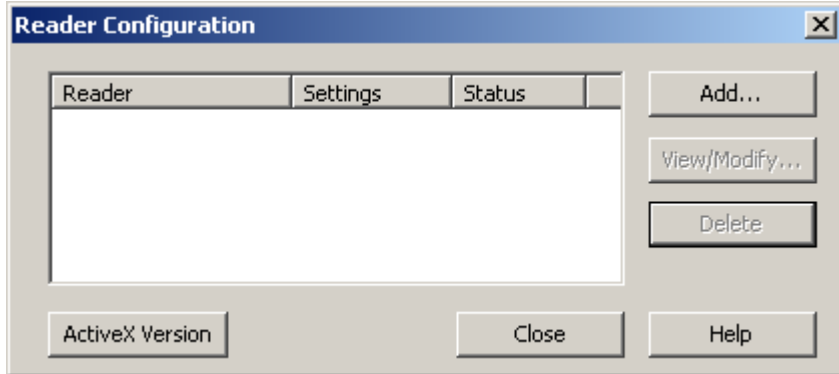


Figure 20: Reader Configuration dialog

4. Click the **Add** button to open the 'Reader Settings' dialog.

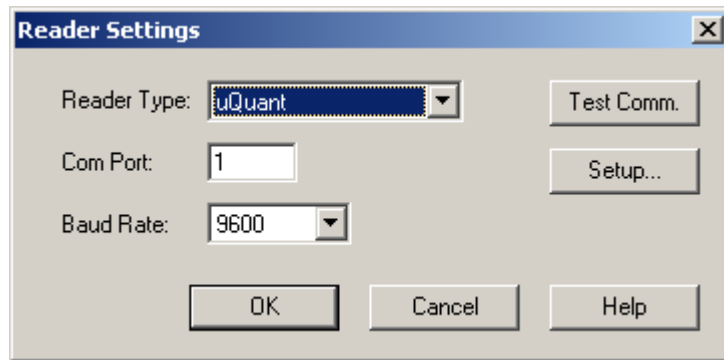


Figure 21: Reader Settings dialog

5. Use the drop-down list to select **uQuant** and enter the appropriate **Com Port**.
 - The **Baud Rate** is set to the default rate of **9600** for the μ Quant™ and may not be changed.
6. Click **Test Comm.** Gen5 will attempt to communicate with the reader.

- If you receive “The Reader is communicating!” message, click **OK**, and then click **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, and you receive an error message, refer to the “Troubleshooting” section of the Help system.
7. At the main screen, select **File|New Experiment**. The ‘New Experiment’ dialog will appear:

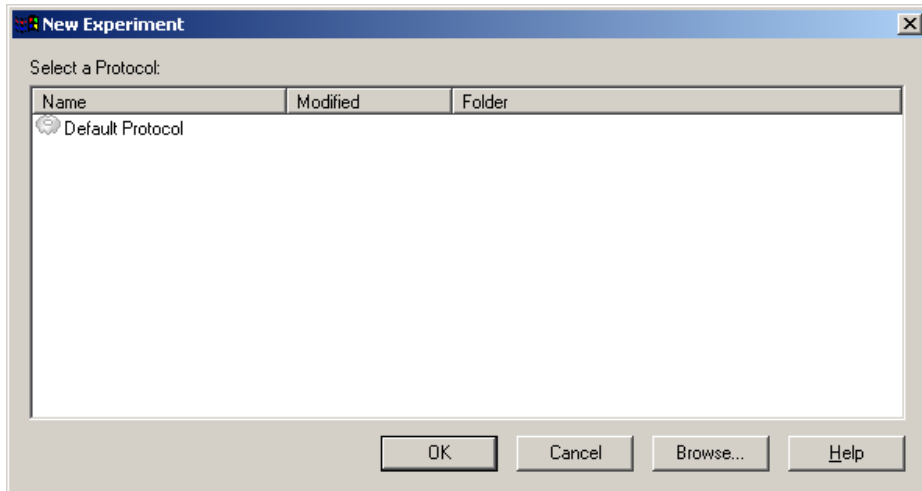


Figure 22: New Experiment dialog

8. Click **Default Protocol**, then click **OK**. Gen5 will open the Experiment workspace, which includes the Protocol menu and Plate screen:

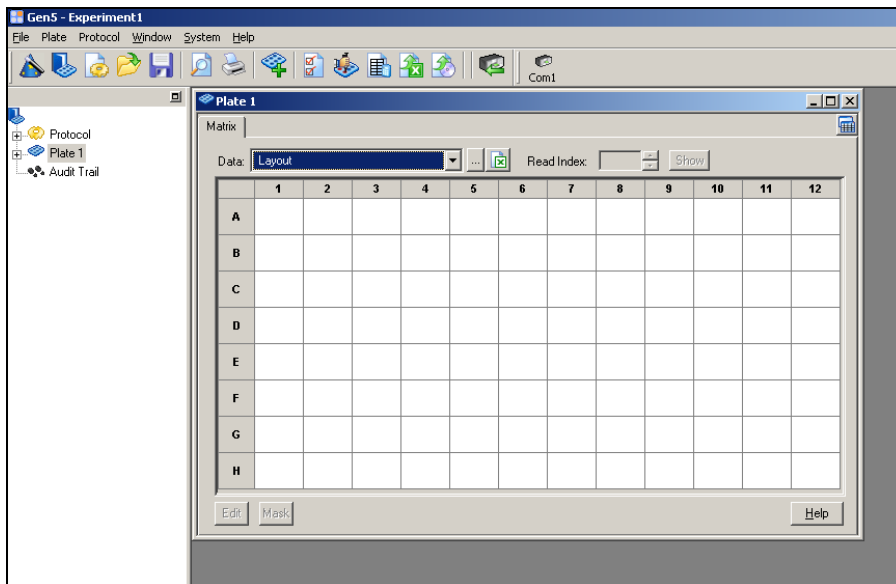


Figure 23: Experiment workspace

9. Select **Plate|Read** or click the **Read Plate** icon to open the 'Procedure' dialog. Use the drop-down arrow in **Plate Type** to select a 384-well plate.

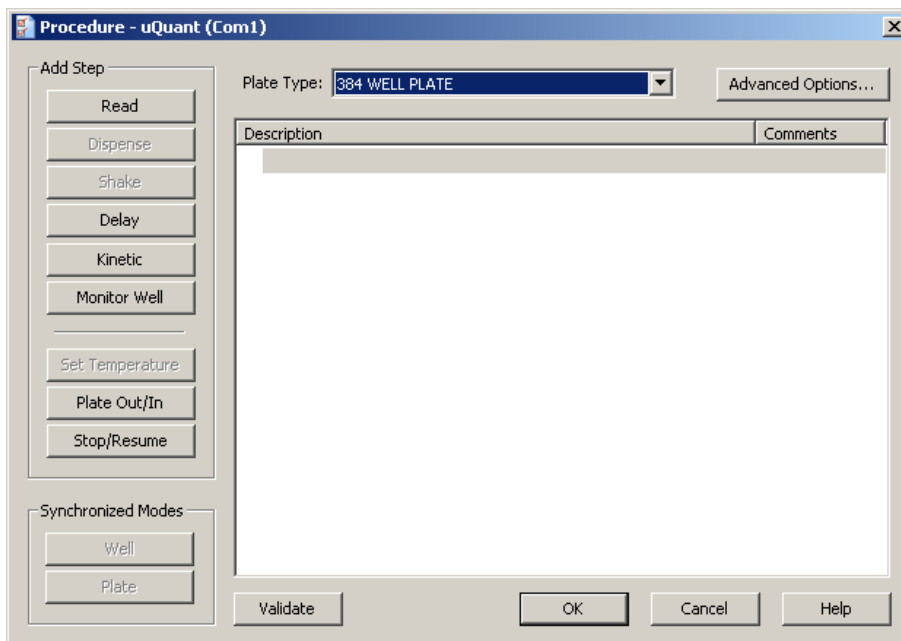


Figure 24: Procedure dialog

10. Click **Read** to open the 'Read Step' dialog. Select a **Read Type**.

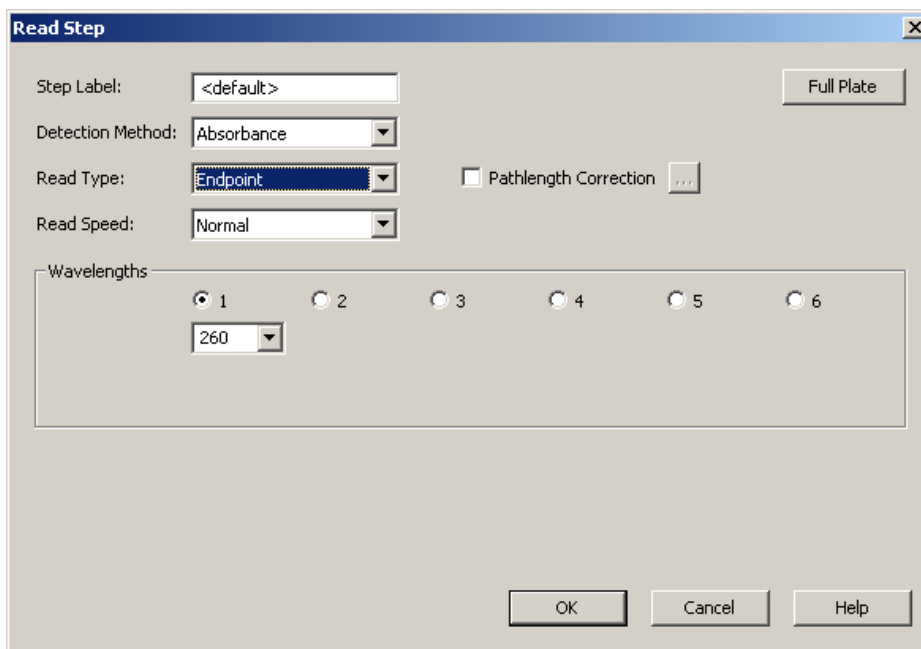


Figure 25: Read Step dialog

11. Define the **Wavelength(s)** at which the plate will be read.
12. Define other reading parameters as desired. Click the **Help** button for assistance.
13. When complete, click **OK** to return to the Procedure dialog.
14. Click **Validate** if you would like Gen5 to verify the defined read parameters. If any parameters are incorrect (or incomplete), follow the instructions provided by Gen5 for correcting the parameters.
15. Click **OK** to save and close the Procedure dialog. The 'Plate Reading' dialog will appear.

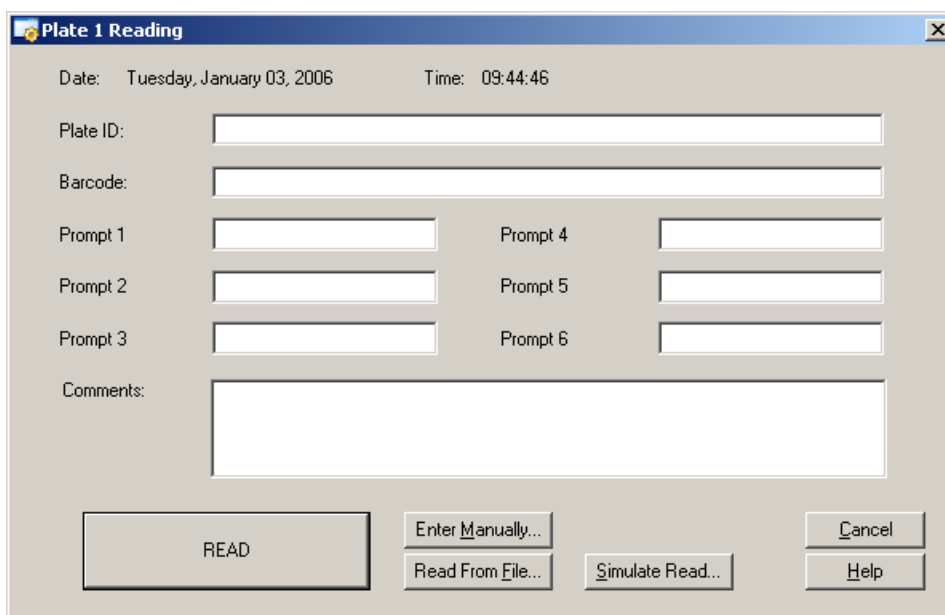


Figure 26: Plate Reading dialog

- If the Plate screen initially opened in 96-well layout (see **Figure 23** on page 179), the screen will change to 384-well layout.
16. Enter any desired information, place a 384-well plate on the carrier, and then click **READ** to begin the plate read. 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 need to be defined. Refer to Gen5's Help system for instructions.

❖ Gen5 Reader Control does not support data reduction.

Reading a 384-Well Plate in KC4™

1. Start KC4. Log in, if prompted.
2. At KC4's main screen, choose **System|Readers**.

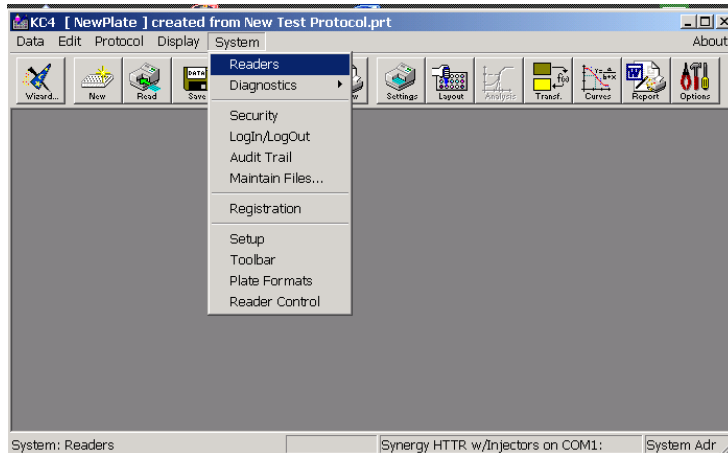


Figure 27: KC4 main menu, System|Readers

3. The 'Reader Selection' dialog will appear. Select the **μQuant**.
4. Click the **Port** button and (subsequent **Setup** button) to define the communications parameters.

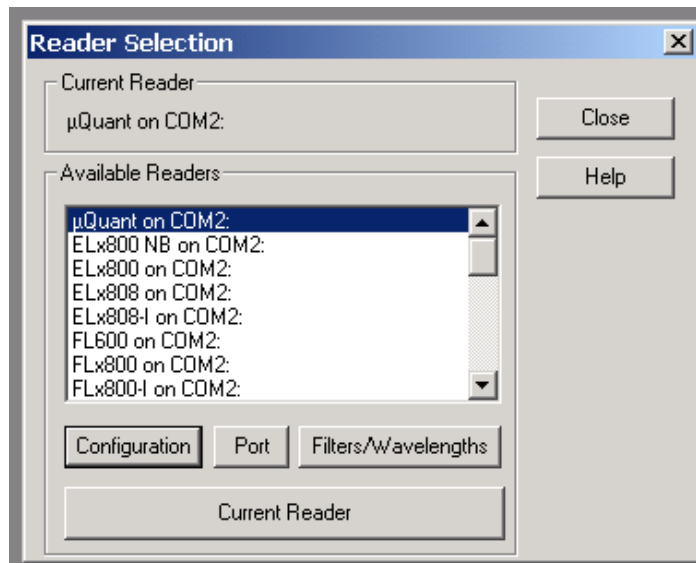


Figure 28: Reader Selection dialog

5. Click **Current Reader** to establish communication with the reader, then click **Close** to return to the main screen. If the test fails, KC4 will provide appropriate instructions for resolving any problems.
6. At the main screen, select **Data|New Plate**. The 'New Data File' dialog will appear:

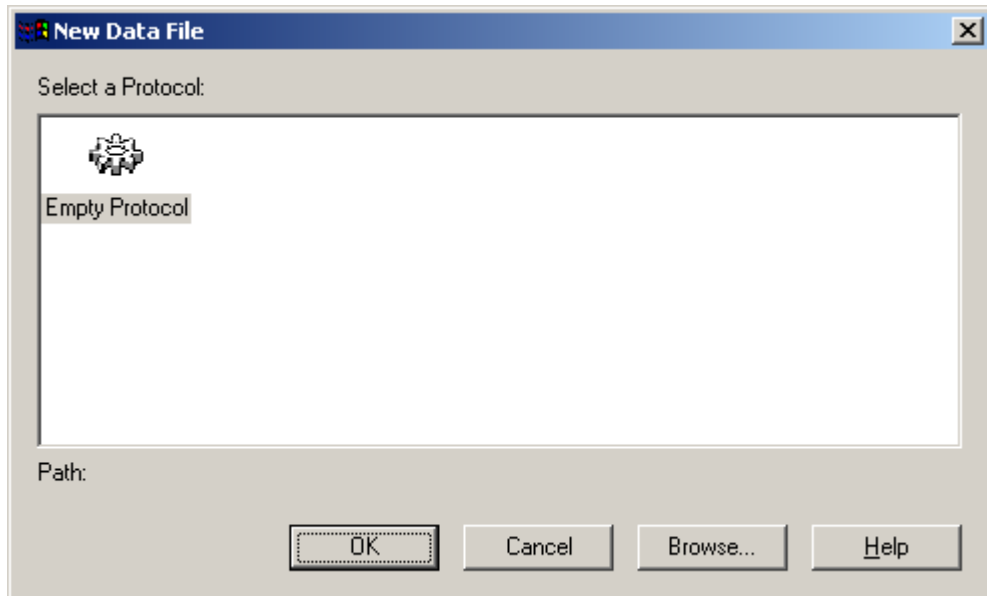


Figure 29: New Data File dialog

7. Select **Empty Protocol** and click **OK**.
8. Select **Protocol|Reading** from the main screen. The 'Reading' dialog will appear.
9. Define the Reading parameters:
 - Select a **Reading Type**.
 - Define the **Wavelength(s)** at which the plate will be read.
 - Set the **Plate Type** to **384 WELL PLATE**.
 - Define other reading parameters as necessary. Click the **Help** button for assistance.

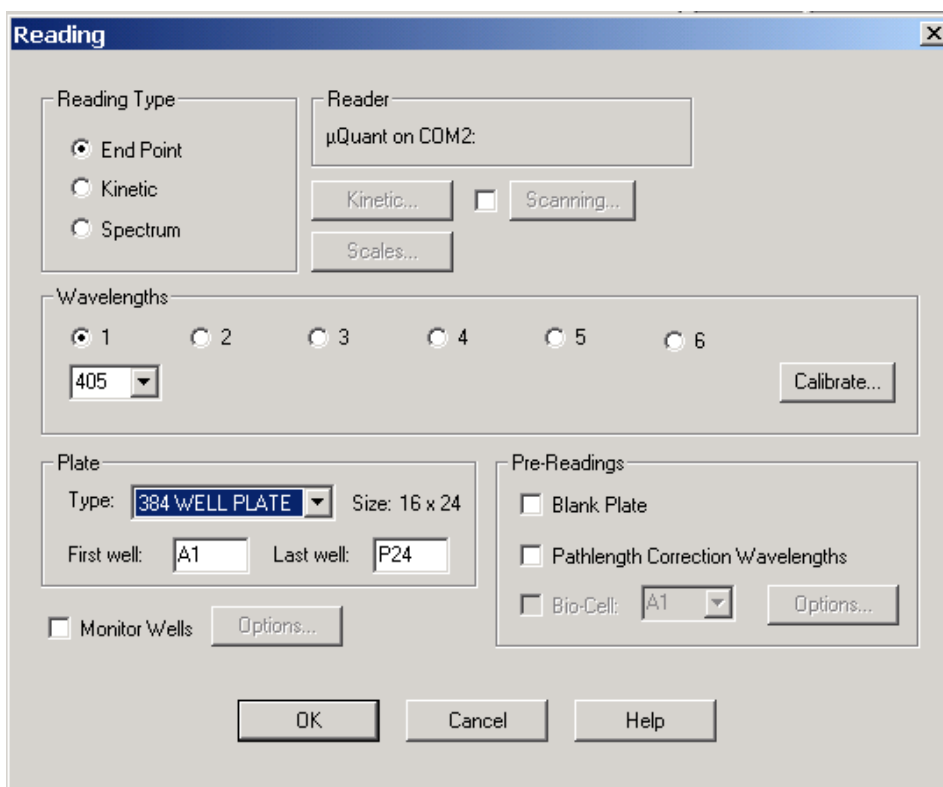


Figure 30: Reading dialog

10. When you have finished defining the parameters, click **OK**. KC4 will automatically validate the defined parameters. If any parameters are incorrect (or incomplete), follow the instructions provided by KC4 for correcting the parameters.
11. Select **Data|Read Plate**. The 'Plate Reading' dialog will appear.
12. Enter any desired information, place the 384-well 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 be displayed in KC4.
 - To analyze, manipulate, or print results, **Protocol** parameters should be defined. Refer to KC4's Help system or User's Guide for instructions.

Reading a 384-Well Plate in KCjunior™

1. Start KCjunior.
2. At KCjunior's main screen, choose **Setup|Reader 1** or **Reader 2**.

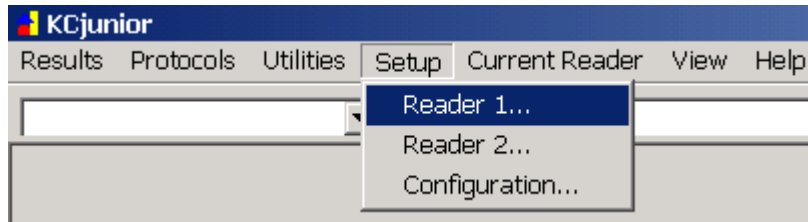


Figure 31: Setup|Reader dialog

3. The 'Reader Setup' dialog will appear. Select the **μQuant** reader for the **Reader Type**.

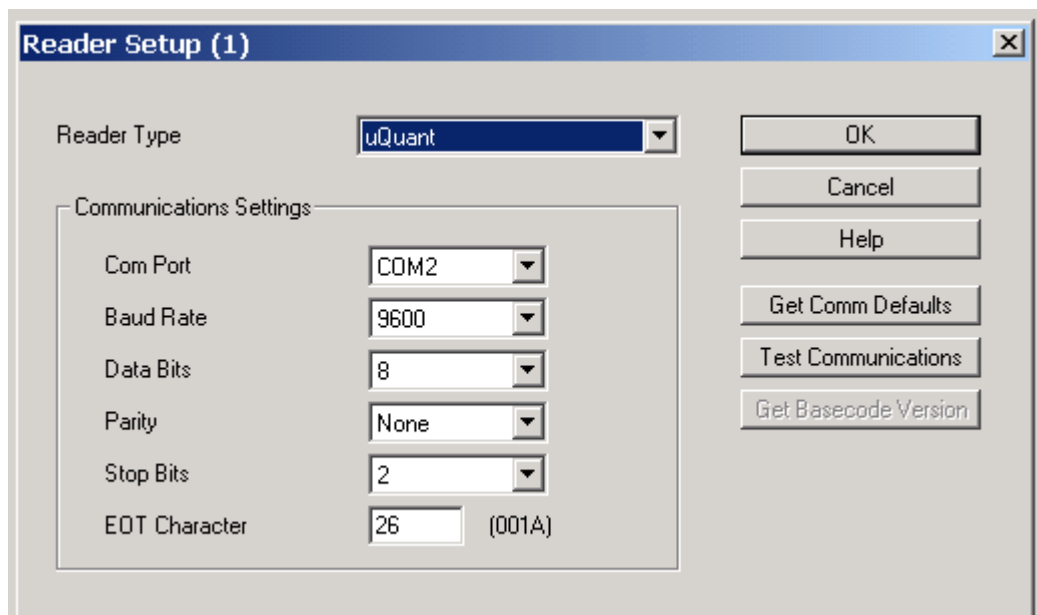


Figure 32: Reader Setup dialog

4. Define the communication parameters.
5. Click **Test Communications** to establish communication with the reader, then click **OK**. If the test fails, follow the instructions provided by KCjunior.

6. Click the **Read Plate** button in KCjunior's main screen. The 'Read Plate Dialog' will appear. If desired, enter the **Results ID** and **Plate Description**.

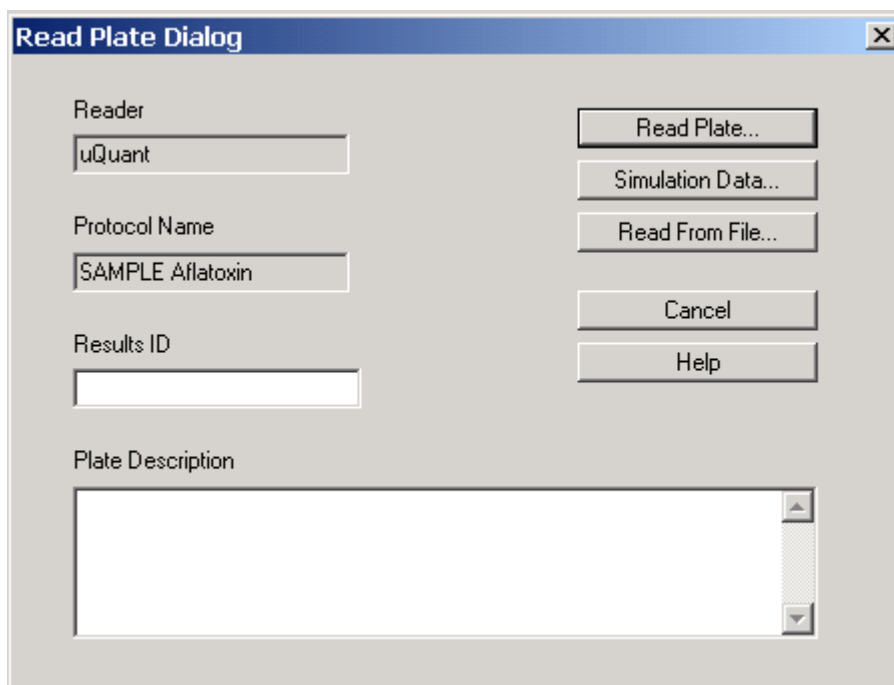


Figure 33: Read Plate dialog

7. Click **Read Plate** to open the 'Protocol Definition' dialog. Define the **Read Method** parameters:
- Select a **Read Method Type**.
 - Define the **Wavelength(s)** at which the plate will be read.
 - Set the **Plate Geometry** to **16 x 24**.
 - Define other reading parameters, as necessary. Click the **Help** button for assistance.

❖ When you have finished defining the parameters, click **Validate Read Method** if you would like KCjunior to verify the defined parameters.

If all parameters are valid, you will receive confirmation: "The read method is valid for the configured reader." If any are invalid, KCjunior will provide instructions for selecting the correct parameters.

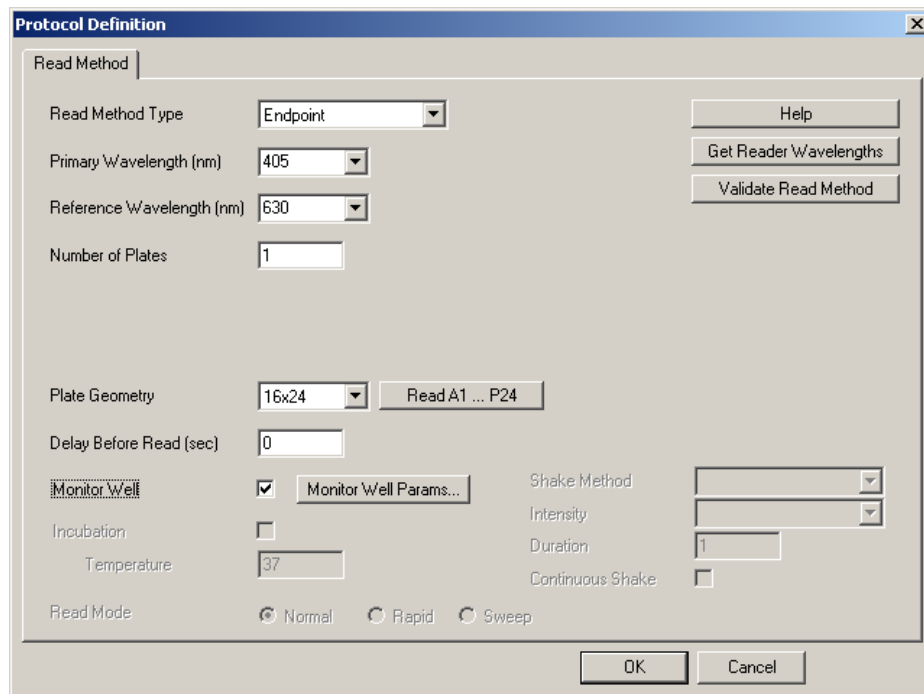


Figure 34: Protocol Definition dialog

8. Place the 384-well plate on the plate carrier, then click **OK** to read the plate. Click **OK** when prompted to “Place the plate on the carrier.”
 - The plate will be read and then the raw data results will be displayed in KCjunior.
 - To analyze, manipulate, or print the results, **Protocol** parameters should be defined. Refer to KCjunior’s 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 μ Quant. Refer to **Printing Reports** in **Chapter 4** for details on how to print these reports. In addition, an Assay List, Assay Definition, Map, and Result can be printed by choosing Report from the Main Menu screen.

Samples With Calls on Matrix Report.....	190
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Spectral Scan.....	197

Bio-Tek Instruments

Assay: ASSAY 01

Date: 03/16/04

Lot: _____

Wavelength: 450

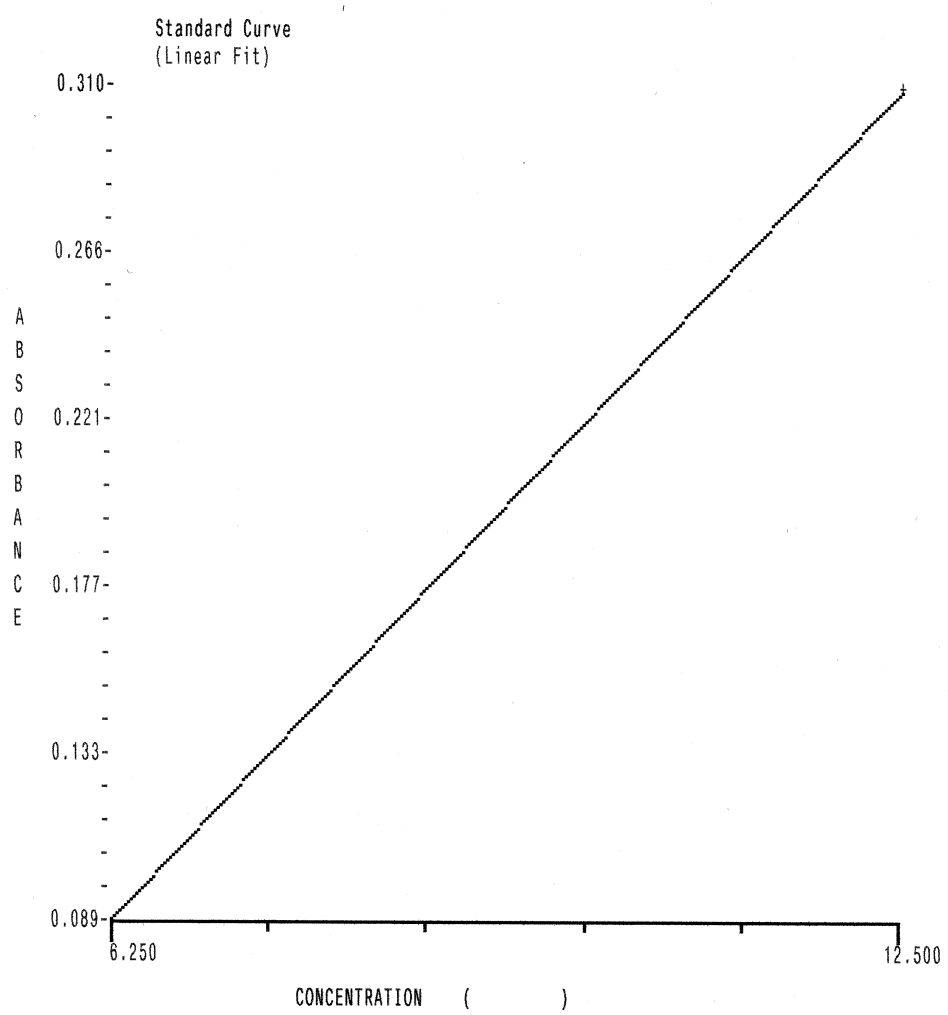
Time: 02:27:57PM

Operator: _____

Temp: _____

Plate ID: _____

COMMENTS



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

Figure 36: Curve Fit Report

Bio-Tek Instruments

Assay: Open Assay 04

Date: 03/16/04

Lot: _____

Wavelength: 405

Time: 01:05:34PM

Operator: _____

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	B1kdOD	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

Figure 37: Samples with calls on Column Report

Bio-Tek Instruments

Panel

Date:07/31/96
Time:02:24:01PM
Temp:Lot: _____
Operator:
Plate ID:**COMMENTS**

Specimen	Assay	Well	BlkdOD	CalcOD	Call	RSLT	Std Dev	CV%	Notes:
SMP0001	ASSAY 01	F01	-0.006	-0.006					
	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	-0.002	-0.002					
	ASSAY 02	F05	0.010	0.010					
SMP0006	ASSAY 01	C02	0.005	0.005					
	ASSAY 02	G05	-0.001	-0.001					
SMP0007	ASSAY 01	D02	-0.001	-0.001					
	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	-0.006	-0.006					
	ASSAY 02	C06	0.010	0.010					
SMP0011	ASSAY 01	H02	-0.005	-0.005					
	ASSAY 02	D06	0.004	0.004					
SMP0012	ASSAY 01	A03	-0.00	-0.00					
	ASSAY 02	E06	0.003	0.003					
SMP0013	ASSAY 01	B03	0.002	0.002					
	ASSAY 02	F06	-0.001	-0.001					
SMP0014	ASSAY 01	C03	-0.005	-0.005					
	ASSAY 02	G06	0.001	0.001					
SMP0015	ASSAY 01	D03	-0.002	-0.002					

Page 1

Figure 39: 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 UNKNOWNNS? 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

Figure 40: Assay Detail Report (Sheet 1 of 2)

Assay List
Version: v2.00.1

0	SPECTRAL SCAN
1	Raw_OD
2	Raw_OD w/blk
3	Bio-Cell Read
4	KFactor Bio-Cell
5	DNA Quant
6	260/280 Ratio
7	RNA Quant
8	Oligo Quant
9	Protein Quant
10	Phenol
11	EDTA Test
12	A320 Scattering
13	Lowry Protein
14	Bradford Protein
15	BCA Protein
16	B-galactosidase
17	NADH
18	HRP 5AS
19	HRP ABTS
20	HRP OPD-S
21	HRP OPD
22	HRP TMB-S
23	HRP TMB
24	Alk phosphatase
25	B-galact ONPG
26	Urease
27	MTT
28	XTT
29	WST-1
30	Methylene Blue
31	Open Assay 01
32	Open Assay 02
33	Open Assay 03
34	Open Assay 04
35	Open Assay 05
36	Open Assay 06
37	Open Assay 07
38	Open Assay 08
39	Open Assay 09
40	Open Assay 10
41	Open Assay 11
42	Open Assay 12
43	Open Assay 13
44	Open Assay 14
45	Open Assay 15
46	Open Assay 16
47	Open Assay 17
48	Open Assay 18
49	Open Assay 19
50	Open Assay 20
51	Open Assay 21
52	Open Assay 22
53	Open Assay 23
54	Open Assay 24
55	Rpen Assay 25
56	Open Assay 26
57	Open Assay 27

Figure 41: Assay Detail Report (Sheet 2 of 2)

Operator ID: _____

Notes: _____

11:21AM 06/29/98 SPECTRAL SCAN
 400nm - 600nm / 2nm AT WELL LOCATION: H06

Min OD: 0.713 @ 530nm
 Max OD: 1.926 @ 402nm

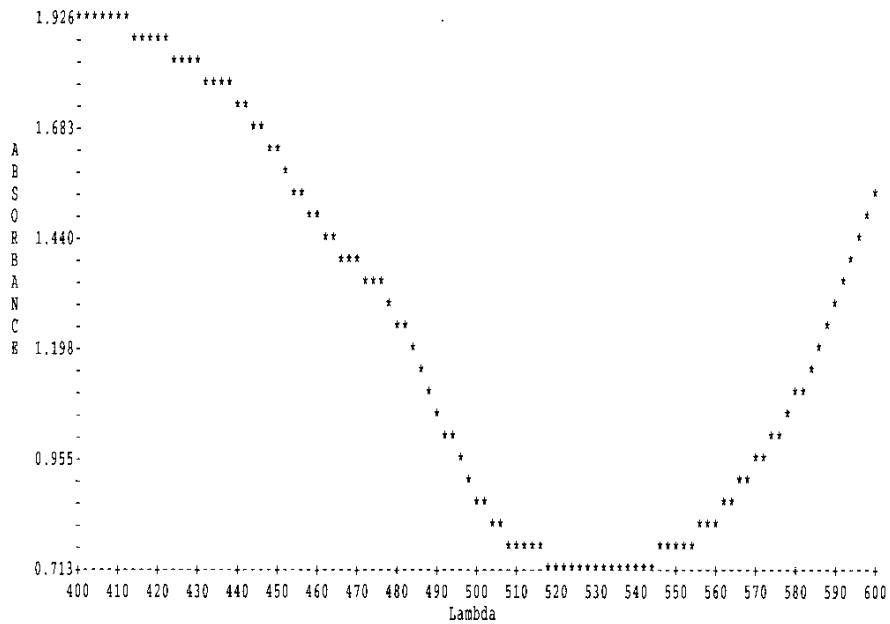


Figure 42: Spectral Scan

