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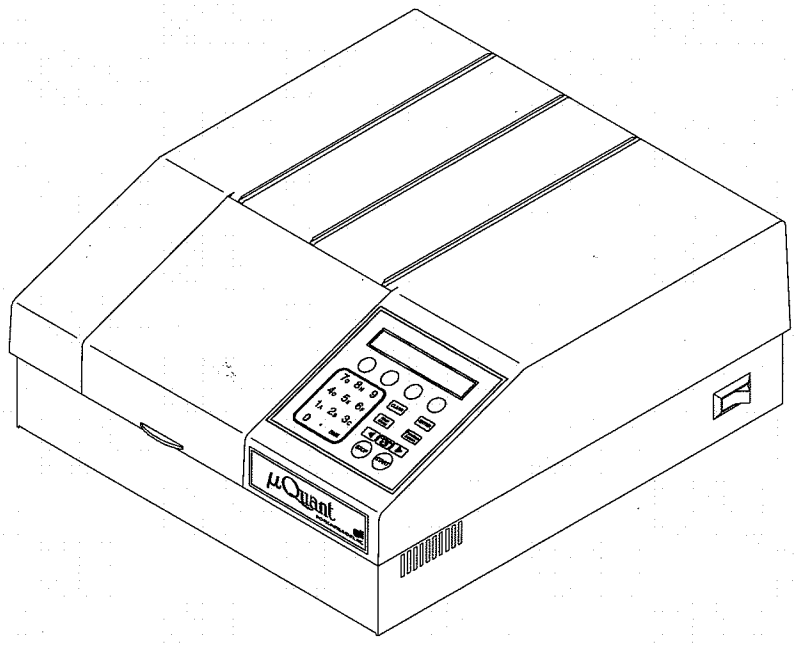
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μQuant

BIO-TEK INSTRUMENTS, INC.



Universal Microplate Spectrophotometer

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μQuant

Universal Microplate Spectrophotometer
Operator's Manual

OCTOBER 2003
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PART NUMBER 7271000
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Revision History

Revision	Date	Page	Change
A	7/98		Release to Production
B	7/98	1-6; 4-16	Changed λ accuracy from ± 3 nm to ± 2 nm.
C	9/98	1-6	Added reference to 384-well microplate in Specifications.
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.

Document Conventions

This manual uses the following typographic conventions.

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

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Warnings



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

Hazards

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

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

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

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

Precautions

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

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

Caution: Environmental Conditions. Do not expose the system to temperature extremes. Ambient temperatures should remain between 15-40°C. System performance may be adversely affected if temperatures fluctuate above or below this range.

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

Caution: Power Supply. Only use the specified power supply to ensure proper operation of the unit. The *µQuant* has a universal power supply that functions from 100 to 240 VAC (± 10%) 50-60 Hz without external switching.

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Electromagnetic Interference and Susceptibility

USA FCC CLASS A

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

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

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

Canadian Department of Communications Class A

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

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

User Safety: North American

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

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

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

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

EC EMC Directive 89/336/EEC Electromagnetic Compatibility

- **EC EN 55022, Class A-Emissions**

  This unit has been type tested by an independent testing laboratory and found to meet the requirements of EC Directive 89/336/EEC for Radiated Emissions and Line Conducted Emissions. Verification was to the limits and methods of EN 55022. This unit is classified as EN 55022, Group 1, Class A.

- **Immunity**

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

  EN 61000-4-2 (1991) Electrostatic Discharge

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

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

  EN 61000-4-5 (1995) Surge Immunity

  EN 61000-4-6 (1996) Conducted Disturbances

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

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

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

- **EN 61010-1 (1993)**

  "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements" (including amendments 1 and 2).

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Environmental Operating Specifications

Safety

This unit is designed to be operated under the following conditions:

- Altitude up to 2,000 meters
- 18° to 40°C
- Relative humidity less than 80% for temperatures up to 31°C to a relative humidity of 50% at a temperature of 40°C
- Mains supply voltage fluctuations not greater than 5% of the nominal voltage
- Transient over-voltage category II
- Pollution degree of 1
- This unit is classified to installation category (overvoltage category) II.
- This unit has a power rating (requirement) of 86 volts/amps (V/A).

Caution:

- To ensure proper cooling, be sure to rest the unit on a hard, flat surface that will not block the air flow beneath.
- The use of this product in any manner that is not specified by Bio-Tek Instruments will void the warranty, and could cause damage to the product as well as cause possible injury to the operator.

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Safety Symbols

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



Alternating current
Courant alternatif
Dreiphasen-Wechselstrom
Corriente Alterna
Corrente alternata



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



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

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



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

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



Caution, risk of electric shock
Attention, risque de choc electrique
Gefährliche elektrische Spannung
Atención, riesgo de sacudida eléctrica
Alta tensione (in questo documento Alta tensione non significa "tensione pericolosa" come definito in IEC 417)

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Intended Use Statement

The *µQuant* is a single-channel, automated, bench-top, general-purpose, Enzyme Immunoassay Analyzer that performs analyses and DNA quantification of a variety of samples.

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

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

This system is designed for use with 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 pre-loaded on every *µQuant*. If using *Extensions*™ Define Protocol software, up to 75 assay protocols and blanking patterns can be defined, 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 Bio-Tek software package, such as KC*junior*™ or *KC4*™ for Windows®.

Specimen Preparation

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

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.

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About This Manual

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

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

Registration Card

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

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Warranty

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

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

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

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

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Chapter 1
Introduction

This chapter introduces the μQuant, and describes its hardware and software features. Also included are instructions on how to contact Technical Assistance, if needed, and information about this manual.

Introducing the μQuant

The *μQuant* is a single-channel reader-assay system for *research and development* and *in-vitro diagnostic use*, designed to automatically perform endpoint analysis. 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 3.000 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 on-board processor, via a 2 X 24 LCD screen and membrane switch. The μQuant 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.

The *μQuant* is backed by a superior support staff. If the Microplate Reader ever fails to work perfectly, please refer to the phone and fax numbers, and Internet site address provided at the end of this chapter to contact the Technical Support Staff.

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.

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Hardware Features

The Microplate Reader's hardware features include:

- Xenon-flash light-source
- Single measurement system with reference channel
- Monochromator with wavelength range of 200-999 nm
- A 2 X 24 LCD display
- A membrane keypad with alphanumeric keys
- X-Y carrier movement
- Capability of reading 6-, 12-, 24-, 48-, 96- and 384-well microplates
- External 24-volt power supply that runs on 100-240 VAC ± 10% @ 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;
- Up to 75 assays that can be programmed into memory and recalled instantly;
- Up to 10 results that can be stored in memory;
- On-board spectral scanning.

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Package Contents

The contents of the *µQuant* package include:

- Microplate Scanning Spectrophotometer
- Power Cord
- Power Supply
- Operator's Manual and Registration Card
- Printer Cable (PN 71072)
- Dust Cover (PN 7332040)

Optional Accessories

- Serial Cable (PN 75053)
- Service Manual (PN 7331005), all instrument types
- Universal Calibration Test Plate (PN 7260522)
- Bio-Cell™ quartz vessel (PN 7272051)
- Bio-Cell™ adapter plate assembly (PN 7270512); holds up to six Bio-Cells.

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Specifications

- **Microplates**

  Accommodates all 6-, 12-, 24-, 48-, 96- and 384-well microplates, and 72- and 96-well Terasaki plates.

- **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 Abs

Accuracy:

0.000 to 2.000 Abs ± 1% ± 0.010 Abs Regular Read Mode

2.000 to 3.000 Abs ± 3% ± 0.010 Abs Regular Read Mode

0.000 to 2.000 Abs ± 1% ± 0.010 Abs Fast Mode or Spectral Scanning

2.000 to 2.5000 Abs ± 3% ± 0.010 Abs Fast Mode or Spectral Scanning

(Measured with neutral density filters calibration plate @ 405, 450, 490, 550, 630, 690 nm)

Linearity:

0.000 to 2.000 Abs ± 1% Regular Read Mode

2.000 to 3.000 Abs ± 3% Regular Read Mode

0.000 to 2.000 Abs ± 1% Fast Mode or Spectral Scanning

2.000 to 2.500 Abs ± 3% Fast Mode or Spectral Scanning

(Measured @ 405 nm using yellow food dye)

Repeatability (STD):

0.000 to 2.000 Abs ± 1% ± 0.005 Abs Regular Read Mode

2.000 to 3.000 Abs ± 3% ± 0.005 Abs Regular Read Mode

0.000 to 2.000 Abs ± 1% ± 0.005 Abs Fast Mode or Spectral Scanning

2.000 to 2.500 Abs ± 3% ± 0.005 Abs Fast Mode or Spectral Scanning

(Measured @ 405 nm using yellow food dye)

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- **Hardware and Environmental Specifications**

Display:	2 X 24 LCD display
Light Source:	Xenon flash
Dimensions:	42 cm X 38 cm X 18 cm (16.5" deep X 15" wide X 7" tall)
Weight:	10.5 kg. (23 lb. maximum)
Environment:	Operational temperature 18-40°C
Humidity:	Relative humidity less than 80% for temperatures up to 31°C to a relative humidity of 50% at a temperature of 40°C
Altitude:	Up to 2000 meters

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Technical Support

If your new Microplate Reader system fails to start or operate successfully, please contact Bio-Tek's Technical Assistance Center immediately.

Whichever method of contact you choose, please provide the following information.

- Product name and serial number
- Revision level of your software
- The specific steps that reproduce your problem
- Any error codes displayed on the screen (see *Appendix E* for information on error codes)
- A daytime phone number
- Your name and company information
- A fax number and/or e-mail address, if available
- If you need to return the instrument to Bio-Tek for service, contact Bio-Tek for a Return Materials Authorization (RMA) number, and be sure to repack the reader properly (see *Chapter 2, Installation*).

Phone Support

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

Bio-Tek Instruments Main Number:	802-655-4040
Technical Assistance Center:	800-242-4685
European Coordination Center	+49 (0) 7136-9680

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Written Communication

If you prefer, you can write a letter with your comments and send it to:

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

Bio-Tek® Instruments GmbH
Kocherwaldstr. 34
D-74177 Bad Friedrichshall
Germany

Facsimile Support

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

Technical Assistance Center:	802-655-3399
European Coordination Center:	+49 (0) 7136-968-111

Electronic Communication

Electronic communication is available via the following:

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

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Chapter 2
Installation

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

The Operating Environment

The *µQuant* 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.
- Excessive ambient light, as it may affect the instrument's optics and readings. Bright sunlight or strong incandescent light can reduce the linear performance range of the instrument.
- Dust — optical density readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

Unpacking and Repackaging the Instrument

- If the shipping box has been damaged, inspect the instrument for visible dents and scratches as you unpack it.
- If the reader is damaged, notify the carrier and your manufacturer's representative. Keep the shipping cartons and the packing material for the carrier's inspection. The manufacturer will arrange for the repair or replacement of your instrument immediately, before the shipping-related claim is settled.
- If the reader is shipped to the factory for repair or replacement, it must be carefully repackaged using the original packing materials. If the original packing materials have been damaged, contact the manufacturer for replacement materials. Shipping with improper packaging materials may void your warranty.

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To Unpack the Instrument

- Carefully open the top of the box, and remove the top foam end cap, manual, and Declaration of Conformity *(Figure 2-1)*.
- Lift the reader out of the box (*Figure 2-2*). Place the reader on a level surface and remove the instrument from the plastic bag.

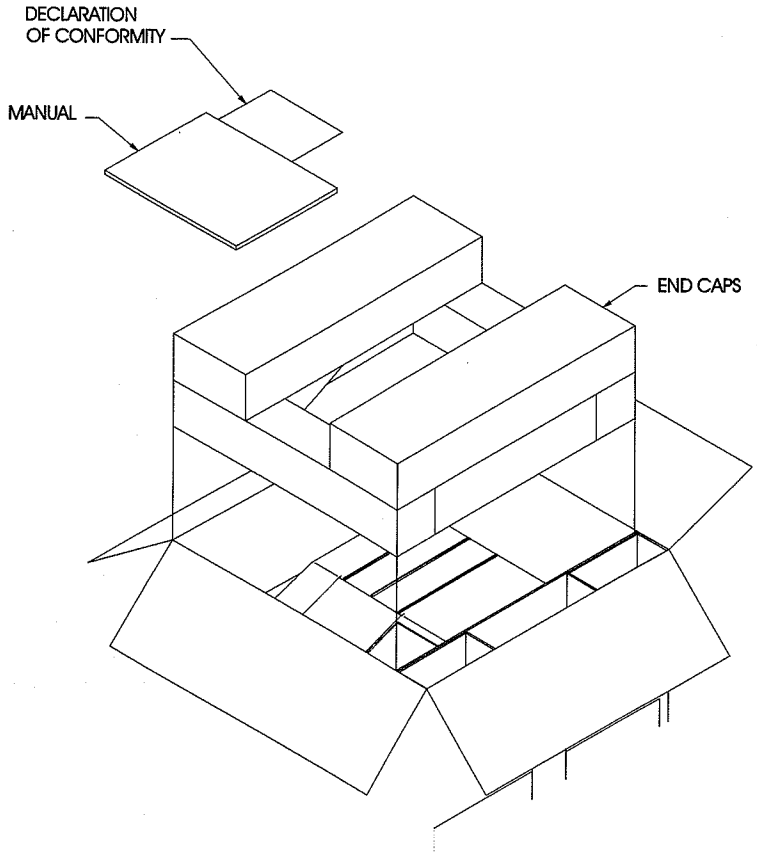


***Figure 2-1**: Removing the top end cap*

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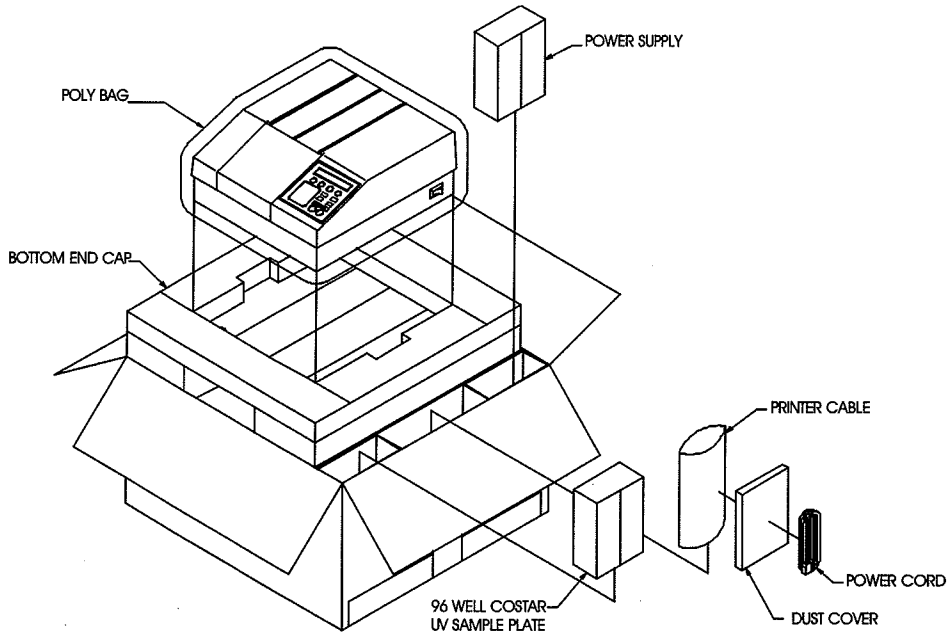


***Figure 2-2**: Removing the foam end caps*

1. After placing the instrument on a stable surface, open the front door of the unit.
2. Remove the shipping block and mounting hardware, while holding the carrier in place. Retain the shipping block and mounting hardware for Step 4.
3. At the back of the unit, remove the two black dress screws, as shown in *Figure 2-3*. Retain these screws for Step 5.
4. Install the carrier shipping block and mounting hardware that was removed in Step 2 to the back of the unit.
5. Insert the two black dress screws removed from the back of the unit into the two mounting holes for the carrier shipping block in the front of the unit.

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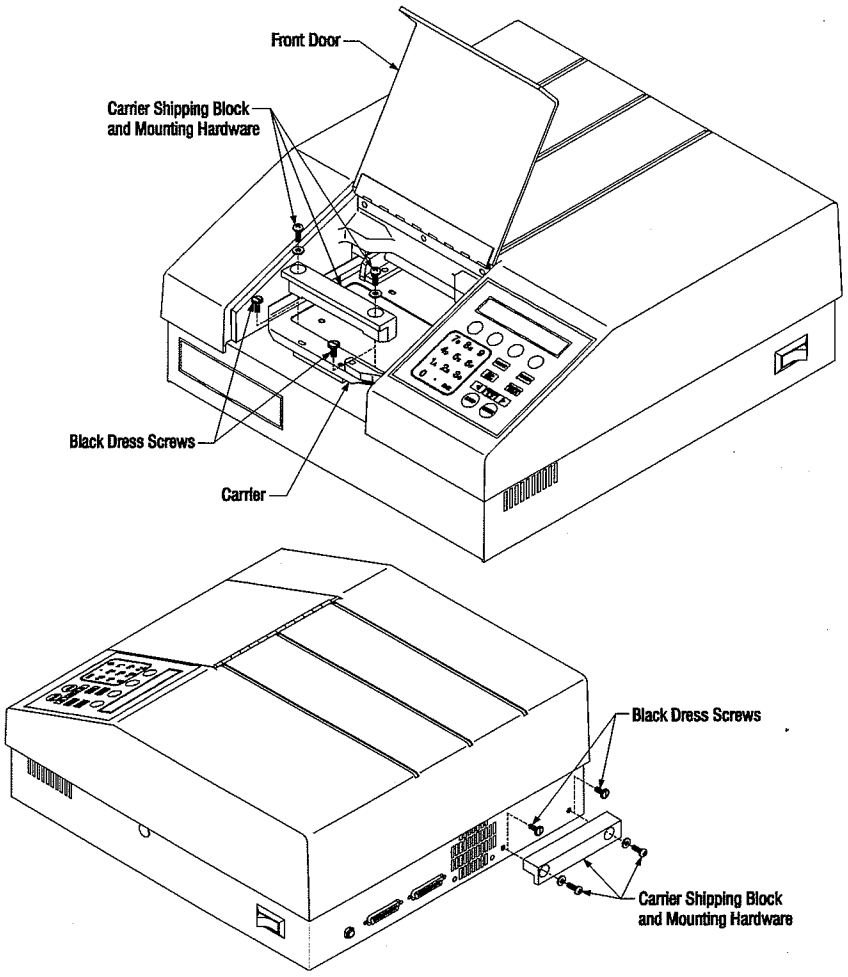


***Figure 2-3**: Removing the shipping block and mounting hardware*

Before Repackaging the Instrument

- Decontaminate the reader prior to shipping. (Refer to the Decontamination procedure in *Appendix A*.)
- Once the instrument is clean, reattach the shipping block.

Follow the instructions on the following page to park the monochromator in a safe position for shipping. The keyboard will be locked once the parking process begins. Power must be recycled to regain control of the instrument.

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- From the Main Menu, select the **UTILITY** option.
- At the **SELECT UTILITY OPTION**, choose **SETUP**.
- Press **Shift 9** to access the Monochromator Park Start screen. Press **<READ>** to continue.

P	**a**	**r**	**k**		**M**	**o**	**n**	**o**	**c**	**h**	**r**	**o**	**m**	**a**	**t**	**o**	**r**			
P	**r**	**e**	**s**	**s**		**<**	**R**	**E**	**A**	**D**	**>**		**t**	**o**		**C**	**o**	**n**	**t**	**i**	**n**	**u**	**e**

Park Monochromator screen

- Return the instrument to its shipping box, using the original packing materials.

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

After Unpacking, Verify Performance

Prior to using the instrument for the first time, verify that the instrument is operating properly and that no damage occurred during shipping by running the Calibration Validation and Verification of Alignment tests. These tests are described in *Chapter 4*.

Power Supply

The *µQuant* comes with a 24-volt external power supply. The input is 100-240 VAC (± 10%) at 50-60 Hz, with an output of 24 VDC at 3.75 A.

1. Plug the power supply's outlet plug into the 24-VDC connector at the rear of the unit (see *Figure 2-4*).
2. Insert the power cord into the power supply and into an appropriate wall outlet.

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Adjusting the Reader's Wavelength Table Setting

To check, or change the software wavelength:

- Power up the reader. At the Main Menu Screen, press the soft key under the **UTIL** menu option.

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

The Main Menu screen

The **Utilities Options** menu appears:

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

The Utilities Option Screen

- From the Select Utility Option menu, press the soft key beneath the menu option **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

The Edit Setup Screen

- From this menu, press the soft key under the **LAMBDA** menu option.
- The wavelength will be displayed. To advance to the next wavelength, press **ENTER**.
- 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. To save the entry and move to the next wavelength on the table, press the **ENTER** key.
- Any wavelength value between 200 and 999 nm may be entered in 1-nm increments.

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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	

The Wavelength Entry screen

- 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

The Edit Setup screen

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

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Installing a Printer

The *µQuant* has a parallel port (LPT1) to allow connection to Epson-compatible printers or HP Deskjet™ printers. (*For more information on compatible Epson and Hp Deskjet printers, refer to page 2-16 under Output Options.*) The port is illustrated in *Figure 2-4.* The parallel port requires a 25-pin D-sub connector.

The *µQuant* comes with a parallel cable designed to connect the reader to a printer.

To attach a printer to the *µQuant*:

- Place the printer in a location adjacent to the *µQuant.*
- Attach one end of the cable to the printer's parallel port.
- Attach the other end of the cable to the Parallel Port on the reader.
- Make sure the securing screws on both ends of the cable are tightened.

---

iii **IMPORTANT:** To avoid system instability, ensure that the printer is connected to the reader *before* the reader is powered up.

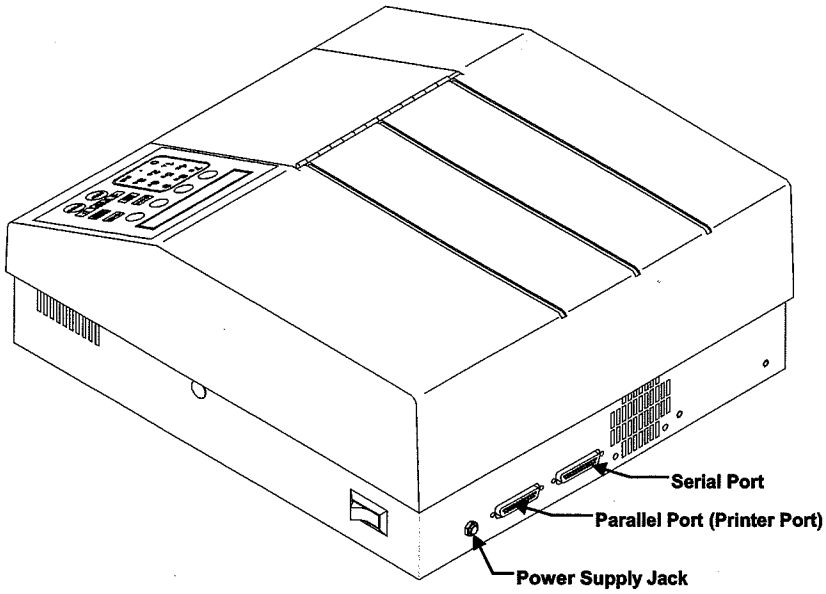


Figure 2-4: *Connectors for printers (parallel), computer (serial) ports, and power supply jack*

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Parallel Port Pin Definition

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

Table 2-1
Parallel Connector Pinouts

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

Setting Up the Serial Port for Communications

The *µQuant* has a 25-pin serial (RS232) 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 RS232 protocols.

The serial port also allows field upgrades of the *µQuant* software.

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

Attaching the Cable

- Power down the computer and the *µQuant*.
- 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. The connector's pinout is illustrated in *Table 2-2*.
- Power up the reader and the computer.
- Ensure that the *µQuant* and the computer are operating with the same communications settings.

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Serial Port Pinout Description

Table 2-2 provides a pinout description of the *µQuant*'s Serial/RS232 connection.

Table 2-2
Serial Pinout Description

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

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Utility Options

The *µQuant* may be configured a number of additional ways, depending on user preferences. These additional configuration options may be accessed via the **Utility Options** menu, and include:

- Under **SETUP**: Setting the date and time and lambda. By pressing ***MORE**, **RS232** and **CALPLATE** become available. By pressing ***MORE** an additional time, **BULB ALIGN** will be displayed.
- Under **OUTPUT**: Selecting whether reports will be output to a Printer, the Computer display or both, choosing a Column or Matrix Report format, and determining if Curve Fits will be printed.
- Under **READ**: Whether prompts are required for Plate IDs, Sample IDs, and Sample Counts, and if Rapid Read is desired.

To set these user-configurable options:

- At the **Main Menu** screen, press the soft key beneath **UTIL** to access the Utility Options menu.

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

Selecting Setup, Output and Read to Access Configuration Options

Test Options

- At the **UTILITY OPTIONS** screen, press the soft key beneath the menu option **TESTS**.
- At the **SELECT TESTS** screen, press the soft key beneath **SYSTEM.**

System Test (System)

The System Test checks that the light levels and electronic noise at all the programmed wavelengths fall within factory acceptance criteria. This is done by measuring the air and dark readings, and evaluating them to ensure they fall within specified ranges. The reader automatically runs an internal System Test each time it is powered on. An error will be displayed if the power-on System Test fails. No printout will occur at the power-on System Test. To obtain a printout of the System Test values for either periodic testing and documentation or troubleshooting, press **SOFT KEY 1,** from the **Tests** submenu.

When **SOFT KEY 1** is pressed, the *µQuant* runs the instrument's System Test, and reports results in a pass/fail format.

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Checksum Test (CHKSUM)

This test also runs automatically when the reader is powered on. The test compares the software to the internally recorded checksum values to ensure that the programming has not been corrupted. If there are any errors during the power-on checksum test, they will be displayed. To verify the checksum, revisions and version of software currently loaded onto your reader, press **SOFT KEY 2** from the Tests submenu.

When the CHKSUM Test is selected, the software versions and the software's checksum appear on the display, as shown in the example below:

Software P/N Software Version

Code Checksum: (3B43)

The second screen shows:

Configuration P/N Configuration Version

(Blank)

Calibration Test Plate Software Utility (CALPLATE)

It is considered good laboratory practice to periodically verify the calibration of the *µQuant*. The *µQuant* includes a utility that when used in conjunction with the Universal Test Plate (an optional accessory, also referred to as the Universal Calibration Test Plate) will test the accuracy, alignment, and repeatability of the reader. Alternatively, the user may choose to run a "Liquid Test" if they do not currently own a Universal Test Plate. Three suggested liquid tests are described later in *Chapter 4*.

Setup Options

- At the **UTILITY OPTIONS** screen, press the soft key beneath the menu option **SETUP**.
- At the **EDIT SETUP INFORMATION** screen, press the soft key beneath **DATE**.

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

Selecting the Date Option

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- Enter the new date, using the **NUMERIC** keys. The cursor will be positioned under the first editable field, and advance automatically. To change the date format , press the soft key beneath **MMDDYY** or **DDMMYY**. The display will be updated to reflect the new format.

D	A	T	E	:			0	3	/	1	6	/	9	5					M	D	Y
M	M	D	D	Y	Y							D	D	M	M	Y	Y					

Entering the Date/Selecting the Date Format

- Press **ENTER** to return to the **EDIT SETUP INFORMATION** screen.
- To edit the **TIME**, press the soft key positioned beneath the **TIME** menu option. At the Time entry screen, use the **NUMERIC** keys to enter the correct time. The cursor is positioned at the first editable field.

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

Entering the Time/Selecting the Format

- Select a **12-** or **24-hour** format, by pressing the soft key positioned beneath these options. The display automatically updates with the new time/format.
- Press **ENTER** to return to the **EDIT SETUP INFORMATION** screen.
- To edit the **Wavelength**, press the soft key positioned beneath the **LAMBDA** menu option. At the **LAMBDA** entry screen, use the **NUMERIC** keys to enter the correct wavelength for each of the six positions.

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

Entering the Wavelength

- Continue to scroll through the 6 available wavelength positions, pressing **ENTER** between each. When all six wavelengths have been defined, or skipped, the software will be returned to the **EDIT SETUP INFORMATION** screen.

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Setting Up Communications Parameters

Before serial communications is initiated between the *µQuant* and another device, the communication parameters (Baud Rate, Data Bit Configuration and Parity Status) must match on both devices.

The reader's default communication parameters are:

- 2400 Baud,
- 8 Data Bits,
- 2 Stop Bit, and
- No Parity.

The user may change the Baud rate on the *µQuant* from the recommended default setting of 2400 to 1200 or 9600. Data bits, Stop Bits and Parity are **not** user configurable. Thus, the communications software operating on the other device (computer, etc.) should be set to 8, 2 and None.

If you would like to change these settings, choose ***MORE** to access the **RS232** (baud rate and parity) and the **CALPLATE** (calibration wavelength) functions.

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

Accessing the RS232 and Calplate functions

Changing the Baud Rate on the µQuant

To change the baud rate from the default of 2400, to either 1200 or 9600:

- Power up the instrument. At the **Main Menu** screen, press the soft key beneath the menu option **UTIL**.

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

The Main Menu screen

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- At the **UTILITY OPTIONS** screen, press the soft key beneath the menu option **SETUP**.

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

Selecting Setup

- At the **EDIT SETUP INFORMATION** screen, press the soft key beneath the ***MORE** menu option to advance to the **EDIT SETUP/RS232** option screen.

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

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

*Select *MORE to advance to the RS232 menu*

- Press the soft key beneath the **RS232** menu option to access the **BAUD RATE** selection menu. The top line of the display shows the Baud rate currently set in memory.

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

Selecting the Baud Rate

- To change the **BAUD** rate, press the soft key positioned beneath the desired baud rate. The display (top line) automatically updates to reflect the new choice.

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- To view the reader's other communication settings, press the soft key beneath the **VIEW** menu option.

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

Viewing Other Communication Settings

Output Options

- At the **SELECT UTILITY OPTIONS** screen, press the soft key beneath **OUTPUT** to set report output preferences.

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

Selecting Report Output

- Any previously defined selection appears on the top line of the display. Press the soft key beneath the desired option (**PRINT, COMPUTER, BOTH**) to change the output device.
- Press **PRINT** or **BOTH** to advance to the **SELECT PRINTER** menu screen. If **COMPUTER** is pressed, all results will be transferred directly to the computer screen via the RS232 serial port.

S	E	L	E	C	T		P	R	I	N	T	E	R						E	P	S	O	N
E	P	S	O	N				H	P													

Selecting Printer Output

Compatible Printers

BTI readers support printers using either HP's PCL3 language, such as the HP DeskJet series, or Epson's LQ language. For the latest list of compatible printers, call Bio-Tek Instruments' Technical Assistance Center (refer to *Chapter 1* for contact information).

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Report Type

- Press **ENTER** to advance to the **REPORT TYPE** menu screen.

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

Selecting Report Type

- Any previously defined selection appears on the top line of the display. Press the soft key beneath the desired option (**COLUMN, MATRIX, BOTH**) to change the Report Type. The display will automatically update with the new type.

NOTE: See *Appendix C* for examples of Reports.

- Press **ENTER** to advance to the **SAMPLES IN COLUMN REPORT** screen.

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

Choosing whether to include samples in Column Report

- Press **ENTER** to advance to the **PRINT CURVE FIT** screen.

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

Choosing to Print Curve Fit data

- Any previously defined selection is displayed on the top line of the screen. To change the report option, press the soft key under **YES** or **NO**. The display updates to reflect the selection.
- Choose **YES** only if there are quantitative assays defined on-board.
- Press **ENTER** to return to the **SELECT UTILITY OPTION** screen.

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Read Options

- At the **SELECT UTILITY OPTIONS** screen, press the soft key beneath **READ** to set up Reader Prompt preferences. Select YES to prompt for identifications and sample counts before a microplate is read. The prompts are shown below.

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

Prompt for Plate ID screen

- **PROMPT FOR PLATE ID** allows the user to enter an alphanumeric name of up to 10 characters.

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

Prompt for Sample ID screen

- **PROMPT FOR SAMPLE ID** allows the user to identify samples with a 4-character alphanumeric name. The starting ID is entered and automatically incremented by the software.

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

Prompt for Sample Count screen

- **PROMPT FOR SAMPLE COUNT** allows the user to enter the total number of samples on the plate at runtime; only those sample results will be printed.

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

Selecting Read Preferences

- **RAPID MODE** reads a 96-well plate in approximately 30 seconds. Answering **NO** means the plate will be read in High Precision or 'Regular' Mode (approximately 50 seconds). Specifications for these modes are outlined in *Chapter 1*.
- Press **ENTER** after each selection to advance the display. When selections are completed, the display returns to the **SELECT UTILITY OPTION** screen.

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Chapter 3
Operation

This chapter includes instructions for operating the µQuant and its software.

Introduction

The *µQuant* features a 25-pad keyboard and a 2 X 24- character LCD display, allowing the user to access the reader's program menus to view test results. The reader's bi-directional serial port allows computer control of the instrument, and provides the means for downloading additional assay definition files to the instrument.

NOTE: The *µQuant* reader may come with pre-programmed assays already installed. This chapter describes the operation of the *open* assays on-board the *µQuant.*

The Keyboard

Figure 3-1 shows the instrument's keyboard layout.

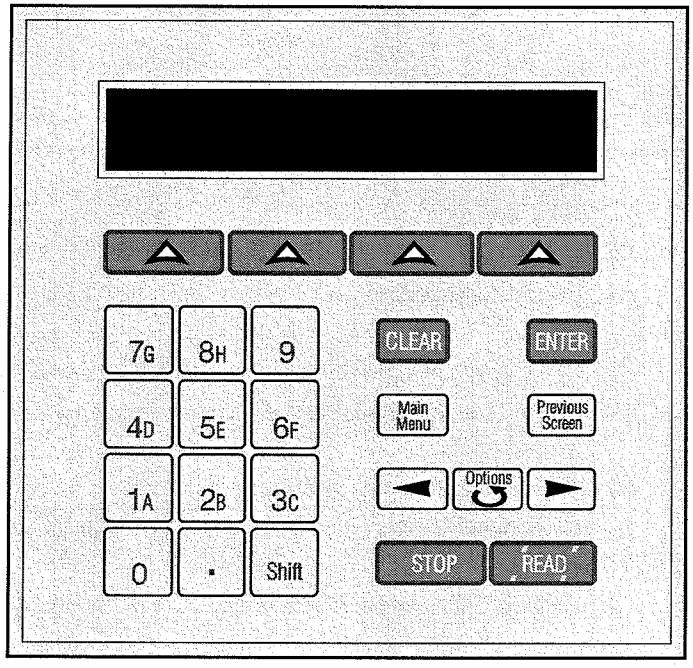


***Figure 3-1**: Keyboard*

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The Startup Screen

The *µQuant* performs a self-test when powered on, displaying the Startup screen until initialization is complete. During this period, no keys are active. Should the instrument fail to pass the self-test, a beep will sound, and an error code will display. For assistance with troubleshooting this error, contact Bio-Tek Instrument's Technical Assistance Center (refer to *Chapter 1* for contact information).

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

The Main Menu Screen

Once the system is initialized, the **Main Menu Screen** is displayed. The keyboard's four "soft keys," located below the on-screen menu options (*Read, Define, Report* and *Util*), are activated, and may be selected.

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

Main Menu Screen

Press the "soft key" that corresponds to a displayed menu option to activate that option:

- **READ** option (or, press the key labeled **READ** on the keyboard) for plate-reading prompts. The user will be asked to select an assay from a list of choices.
- **DEFINE** option: Allows the creation of a reading and data reduction protocol. The user will be prompted to select an assay from an assay definition list.
- **REPORT** option: Allows printing of results and protocol descriptions. The user will be prompted for the name of a previously run assay with valid data.
- **UTIL** option: The user will be prompted to select options from the list of on-board utilities.

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Define

The Main Menu option, **DEFINE**, allows the user to edit previously defined assays stored in memory.

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

Select Assay

At the Select Assay Number screen,

- Use the **NUMERIC** keys to enter the number of any predefined Assay Definition Files stored in the reader's memory, or the **OPTION** key to advance one assay at a time. The cursor is positioned at the first editable field, and advances automatically. The numeric range depends on the number of assays programmed in the reader's memory.

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

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

Assay Selection Screen

NOTE: The *µQuant* reader may come with pre-programmed assays already installed, or new ones may be created.

Press **ENTER** to advance to the **EDIT ASSAY NAME** screen. You may change the default assay number to a more descriptive one (see assay name below):

- **CLEAR**: Clears the reader's LCD display.
- **MAIN MENU**: Returns the display to the Main Menu screen.
- **PREVIOUS SCREEN**: Returns the display to the previous screen.
- **ENTER** key: Advances the display to the next screen.

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Assay Name

At the **EDIT NAME** screen, it is possible to edit the name assigned to the Assay. The Assay name can be up to 16 characters.

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

Assay Name screen

- Use the **ALPHA** and **NUMERIC** keys to change the Assay name. The cursor is positioned at the first editable field.
- Use the **OPTION** 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 **CLEAR** key to clear the Assay name from the display.
- Use the **LEFT & RIGHT ARROW** keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Use **SOFT KEYS 1, 2, 3 & 4** when using alphanumeric characters on the display above the soft key in the assay name.

---

iii **NOTE:** When assay number 99 is chosen, a panel can be programmed (*refer to page 3-29*). When assay number 00 is chosen, the *µQuant* will begin the **Spectral Scanning** sequence of prompts. (*Refer to page 3-33.*)

Select the number of samples that will be run on the specific assay within the panel. The number of samples that have been defined previously for the assay, will be ignored. All information about the other well types (positive controls, negative controls, standards and blanks) will be copied and stored from the original assay into the panel definition.

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

Entering the number of samples

- Use the **MAIN MENU** key to return to the Main Menu screen saving only the panel name.
- Use the **ENTER** key to save the contents of the display and advance to the next screen.
- Use the **NUMERIC** keys to enter the number of samples. The valid range is 0 to the number of remaining undefined locations on the plate.

At this point the instrument will repeat all the prompts previously seen for the remaining assays.

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Define (Method, Map, Formula and Curve)

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

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

Define Option screen

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

- **SOFT KEY 1: METHOD** is selected, and the user is prompted to select the wavelength, filters, and specify plate geometry.
- **SOFT KEY 2: MAP** is selected and the user is prompted for mapping information.
- **SOFT KEY 3: FORMULA** is selected and the user can access the formula entry screens.
- **SOFT KEY 4: CURVE FIT** is selected and the user is prompted for curve-fit options.
- In addition, the **MAIN MENU, PREVIOUS SCREEN** and **ENTER** keys are active, allowing the user to move back and advance through the menu structure.

Defining the Method

The definition of a method includes selecting:

- single or dual wavelength
- filters, and
- plate geometry

The screens appear on the display in the order that they were programmed in the assay. If the assay contains a closed variable, the entry screen is skipped.

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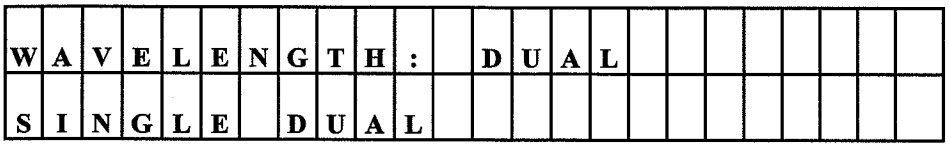
Single or Dual Wavelength

The **Wavelength** selection screen allows the user to select **SINGLE** or **DUAL** wavelength for the assay.

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

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

Any previously defined wavelength appears on the top line of the display, and the options on the second.



Specifying the Wavelength

- Press **SOFT KEY 1** to select **SINGLE** wavelength. The top line of the display is updated to reflect the selection.
- Press **SOFT KEY 2** to select **DUAL** wavelength. The top line of the display is updated to reflect the selection.
- Press **ENTER** to save the selection and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

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Meas Selection

The **MEAS** selection screen allows the user to select the filter(s) for the assay. Any previously defined filter appears on the top line of the display and the options on the second.

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

Specifying the Filters when using Dual Wavelength

- Use **SOFT KEYS 1, 2, 3 & 4** to select the filter option displayed above the soft key. The display updates to reflect the selection.
- Use the right arrow key to select the Reference Filter.
- Press **ENTER** to move to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

Plate Type

The **PLATE TYPE** selection screen allows the user to select the type of plate that will be used for the assay. Any previously defined plate is shown on the top line of the display, and plate options are on the second.

P	L	A	T	E		T	Y	P	E	:		9	6		W	E	L	L	S				
	2	4					4	8					9	6					*	M	O	R	E

P	L	A	T	E		T	Y	P	E	:		9	6		W	E	L	L	S				
	3	8	4				7	2	T				9	6	T				*	M	O	R	E

Selecting the Plate Type. Note: Only the NB reader models will include 384-well plates as an option.

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- Use **SOFT KEYS 1, 2, 3 & 4** to select the plate type displayed above the soft key. The display updates to reflect this selection.

- Use the **ENTER** key to save the selection. The **DEFINE** menu screen will be displayed.

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

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Map Definition

MAP Definition screen allows the user 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. Selecting 6-, 12-, 24-, 48-, 64-, 72- or 96-well options enables data reduction capabilities.

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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

Selecting the Map option on the DEFINE screen

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

Map Generation

This option allows the user to select manual or automatic plate map generation for the assay. Any previously defined value is shown on the top line of the display and the available options on the second line.

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										

Selecting Manual or Automatic Map Generation

Automatic Plate Map Generation refers to the ability of the software to automatically generate a Plate Map after the number of blanks, controls, standards and samples have been identified.

Manual Plate Map Generation refers to the ability of the user to identify where each blank, control, standard, or sample will be located.

- Press **SOFT KEY 1** for sample plate map generation. The display will update to reflect the selection.
- Press **SOFT KEY 2** for **MANUAL** plate map generation. The display updates to reflect the selection.
- Press **ENTER** to save the selection and move to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

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

Mapping Direction

This option allows the user to select how the wells are mapped on the plate. The well types can be listed in column format (down) or in row format (across). Any previously defined Mapping Direction appears on the top line of the display; the available options appear on the second line.

M	A	P	P	I	N	G		D	I	R	E	C	T	I	O	N	?	D	O	W	N		
D	O	W	N			A	C	R	O	S	S												

Mapping Direction Entry screen

- Press **SOFT KEY 1** to map **DOWN** the column.
- Press **SOFT KEY 2** to map **ACROSS** the row.
- Press **ENTER** to save the selection and move to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

Replication Direction

This option allows the user to specify how replicates are mapped on the plate. Any previously defined Replication Direction appears on the top line of the display and available options on the second.

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												

Mapping the Direction of the Replicates

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- Press **SOFT KEY 1** to map the replicates **DOWN** the column, following the direction of the map listing.

- Press **SOFT KEY 2** to map the replicates **ACROSS** (in a paired format). As an example, two replicates can be placed in A1 and A2 wells. The third replicate would follow in B1. The next standard control, or sample, would follow in B2.

- Press **ENTER** to save the selection and advance.

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

Examples of mapping directions are shown in *Figure 3-2*.

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	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										

The map above shows Map Direction DOWN, Rep Direction DOWN

	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												

The map above shows Map Direction ACROSS, 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										

The map above shows Map Direction DOWN, Rep Direction ACROSS

	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												

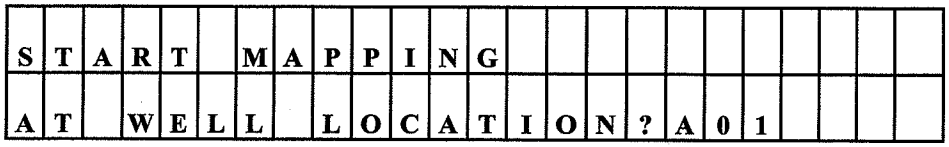
The map above shows Map Direction ACROSS, Rep Direction DOWN

Figure 3-2: *Examples of Mapping Directions*

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Start Mapping at Well Location

The Start Mapping at Well Location screen is only shown if automatic mapping is selected. This option allows the user to enter the location of the well that will be the starting point for automatic mapping. Previously defined Starting Well Location appears on the top line of the display.



The Starting Well Location screen

- Use the **LEFT & RIGHT ARROW** keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Use the **NUMERIC & ALPHA** keys to enter a letter or number at the cursor location. For all prompts of a well location, only the ALPHA keys are active for the first character and NUMERIC for the second and third character. The plate geometry determines which characters and numbers are valid for entry. The valid range for each plate type is listed below.

  ⇒ **Valid range for 24-well plates**: A1-A6, B1-B6, C1-C6 and D1-D6

  ⇒ **Valid Range for 48-well plates**: A1-A8, B1-B8, C1-C8, D1-D8, E1-E8 and F1-F8

  ⇒ **Valid range for 96-well plates:** A1-A12, B1-B12, C1-C12, D1-D12, E1-E12, F1-F12, G1-G12 and H1-H12.

- Press **ENTER** to save the well location and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back and advance through the menu structure.

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Blank Map

This option allows the user to select which blanking method to apply to the assay. Any previously defined Blank Map value appears on the top line of the display, with available options on the second.

The blanking options, AIR, FULL and CONSTANT, ROW and COLUMN, and P-ACROSS and P-DOWN are displayed on three screens and are only valid for automatic mapping methods.

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

BLANK  MAP: FULL
ROW  COLUMN      *MORE

BLANK  MAP: FULL
P-ACROS  P-DOWN  *MORE
```

The Three Blank Map Screens

- **Use SOFT KEYS 1, 2 or 3** to select the **BLANK MAP** type above the soft key. The display updates to reflect the selection.
- Use **SOFT KEY 4** to access **MORE** options: ROW or COLUMN, and P-ACROSS or P-DOWN.
- Use the **MAIN MENU** or **PREVIOUS SCREEN** keys to move back through the menu structure.

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Blank Map Definitions:

- **AIR** uses the initial blanking value on air as a blank value.

- **FULL** enables a single blank well or an average of blank wells (up to 12) to be subtracted from the whole plate.

- **CONST** (Constant) allows entry of a user-specified absorbance value from the whole plate. Use a blank value from the first plate, or a blanking plate to save space on subsequent assay plates.

- **COLUMN** enables a single blank well or an average of blank wells to be placed in each row. 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.

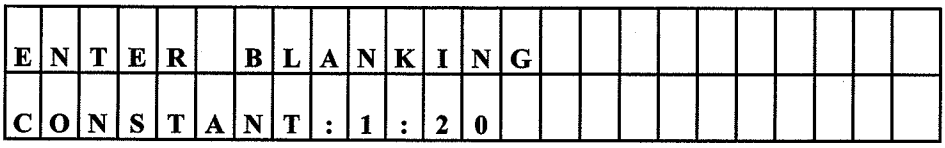
- **P-DOWN** enables a blank in the B, D, F and H rows to be subtracted from the well above in the A, C, E and G rows. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the A, C, E, and G rows.

- **ROW** enables a single blank well or an average of blank wells to be selected for each row. The maximum number of blanks is 48. Use manual mapping to position blanks, standards, controls, and samples.

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Constant Blank Value

This entry screen only appears when a Constant Blank map is selected. The user enters a value that is subtracted from each well on the plate.

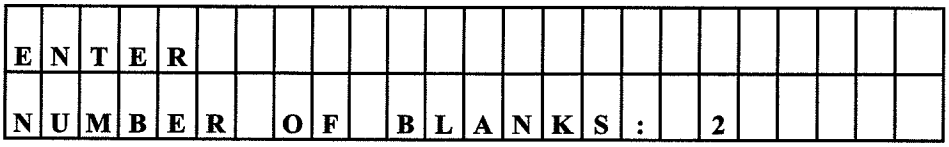


Constant Blank Value screen

- 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.
- Use the **CLEAR** key to clear the value on the display.
- Use the **ENTER** key to save the value and move to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** to move back through the menu structure.

Number of Blanks

The Number of Blanks field allows the user to enter the number of blanks in the Assay. This entry screen is only displayed when Full, Column or Row Blank maps are selected. Any previously defined value is displayed.



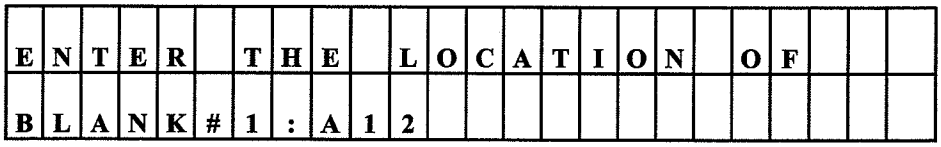
Number of Blanks entry screen

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

Blank Location

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



Defining the Location of Blanks

- Use the **NUMERIC & 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.
- Use the **ENTER** key to save the value, and move to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** to move back through the menu structure.

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Number of Standards

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

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

Number of Standards entry screen

- 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.
- Use the **ENTER** key to save the value, and move to the next screen.
- Use the **CLEAR** to clear the value on the display.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

Number of Standard Replicates

This option allows the user to enter the number of replicates per standard in the assay. Any pre-defined value appears on the display.

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

Number of Standard Replicates screen

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- 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.

- Use the **CLEAR** key to clear the value on the display.

- Use the **ENTER** key to save the value and move to the next screen.

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

Average Standards

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

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

Average Standards entry screen

The active keys are:

- Use **SOFT KEY 1** to select **YES** (average the replicates). The top line of the display updates to reflect the selection.

- Use **SOFT KEY 2** to select **NO** (do **not** average the replicates). The top line of the display updates to reflect the selection.

- Use the **ENTER** key to save the selection, and advance to the next screen.

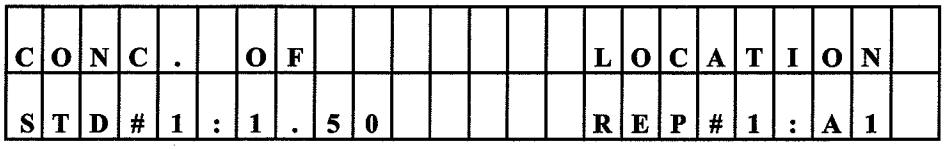
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

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Standard Concentration

The Standard Concentration field allows the user to enter a concentration value for each standard. Any previously defined value is displayed.

If automatic map generation is selected, the location field is only editable for the first replicate of the first standard.



Standard Concentration screen

- Use the **NUMERIC & ALPHA keys** and the **DECIMAL POINT** key to enter Standard Concentration values. The range is .00001 to 999999, but the total field cannot exceed 6 characters for any concentration. Valid well locations for the defined geometry are listed below.
- Use the **RIGHT ARROW** and **LEFT ARROW** keys to move to the next or previous editable field.
- Use the **CLEAR** key to clear the Standard Concentration value from the display.
- Use the **ENTER** key to save the value on the display and move to the next screen.
- Use the **MAIN MENU**, and **PREVIOUS SCREEN** keys are to move back through the menu structure.

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Valid Well Locations:

For all prompts of a well location, only the ALPHA keys are active for the first character and numeric for the second and third characters. Valid characters and numeric entries are based on the selected plate geometry. Listed below are definitions of acceptable entries for well locations based on plate geometry:

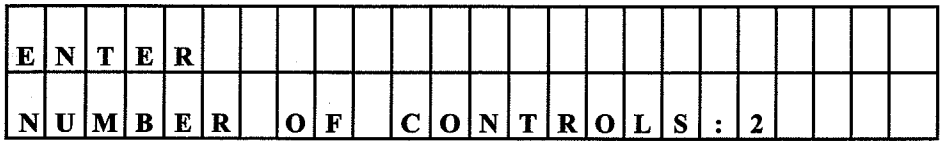
⇒ **24-well plate range:** A1-A6, B1-B6, C1-C6 and D1-D6

⇒ **48-well plate range:** A1-A8, B1-B8, C1-C8, D1-D8, E1-E8 and F1-F8

⇒ **96-well plate range:** A1-A12, B1-B12, C1-C12, D1-D12, E1-E12, F1-F12, G1-G12 and H1-H12.

Number of Controls

The Number of Control screen allows the user to enter the number of controls that will be used in the assay. Any previously defined value will appear on the display.



Number of Controls screen

- Use the **NUMERIC** keys to enter the Number of Controls. The range depends on the number of locations on the plate that are undefined. The maximum number of controls is 8.
- Use the **CLEAR** key to clear the value on the display.
- Use the **ENTER** key to save the value and advance to the next screen.
- Use the **MAIN MENU**, and **PREVIOUS SCREEN** keys to move back and through the menu structure.

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Type of Control

This option allows the user to enter the type of control used in the assay. Any previously defined Control Type will be displayed on the screen. The user can either select the Control Type displayed above each soft key, or define a new one.

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

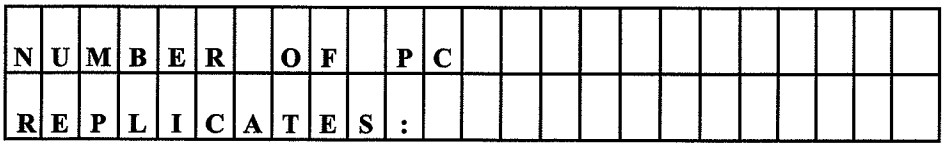
Types of Control screen

- Press the soft keys under the displayed Control Type to select the option (**P**ositive **C**ontrol, **N**egative **C**ontrol, **H**igh **P**ositive **C**ontrol, **L**ow **P**ositive **C**ontrol, CTL1, CTL2, CTL3, CTL4.
- Use the **CLEAR** key to clear the Control Type from the display.
- Use the **ENTER** key to save the displayed Control Type and advance to the next screen.
- Use the **MAIN MENU**, and **PREVIOUS SCREEN** keys to move back through the menu structure.

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Number of Control Replicates

The Number of Control Replicates screen allows the user to enter the number of replicates per control in the assay. Any previously defined number will be displayed.



Number of Control Replicates screen

- Use the **NUMERIC** keys to enter a value for Number of Control Replicates. The range is 1 to 12 replicates. The software performs a check to ensure the number of replicates, multiplied by the number of controls, does not exceed the number of undefined wells remaining on the plate.
- Use the **CLEAR** key to clear the displayed Number of Replicates value.
- Use the **ENTER** key to save the displayed value and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

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Location of Controls

This option allows the user to enter the location of the controls in the assay. If automatic map generation is selected, only the location field that is displayed is editable.

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	2	

Location of Controls screen

- Use the **CLEAR** key to clear the value on the display.
- Use the **NUMERIC & ALPHA** keys: Use these keys to enter characters and numeric values for valid well locations, depending on the plate geometry. Valid locations are as follows.

Valid Well Locations:

For all prompts of a well location, only the **ALPHA** keys are active for the first character and numeric for the second and third character. Valid characters and numeric entries are based on the selected plate geometry. The definitions below define acceptable entries for well locations based on plate geometry:

⇒ **24-well plate range:** A1-A6, B1-B6, C1-C6 and D1-D6

⇒ **48-well plate range:** A1-A8, B1-B8, C1-C8, D1-D8, E1-E8 and F1-F8

⇒ **96-well plate range:** A1-A12, B1-B12, C1-C12, D1-D12, E1-E12, F1-F12, G1-G12 and H1-H12.

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Number of Samples

This option allows the user to enter the number of samples that will be used in the assay. Any previously defined value appears on the display. If the number of samples is altered, the number of replicates for the sample defaults to a value of 1.

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

Number of Samples screen

- Use the **NUMERIC** keys to enter the Number of Samples in the Assay. The range is 0 to the number of undefined well locations remaining on the plate. 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.
- Use the **CLEAR** key to clear values on the display.
- Use the **ENTER** key to save the displayed value and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

Number of Sample Replicates

This option allows the user to enter the number of replicates per sample that will be run in the assay. Any previously defined value will be displayed.

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	:		2	

Number of Sample Replicates screen

- Use the **NUMERIC** keys to enter the Number of Sample Replicates. The range is 1 to 12 replicates. The software ensures that the number of replicates multiplied by the number of samples, multiplied again by the number of dilutions, does not exceed the number of undefined wells remaining on the plate.

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- Use the **CLEAR** key to clear the value on the display.
- Use the **ENTER** key to save the displayed value and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move back through the menu structure.

Sample Location

This screen allows the user to select the well location of the sample on the plate. Any previously defined Sample Location appears on the display.

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

Sample Location screen

Use the **NUMERIC, ALPHA** keys and **DECIMAL POINT** keys to enter the sample and its location on the plate. Valid characters and numeric entries are based on the selected plate geometry.

The definitions below define acceptable entries for well locations based on plate geometry:

⇒ **24-well plate range:** A1-A6, B1-B6, C1-C6 and D1-D6

⇒ **48-well plate range:** A1-A8, B1-B8, C1-C8, D1-D8, E1-E8 and F1-F8

⇒ **96-well plate range:** A1-A12, B1-B12, C1-C12, D1-D12, E1-E12, F1-F12, G1-G12 and H1-H12.

- Use the **CLEAR** key to clear the displayed value.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

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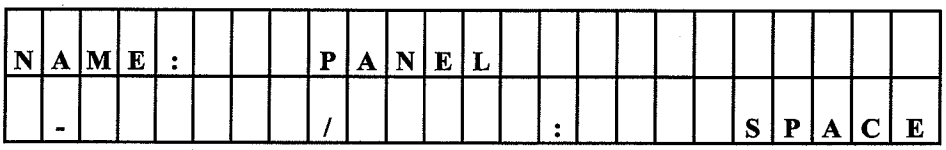
Panel

A Panel is a collection of assays to be run on one plate. The most common reason to use a panel test 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 instrument can then be programmed to run up to 8 assays within this panel. The assays must have been originally defined in any of the "full" assay positions 1-55 on the reader before they can be used in a panel

Other Important Notes:

- The Panel assays must be endpoint method only.
- Any curve fit, 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. If the user wishes to change any of the map or assay parameters, they must be changed in the "full" assay first.

To use the Panel option, press **DEFINE**, and choose Assay Number **99**. When defining a panel, the first screen will prompt for the name of the panel.



Panel Name Screen

- Use the **ALPHA** and **NUMERIC** keys to update the Assay name, if desired. The cursor is positioned at the first editable field.
- Use the **OPTION** 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 **CLEAR** key to clear the Assay name from the display.

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- Use the **LEFT & RIGHT ARROW** keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.

- Use **SOFT KEYS 1, 2, 3 & 4** when using alphanumeric characters on the display above the soft key in the assay name.

In addition:

- Use the **MAIN MENU** key to return to the Main Menu screen.

- Use the **PREVIOUS SCREEN** key to save the contents of the display and return to the previous screen.

- Use the **ENTER** key to save the contents of the display and advance to the next screen.

The next screen will prompt for the number of assays comprising this panel.

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

Number of Assay Screen

- Use the **MAIN MENU** key to return to the Main Menu screen saving only the panel name.

- Use the **ENTER** key to save the contents of the display and advance to the next screen.

- Use the **NUMERIC** keys to update the display and the cursor to advance to the next field. The limit is 8 assays.

The next screen, when defining a panel, prompts to select the mapping direction of the assays. The available options are **DOWN** or **ACROSS**.

NOTE: All original map directions for the assays are overwritten in the panel. If the assay includes replicates, they will follow the panel map direction.

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

Choosing the Mapping Direction

- Use the **MAIN MENU** key to return to the Main Menu screen saving only the panel name.
- Use the **ENTER** key to save the contents of the display and advance to the next screen.
- **SOFT KEY 1** maps the assays and replicates **DOWN**.
- **SOFT KEY 2** maps the assays and replicates **ACROSS**.

After selecting the mapping direction of the assays, it is necessary to choose which assays will be used in the panel. **It is important to note that these assays must have been previously defined to be used in the panel definition, and must all use the same wavelength.**

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

Selecting the Assay Number

- Use the **MAIN MENU** key to return to the Main Menu screen saving only the panel name.
- Use the **ENTER** key to save the contents of the display and advance to the next screen.
- The **OPTION** key will increment the assay number by one. The display will be updated as the keys are pressed.
- The **NUMERIC** keys are used to choose the assay numbers of the tests to be run. The range is 1 to 55.

Once the first assay has been chosen, the software will prompt for the location where the reader should start mapping the assay.

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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

Well location to begin Mapping

- Use the **MAIN MENU** key to return to the Main Menu screen saving only the panel name.
- Use the **ENTER** key to save the contents of the display and advance to the next screen.
- Use the **LEFT** and **RIGHT ARROW** keys to move the cursor in the editable field to the previous or next field. The cursor will wrap around the edit field.
- Use the **ALPHA & NUMERIC** keys to choose the well location to begin the assay. Wells A-1 through H-1 are valid for ACROSS; A-1 through H-12 are valid for DOWN.

This process will be repeated for each assay within the panel. The user must be aware of the total number of controls, standards and blanks that were originally mapped in the "full" assay as they map the panel assay. It is recommended that the user first print the Assay setups for reference before mapping them in the panel.

For example, if the user wishes to run Assays 1, 8 and 22 within a Panel:

- Assay 1 has a total of 12 wells defined for controls, blanks and standards, and in the panel , the mapping for Assay 1 begins in well A01, with the map direction DOWN. The user wants to run 6 samples in Assay 1. Assay 1 now fills wells A01 through B03.
- The mapping for Assay 8 in the panel can begin in well B04, or any next available well the user chooses. The reader will "beep" if the user tries to map into a well that has been previously used in the panel.
- The mapping for Assay 22 should begin at the next available well location after Assay 8 mapping is complete.

After all the assays have been entered into the panel, the user may print the Panel Assay Map. Choose **Report** (from the Main Menu), **Map, Assay 99**. The reader will print the map of each assay configured in the panel.

The Panel Assay results are sorted by Sample (unless a custom assay has been programmed by Bio-Tek).

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Spectral Scanning

If assay 00 is selected, the **Spectral Scan** assay name is displayed.

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			

Select Assay Number 00-Spectral Scan

Pressing **Enter** will bypass the **Assay Name** and **Define** option selections, and will display the **Select Wavelength Start** screen. This name is not modifiable.

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

Select Wavelength Start screen

Select the wavelength to start the spectral scan. The previously defined selection will be displayed. The following keys are active during this screen and function as follows:

- **MAIN MENU** key: Returns the user to the MAIN SCREEN, saving the selection made.
- **PREVIOUS SCREEN** key: Returns the user to the previous screen, saving the selection made.
- **ENTER** key: Advances the user to the next screen, saving the selection made.
- **NUMERIC** keys: Valid range is 200-999, depending on instrument configuration.
- **LEFT & RIGHT ARROW** keys: The left and right arrow keys move the cursor in the edit field to the previous or next field. The cursor will wrap around the edit field.
- **CLEAR** key: Clears the selection to the default value (200 or 340).

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The next screen that will be displayed asks the user to select the wavelength the scan should stop at. The valid range is 201 to 999, depending on the instrument configuration.

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 Wavelength Stop screen

The user will now be prompted to select the increment between successive wavelengths for the spectral scan. The valid range is 1-799 nm. For example, have the instrument scan from 340 nm to 800 nm in increments of 50 nm (340, 345, 350, 355, 360, etc.).

S	E	L	E	C	T		W	A	V	E	L	E	N	G	T	H				
S	T	E	P		:												5	0	N	M

Select Wavelength Step screen

After selecting the scanning increments, the user will be asked to choose the plate type.

P	L	A	T	E		T	Y	P	E								9	6	W	E	L	L	S
2	4		4	8		9	6												*	M	O	R	E

Select Plate Type screen

P	L	A	T	E		T	Y	P	E								9	6	W	E	L	L	S
3	8	4		7	2	T		9	6	T									*	M	O	R	E

*Select Plate Type * More screen*

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The final screen that will be displayed asks the user to select the well location of the scan. The valid range depends on the plate geometry.

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

Select Well Location for scan

Pressing **ENTER** will bring the user to a screen asking whether they would like to pre-calibrate the instrument before the scanning data is collected. This procedure will collect the new dark and air-blank values. The following screen will be displayed.

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

Pre-calibrate screen

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Formula Definition

Defining the Formula involves identifying the **formula type** and entering the actual **formulas**. Formula definition screens are displayed on the *µQuant* in the order detailed below. Formulas created using Bio-Tek's Extensions (Define Protocol software) cannot be edited by using the reader. Formulas are processed in the following order, with the number of permitted formulas of each type:

- Blank Validation 0-1
- Control Validation 0-4
- Assay Validation 0-4
- Transformations 0-1
- Cutoff Formulas 0-1
- General Formulas 0-4

NOTE: It is not possible to create formulas when using the 384-well format. In order to manipulate the raw data, you must use either the Capture Data for Excel® software that was supplied with the reader, or use computer control via KC*junior*™ or KC4™ PC software. (Refer to *Appendix D* for additional information on using the 384-well format.)

If a curve fit method is also specified, here's the order in which the processing occurs:

- Blank Validation
- Control Validation
- Assay Validation
- Transformations
- Curve-Fit Analysis
- Cutoff Formulas
- General Formulas

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

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Validation Formula Examples

- **Blank Validation:** An assay protocol states that the blank wells on a plate should have an OD of less than 0.050. The formula is entered on the reader as a Blank Validation Formula:

  BLK < 0.050.

- **Negative Control Validation:** An assay protocol states that the Negative Control wells must have an OD of less than 0.100. The formula is entered as a Control Validation Formula:

  NC < 0.100.

- **Positive Control Validation:** An assay protocol states that the Positive Control wells must have an OD higher than 1.000, but less than 2.500. Two Control Validation Formulas can be entered:

  PC > 1.000 and PC < 2.500.

- Or, one formula can be used if the formula is 24 characters or less:

  PC>1.000ANDPC<2.500.

- **Assay Validation**: An assay protocol states that in order for an assay to be valid, the mean of the Negative Control well ODs must be less than 0.100. The Assay Validation formula that should be entered:

  NC;x < 0.100 (the map identifier NC;x indicates the mean of the NC's)

Cutoff Formulas

If a user wishes to define a cutoff value that determines whether a well would be called POS or NEG or EQUIV, then the user would define a **Cutoff Formula**.

- For example, an assay states that the cutoff value is the mean of the Negative Controls plus 0.050 OD. Any wells higher than this result will report as POS. Any ODs less than the result will be called NEG. The Cutoff Formula to be entered is: NC;x + 0.050.

- Note that it is not necessary to use the > and < operators in a Cutoff Formula. A cutoff can be defined just as a map identifier also, for example: All wells whose OD is higher than the mean of STD2 on a plate will be called POS. This formula would be entered as STD2;x in the Cutoff formula definition screen.

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Transformations

Transformation formulas change the absorbance data of all wells defined in the **Map**, to another format, in preparation for further data reduction. (See page 3-44, Transformation Formula for more detail.)

From the assay **Define Menu**, press the arrow corresponding to **Formula.**

Formula Type

The Formula Type screen allows the user to choose the formula type (Cutoff, Transformation, Assay Validation or General formula) that will be entered for the Assay.

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

Formula Type selection screen

S	E	L	E	C	T		F	O	R	M	U	L	A		T	Y	P	E	?	
G	E	N	E	R	A	L		T	R	A	N	S	-	V	A	R			*	M	O	R	E
											↑									

Formula Type selection screen (continued)

- Press **SOFT KEY 1** to select Cutoff formula.
- Press **SOFT KEY 2** to select Transformation Formula.
- Press **SOFT KEY 3** to select Assay validation formula.
- Press **SOFT KEY 4** to select General Formula.
- Use **ENTER** to save the selected formula type and advance to the next screen.

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Validation Type Selection

This option allows the user to select which Validation Formula type (Control, Assay or Blank Validation formulas) to enter for the assay.

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

Validation Type selection screen

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

Formula Entry

This screen allows the user to enter the formula used in the assay.

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

Formula Entry screen

- Each formula can contain a maximum of 24 characters. Spaces are unnecessary.
- Use the **LEFT and RIGHT ARROW** keys to move the cursor to the previous or next editable field.
- Press **SOFT KEY 1** to place the next item on the **MATH** list at the cursor position. See the table that follows for the order of items on the **MATH** list:

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

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

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

- Press **SOFT KEY 3** to place the next defined item on the plate map list (i.e., STD, NC, PC, BLK) at the cursor position.

- Press **SOFT KEY 4** to place the next option on the **FUNCTION** list at the cursor position. See the table that follows for the order of items on the **FUNCTION** list:

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

- Functions are followed by wells, well types or numbers in parentheses.
- Use the **CLEAR** key to clear the item displayed at the cursor position.
- Use the **SHIFT** and **CLEAR** keys to clear the entire entry.
- Use the **ENTER** key to save the displayed value and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

Number of Required Controls

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

E	**N**	**T**	**E**	**R**		**N**	**U**	**M**	**B**	**E**	**R**										
R	**E**	**Q**	**U**	**I**	**R**	**E**	**D**		**P**	**C**	**'**	**S**	**?**								

Number of Required Controls screen

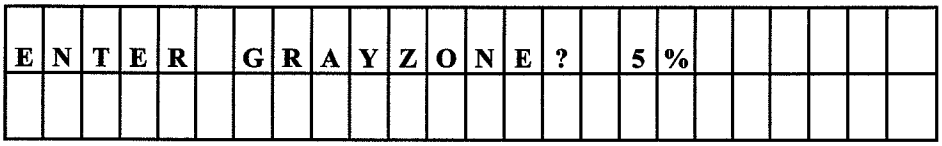
- Use the **NUMERIC** keys to enter the **Number of Required Controls**. The range is 1 through the number of defined replicates of a control.

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- Use the **CLEAR** key to clear the value on the display.
- Use the **ENTER** key to save the displayed value and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

Grayzone

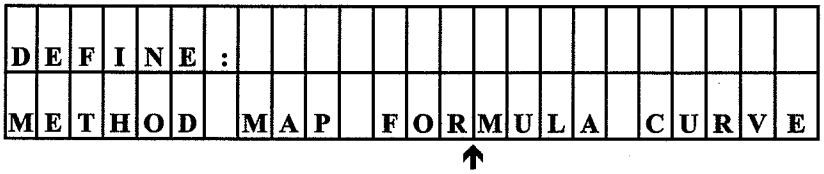
If a cutoff formula is defined, the grayzone screen allows the user to enter the grayzone percentage for the cutoff. The grayzone is the area around the cutoff, that is, samples that fall within an area defined by the cutoff (± 5%) would be considered equivocal or questionable. Any previously defined grayzone value is displayed.



Grayzone Percentage screen

- Use the **NUMERIC** keys to enter the grayzone value. The range is 1 to the number of defined replicates of a control.
- Use the **CLEAR** key to clear the displayed value.
- Use the **ENTER** key to save the displayed value and advance to the next screen.

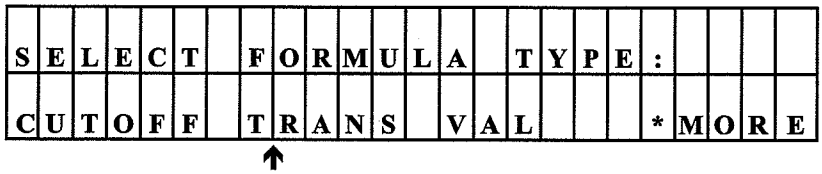
Transformation Formula



The Define screen

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

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Select Formula Type screen

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

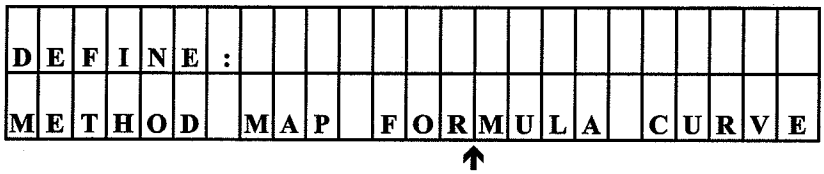
Enter the formula: **(OD/2)*100**

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

Transformation Scope Variable

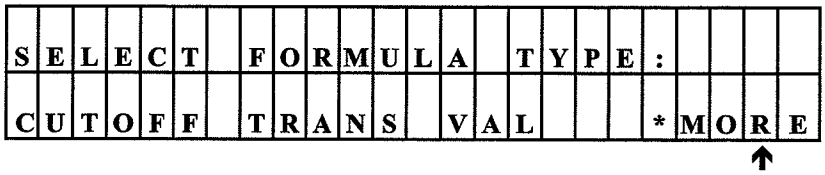
For more complicated transformations, a new Transformation Scope Variable (TVar) feature has been added. The TVar will allow the user to define a variable to be used in the transformation formula. This variable will define the scope of the transformation: whether to apply the transformation to just the samples (SMP) or to all wells defined on the plate (OD).

From the assay **Define** Menu, press the arrow corresponding to ***Formula***.



The Define menu screen

This will bring the user to a screen asking to **Select Formula Type**. At this screen, press ***MORE.***



The Select Formula Type screen

The options displayed now include Trans-Var. Select the arrow that corresponds to this.

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

The Select Formula Type screen

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

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

The Scope Variable screen

If **SMP** is chosen, the transformation formula defined will apply *only* to the samples defined in the plate map. If **OD** is chosen, the formula definition screen will appear. Use the formula keys (Math, Other, Map and Function) to define the ***transformation variable*** *(TVar)*. Once the variable has been defined, it can be used in a transformation formula. The TVar will be available as a MAP option when writing the transformation formula.

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

The assay data reduction states:

- Subtract the mean of the NC minus the mean of the CTL1, from all ODs on the plate.
- Divide by the mean of the NC minus the mean of the CTL1 then multiply by 100.

On paper, the formula reads:

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

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

At the SCOPE VARIABLE selection, choose **OD** and press **Enter**. Now enter the formula **(NC;x-CTL1;x)** by using the **Math, Other, Map** and **Function keys**. Press **Enter**.

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

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Enter the following formula: **(OD-(TVar))/(TVar)*100,** using the Math, Other, Map and Function keys. (TVar is included in Map options on the formula entry screen.) The transformation formula has been added to the assay definition.

Another Transformation Example:

In the case of competitive reactions, converting absorbance data to percent B/B_0 can be: (OD/Std1)*100. This would divide all the wells by Std1, presumably the 0 standard, and multiply by 100.

Go to **TVAR**, choose **OD**. Select **STD1** from **MAP**. At transformation formula screen, enter: **(OD/TVAR)*100**.

General Formula:

A general formula is any desired data reduction that may accompany other reports.

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Defining the Curve Fit

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

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

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

Curve Fit

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

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

- **Linear curve fit:** A simple best fit straight line is plotted using the values of the standards.
- **Quadratic or "Quad" curve fit:** A curve fit which uses the Quadratic equation "$ax^2 + bx + c = y$" to plot the standards 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 which uses the equation "$ax^3 + bx^2 + cx + d = y$" to plot the standards values. This type of curve fit is affected even less than the quadratic fit when any particular standard has a poor value.
- **2-P (LOGIT/LOG):** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The logistic equation is algebraically transformed to a simpler form in which experimentally determined values are used for the responses at concentrations of zero and infinity.

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

- **4-Parameter Logistic or "4-P":** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The 4 parameters are: Left asymptote, Right asymptote, Slope and Value at the Inflection point. This fit is most recommended for immunoassay data, and is more exact than Logit/Log.

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

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

C	U	R	V	E	-	F	I	T		T	Y	P	E	:		C	-	S	P	L	I	N	E
	C	U	B	I	C			4	-	P			L	O	G	I	T		*	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

Curve-Fit screens

- Press **SOFT KEYS 1, 2, 3** or **4** to select the curve-fit type that is displayed above the soft key. Select the soft key below the menu option **MORE** to display additional options. The top line of the display updates to reflect this selection.

- Press **ENTER** to save the selection and advance to the next screen

- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

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Edit Standard Outliers

This screen allows the user to select which method (**None** or **Manual**) will be used to edit Standard Outlier values. Any previously defined edit method is displayed.

E	D	I	T		S	T	D		O	U	T	L	I	E	R	S	?	M	A	N	U	A	L
	N	O	N	E		M	A	N	U	A	L												

Standard Outliers screen

- Press **SOFT KEY 1** or **2** to select the edit option displayed above the soft key. The display updates to reflect your selection.
- Use the **ENTER** key to save the selection and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

Axis Selection

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

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

Axis Selection screen

- Press **SOFT KEY 1, 2, 3** or **4** to select the axis identification option displayed above the soft key. The top line of the display updates to reflect the selection.
- Press **ENTER** to save the selection and advance to the next screen.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

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Extrapolation of Unknowns

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

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

Extrapolation of Unknowns screen

- Press **SOFT KEY 1** to select **YES** (extrapolate the unknowns). The top line of the display updates to reflect this selection.
- Press **SOFT KEY 2** to select NO. The top line of the display updates to reflect this selection.
- Use the **MAIN MENU** and **PREVIOUS SCREEN** keys to move backwards through the menu structure.

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Reading a Microplate

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

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

Select Assay

At the Select Assay Number screens,

- Use the **NUMERIC** keys to enter the number of any predefined Assay Definition Files stored in the reader's memory, or the **OPTION** key to advance one assay at a time. The cursor is positioned at the first editable field, and advances automatically. The numeric range depends on the number of assays (1-70) programmed in the reader's memory.

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

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

Assay Selection Screen

- Press **ENTER** to advance to the **EDIT ASSAY NAME** screen.
- Labeled keys may be used to move around the reader's menu structure; specify the assay number or clear the display.
- **CLEAR**: Clears the reader's LCD display.
- **MAIN MENU**: Returns the display to the Main Menu screen.
- **PREVIOUS SCREEN**: Returns the display to the previous screen.
- **ENTER** key: Advances the display to the next screen.

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Run-Time Prompts

After the assay is selected, the user may be prompted for information, depending on preferences selected in the reader's Utilities, whether a manual map was set up, or if the assay was created in, and downloaded from Bio-Tek's Extensions™ Define Reader Protocol software.

If the assay 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.

Refer to the Extensions™ Reader Protocol Manual for more information on user prompts that might be encountered.

Utility prompts might include:

- Enter number of samples
- Plate ID
- Enter Sample ID

If a **Manual Map** is used, prompts for information might include:

- Well locations for each sample

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Enter Number of Samples

The user can enter from 00 to the maximum number of samples permitted by the previously created well map. This value controls the number of samples reported if Matrix or Column reports are requested.

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

Enter Samples screen

Enter Plate ID

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

NOTE: Use caution when creating multiple Plate IDs. The reader does not warn the user who is about to exceed the maximum of 10 plate IDs stored in memory. If an 11th Plate ID is added, it will overwrite the first Plate ID stored in memory.

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

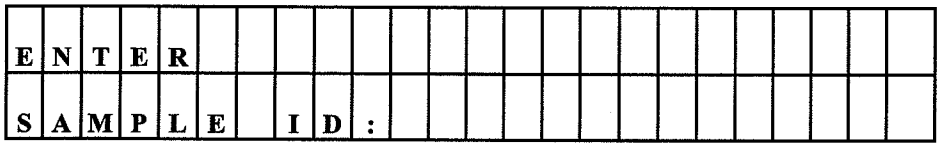
Entering a Plate ID

- Use the **KEYPAD** to enter numbers, and the **Shift-Letter** sequence, or **OPTION** key to enter letters.
- Use the **CLEAR** key to clear the display.
- Use the **LEFT & RIGHT ARROW** keys to move the cursor to the previous or next editable field.
- Use **SOFT KEYS 1, 2, 3 & 4** when using alphanumeric characters on the display in the field above the soft key.

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Enter Sample ID

The user can enter a starting sample identification from 00001 to 99999. The software will automatically increment each subsequent sample identification by 1. The sample IDs will be assigned by following the mapping order previously defined.

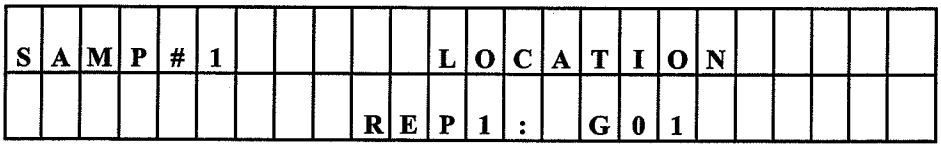


Enter Sample ID screen

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

Prompts for Well Location

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



Enter Well Location screen

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

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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				

Plate and Read prompts

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

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Printing Reports and Assay Lists

Reports are automatically generated after a plate has been read if **PRINT** (or BOTH) has been selected as an output option in the **UTILITIES** menu.

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

The Main Menu with Utilities option

- For additional reports, press the third soft key, **REPORTS.**

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	

Print Report Menu

- Select the **RESULT** option to obtain an exact copy of results from the plate reading. The form in which the results are presented is determined by the report settings (Matrix, Column, Curve-Fit) specified in the **UTILITIES** menu option.
- Select **MAP** to provide a location matrix of the Blanks, Standards, Controls and Samples for a selected assay.
- Select **ASSAY** to provide a map and a listing of all of the assay's settings, such as wavelengths, numbers of well types, all validation, cutoff, transformation, and general formulas and curve-fit parameters.
- Select **LIST** to provide a list of all assays in the *µQuant.*

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Result

R	E	P	O	R	T	:	H	B	S	-	A	G								
I	D	:		0	0	1					0	7	/	1	7	/	9	5		

Selecting RESULT

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

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

Edit Standard Outliers screen

- If **YES** is selected for this screen, the replicates for each standard can be edited. If **NO** is selected, the software will ask if reports should be printed.

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

Edit Replicate screen

- If **YES** was previously selected, the software will ask if each replicate should be edited. Select **YES** to remove a replicate.

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

Print Results screen

- Press **YES** to compile reports, or **NO** to return to the Main menu.

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Map

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

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

Select Assay Number screen

- Use the keyboard to type the assay number, or the **OPTION** key to cycle through the list of available assays. Press **ENTER** to enter the assay and begin printing the map of programmed well locations in the selected geometry.

Assay

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

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

Select Assay screen

- Use the keyboard to type the assay number, or the **OPTION** key to cycle through a list of available assays. Press **ENTER** to enter the assay and begin printing the map and other assay parameters.

List

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

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Chapter 4
Performance Verification/ IQ-OQ-PQ Tests

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

Recommendations for Achieving Optimum Performance

- Microplates should be perfectly clean and free of dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.
- Although the *µQuant* supports all flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with optically clear, flat-bottomed wells.
- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µl per well in a 96-well plate and 25 µl in a 384-well plate.
- Dispensing solution into 384-well plates often traps air bubbles in the wells. Dual wavelength reads will cancel most of these errors; however, for best results, they should be removed by degassing the plate in a vacuum chamber prior to reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Agitate the microplate before reading to help bring this problem within acceptable limits. Use Tween® 20, if possible (or some other wetting agent) to normalize the meniscus. 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.

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Recommended Test Schedule

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

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

Table 4-1
Recommended Test Schedule

	Performance Qualification / *Monthly*	Performance Qualification / *Semi-Annually*	Operational Qualification / *Initially & Annually*
System Self-Test, p. 4-4	✓		✓
Universal Plate Test, p. 4-7	✓		✓
Liquid Test 1, p. 4-17		✓	✓
Liquid Test 2, p. 4-19		✓*	✓*
Liquid Test 3, p. 4-22 Optional for 340 nm			✓

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

Qualification Procedures

Tests outlined in this section may be utilized to confirm initial and ongoing performance of the *µQuant*. Set up the device per *Chapter 2* and confirm that it powers up and communicates with peripherals for the Installation Qualification. After Installation Qualification, conduct the Operational Qualification Tests.

Your *µQuant* reader was fully tested at Bio-Tek prior to shipment and should operate properly upon initial setup. If it is suspected that problems may have occurred during shipment, if the device was reshipped by you, or if regulatory requirements dictate that Performance Qualification Testing is necessary, the following tests should be performed. After the initial confirmation of operation, the Universal Plate Test is performed monthly and Liquid Testing should be performed semi-annually.

- **System Self-Test:** Verifies proper gains, bulb operation and low electronic noise.
- **Universal Plate Test:** Confirms the optical accuracy, linearity, alignment, and wavelength accuracy of the instrument.
- **Liquid Testing:** Quantifies the instrument using liquids, which verifies operation in a way that glass test filters cannot.

iii	**IMPORTANT:** All verification procedures should be run with *µQuant* set to High Precision read mode (page 2-19).

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Accessing Tests and Calibration Using the Utility Option

From the Main Menu screen, press the soft key to select **UTIL**.

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

Select UTIL from the Main Menu

Test Options

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

Selecting Tests from the Utility Options screen

- Press **SOFT KEY 1** to select TESTS.

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

The Utility Tests screen

The active keys are:

- Press **SOFT KEY 1** to run the **SYSTEM TEST**.
- Press **SOFT KEY 2** to select **CHECKSUM**.
- Press **either SOFT KEY 3 and 4** to select **UNIVERSAL PLATE TEST**.

Note: Before running the Universal Plate test, ensure that the Calibration Plate values are entered. See the Calibration Plate section in this chapter for more information.

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Routine Procedure

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

- Select Reader System Test to verify that the light levels and electronic noise at all set wavelengths fall within factory acceptance criteria.
- Select Universal Test Plate to run the calibration plate test to confirm the alignment, repeatability, and accuracy/linearity of the reader.
- Perform a wavelength scan to confirm wavelength accuracy of the reader.

"System" (System Self Test)

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

The reader automatically runs an internal System Test each time it is powered on. An error will be displayed if the power-on System Test fails. No printout will occur at the power-on System Test. To obtain a printout of the System Test values for either periodic testing and documentation or troubleshooting, press **SOFT KEY 1** from the Test's submenu (UTIL, TEST, SYSTEM).

When **SOFT KEY 1** is pressed, the *µQuant* runs the instrument's System Test, and reports results in a pass/fail format (page 4-6) via the printer attached to the reader.

Photodiodes

The Optics portion of the System Self-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.

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"CHKSUM" (Checksum Test)

This test also runs automatically when the reader is powered on. The test compares the software to the internally recorded checksum values to ensure that the programming has not been corrupted. If there are any errors during the power-on checksum test, they will be displayed. To verify the checksum, revisions, and version of software currently loaded onto your reader, press **SOFT KEY 2** from the Tests submenu.

When the CHKSUM Test is selected, the software versions and the software's checksum appear on the display, as shown in the example below:

Software P/N Software Version

Code Checksum: (1ED3)

The second screen shows:

Configuration P/N Configuration Version

(Blank)

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Operator ID: 9219

Notes: 15798

11:38AM 02/04/02 SYSTEM SELF TEST

Lambda: 200	Gain:	1.32	Resets: 1
Channel:	Ref	1	
Air:	9367	39471	
Dark:	4968	4962	
Delta:	4399	34509	
Lambda: 352	Gain:	1.29	Resets: 2
Channel:	Ref	1	
Air:	8576	39469	
Dark:	4968	4963	
Delta:	3608	34506	
Lambda: 620	Gain:	2.12	Resets: 1
Channel:	Ref	1	
Air:	8314	39890	
Dark:	4966	4953	
Delta:	3348	34937	
Lambda: 790	Gain:	1.94	Resets: 1
Channel:	Ref	1	
Air:	8172	39902	
Dark:	4966	4957	
Delta:	3206	34945	
Lambda: 860	Gain:	2.72	Resets: 1
Channel:	Ref	1	
Air:	8105	39684	
Dark:	4963	4948	
Delta:	3142	34736	
Lambda: 962	Gain:	3.24	Resets: 1
Channel:	Ref	1	
Air:	8039	39804	
Dark:	4961	4942	
Delta:	3078	34862	
Channel:	Ref	1	
Noise Max:	4925	4899	
Noise Min:	4925	4899	
Delta:	0	0	

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

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```
AUTOCAL ANALYSIS


Upper Left Corner:  x=9104  y=10772
Lower Left Corner:  x=9096  y=16284
Lower Right Corner: x=416  y=16284
Upper Right Corner: x=424  y=10764
Delta 1 : 9104-9096=8
Delta 2 : 424-416=8
Delta 3 : 10764-10772=-8
Delta 4 : 16284-16284=0


SYSTEM TEST PASS
```

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

Calibration Verification

It is considered good laboratory practice to periodically verify the calibration of the *µQuant*. Verification should be performed per the schedule shown in *Table 4-1*, using the tests in this section:

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

Universal Plate Test

The Universal Plate Test (also referred to as the Calibration Plate Test) confirms the alignment, repeatability, accuracy/linearity, and wavelength accuracy of the *µQuant*. An alternative method that may be used to determine accuracy, repeatability, and linearity is Liquid Test 2, described on page 4-19.

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

The Universal Plate Test confirms the following:

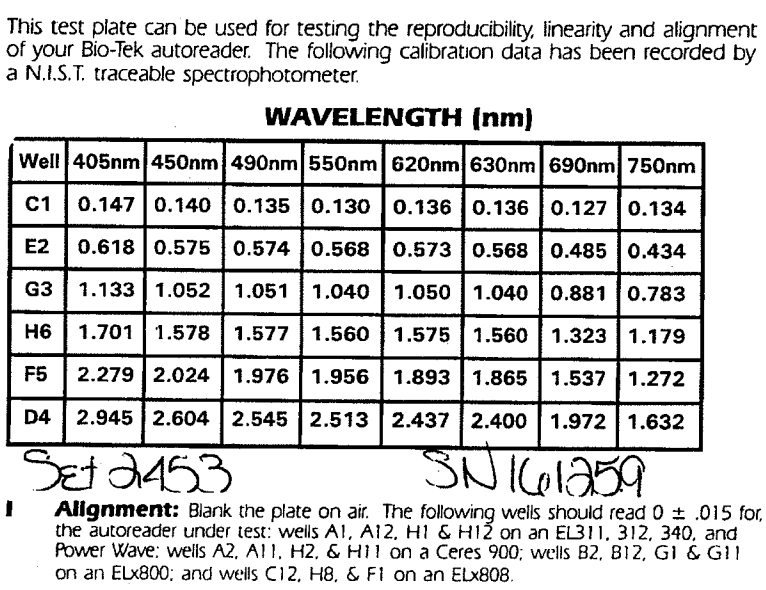
- **Accuracy.** Accuracy of the Optical Density readings – the comparison of the optical density readings with those given with the Universal Test Plate insert will confirm the accuracy of the optical density readings at specific wavelengths.
- **Linearity.** Linearity of the Optical Density readings is confirmed by default if the optical density readings are accurate.

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- **Alignment.** Alignment of the plate carrier and standard microplates is confirmed by the four-corner positional accuracy check.
- **Wavelength accuracy.** Wavelength accuracy is confirmed by using Bio-Tek's Universal Test Plate, Part Number 7260522. The Universal Test Plate provides a multiband test filter (Didymium glass V10), in location C6.

Requirements

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



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

WAVELENGTH (nm)

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

Set 2453 SN 161259

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

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

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Entering the Universal Plate Specifications

Using the Data Sheet provided with the Universal Plate, enter the calibration values. To enter the values into the reader:

From the Main Menu:

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

The Main Menu screen

- Select **UTIL.**

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

The Utility Options screen

- Select **SETUP.**

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

The Edit Setup screen

- Select **MORE.**

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

The Edit Setup Info screen

- At the second EDIT **SETUP** menu, press soft keys 2 or 3 to access the **CALIBRATION FILTER** menu.

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

Choosing the Calibration Wavelength

- Using the insert packaged with the calibration plate, press the soft key beneath the desired filter wavelength to select it.
- Then press the **ENTER** key to advance to the menu that allows entry of Well Location and calibration values.

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

Entering Calibration Values using the CalPlate Insert

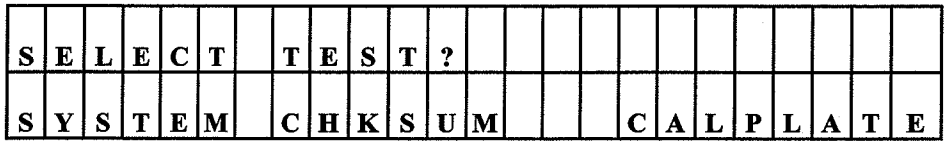
- After each entry, press **ENTER** to advance to the next consecutive well location. Enter the values listed on the Calibration insert packaged with the plate. The cursor will be positioned under the first editable field.
- When all values have been entered, press the **Main Menu** key.

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Running the Universal Plate Test

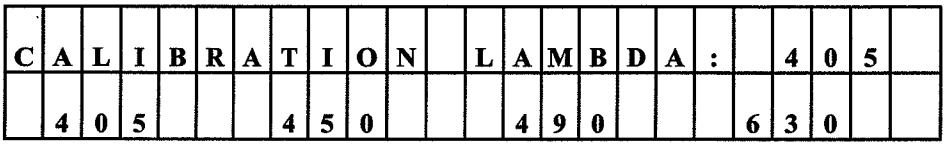
From the reader's **Main Menu** display:

- Press UTIL.
- Press TESTS.
- Select CALPLATE.



Selecting the Calibration Plate Test

- Select the appropriate wavelength at the **CALIBRATION LAMBDA** screen by pressing the soft key beneath the desired value. Press **ENTER** to save the value and advance to the next screen.



The Calibration Lambda Selection screen

- When prompted, insert the calibration plate into the *µQuant*'s plate carrier, and press the **READ** key to begin the calibration program.

The Calibration Plate Analysis Report (*page 4-12*) will be sent to a printer when the test is run.

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Calibration Plate Analysis

CALIBRATION PLATE ANALYSIS

12:28PM 02/04/02 Read Mode:Normal Lambda: 405

Operator ID: 929

Notes: 151998

Alignment Results

B02=-0.000 PASS	B12=-0.000 PASS	G01=-0.000 PASS	G11=-0.000 PASS

Accuracy Results

	C01	D04	E02	F05	G03	H06
STANDARD	0.156	2.841	0.609	2.437	1.170	1.746
DATA	0.157	2.838	0.607	2.412	1.165	1.737
RESULT	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

	C01	D04	E02	F05	G03	H06
READ 1	0.157	2.838	0.607	2.412	1.165	1.737
READ 2	0.157	2.842	0.607	2.413	1.165	1.737
RESULT	PASS	PASS	PASS	PASS	PASS	PASS

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

The Calibration Plate Analysis Report contains results for the following:

- **Alignment:** This portion of the test measures the alignment of the microplate carrier with the optical path. A reading > 0.015 represents an out-of-alignment condition. Wells B02, B12, G01, and G11 are the only valid alignment holes for the reader on the Part Number 7260522 calibration test plate.
- **Accuracy/Linearity:** Accuracy is a measure of the absorbance (optical density) of Calibration Test Plate wells C01, D04, E02, F05, G03, and H06 with known standard values contained in the Specification Sheet that accompanies each Calibration Test Plate. If the accuracy specifications are met, then the reader also proves to be linear.
- **Repeatability:** Repeatability is a measure of the instrument's ability to read the same well with minimum variation between two reads with the well in the same location.

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Wavelength Accuracy

The C6 filter should be scanned between 580 and 590 nm in 1-nm increments using KC4 or KC*junior*™ software. If neither KC4 nor KC*junior* is available, use the on-board software to select six wavelengths at 1-nm increments near the expected peak. The wavelength of the maximum absorbance should be compared with the wavelength written on the sheet supplied with the test plate (see *Figure 4-4*). The accuracy of the wavelength should be ± 3 nm.

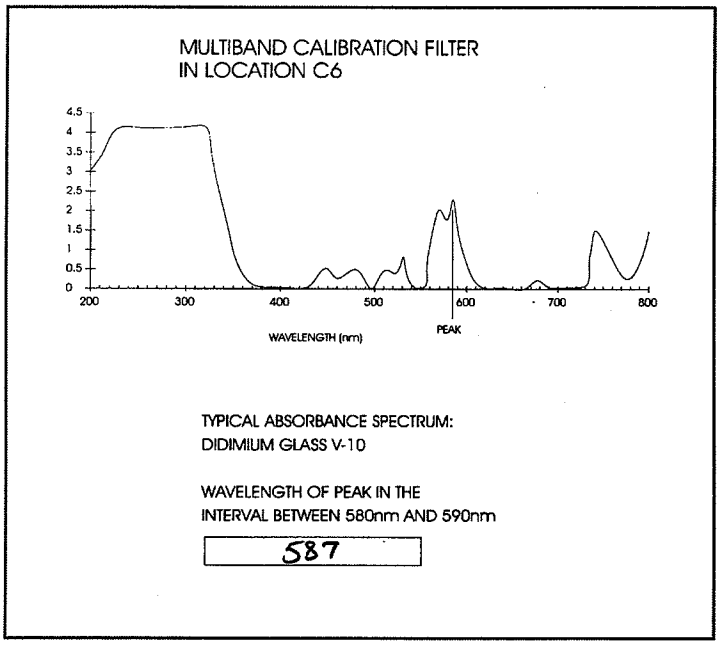


Figure 4-4: *Sample data sheet showing wavelength of peak in the interval between 580 and 590 nm*

iii **IMPORTANT:** The Universal Test Plate and software utility in the reader are used only to verify the reader's performance. The Test Plate and utility DO NOT correct or calibrate the instrument.

Test Failures

If any of the test parameters report as "FAIL," confirm that the standard values on the test plate certificate match the values on the printout. If not, correct and retest. If the test still fails, contact Bio-Tek'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.

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Liquid Testing

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

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

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

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

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Stock Solution Formulation

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

Procedure A

Required Materials:

- Deionized water
- FD&C Yellow No. 5 dye powder (typically 90% pure)
- Tween® 20 (Polyoxyethylene sorbitan monolaurate, a wetting agent)
- Analytical balance
- 1-liter volumetric flask
- 5-ml Class A volumetric pipette

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

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

Preparation of Stock Solution:

1. Weigh out 0.092 gram of FD&C No. 5 yellow dye powder into a weigh boat.
2. Rinse the contents into a 1-liter volumetric flask.
3. Add 0.5 ml of Tween 20.
4. Make up to 1 liter with DI water; cap and shake well.
   - ➢ This should create a solution with an absorbance of about 2.000 when using 200 µl in a flat-bottom microwell. The OD value result will be proportional to the volume in the well and the amount of FD&C No. 5 dye used. You can use a larger or smaller well volume, or add more dye or water to adjust the solution. Note that too small a well volume may result in increased pipetting-related errors.

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Procedure B

Required Materials:

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

Preparation of Stock Solution:

1. Pipette a 5-ml aliquot of Bio-Tek QC Check Solution No. 1 into a 100-ml volumetric flask.
2. Make up to 100 ml with DI water; cap and shake well.
   - ➢ This should create a solution with an absorbance of about 2.000 when using 200 µl in a flat-bottom microwell. The OD value result will be proportional to the volume in the well and the amount of QC Check Solution No. 1 used. You can use a larger or smaller well volume, or add more Check Solution or water to adjust the stock solution. Note that too small a well volume may result in increased pipetting-related errors.

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Liquid Test 1

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

1. Using a freshly prepared stock solution (see Procedure A on page 4-15 or Procedure B on page 4-16), prepare a 1:2 dilution using deionized water (one part stock, one part deionized water; the resulting solution is a 1:2 dilution).
   - The concentrated stock solution will have an optical density of approximately 2.000 Abs. This value is not critical, but it should be at the higher end of this absorbance range. Should it exceed the range, simply reduce the volume in the microwell. The diluted solution should have ODs of half of the concentrated solution.
2. Pipette 200 µl of the concentrated solution into the first column of wells of a new 96-well, flat-bottom microplate (Costar® #3590 is recommended). A new microplate is required for this test, as any scratches may cause variations.
3. Pipette 200 µl of the diluted solution into the second column of wells.
4. Read the microplate five times at 405 nm using High Precision read mode (page 2-19), single wavelength, no blanking.
5. The plate data can be printed or exported to an Excel spreadsheet using KC4 or KC*junior*. The mathematical computations described below may then be performed and the template kept for future data reduction.

Repeatability:

6. 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.
7. For each mean below 2.000 Abs, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 1.0% ± 0.005 Abs. If above 2.000 Abs, apply the ± 3% ± 0.005 specification.
8. The standard deviation for each set of readings should be less than the allowed deviation.

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For example:

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

Repeatability Specification:

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

± 1% ± 0.005 Abs from 0.000 to 2.000 Abs

± 3% ± 0.005 Abs from 2.000 to 3.000 Abs

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Liquid Test 2

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

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

Preparation of Dilutions:

1. Set up a rack containing 10 tubes, numbered consecutively.
2. Prepare a concentrated stock test solution insert using either Procedure A on page 4-15, or Procedure B on page 4-16.
3. Create a percentage dilution series, beginning with 100% of the original concentrated stock solution in the first tube, 90% of the original solution in the second tube, 80% in the third tube, and all the way to 10% in the last tube. Use Class A volumetric pipettes for better accuracy.
4. Dilute using amounts of the remaining 0.05% solution of deionized water and Tween 20, as shown in *Table 4-4*.

Table 4-4
Test Tube Dilutions

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

Plate Preparation:

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

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

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Linearity – Test A

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

   For example:

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

   Expected results:

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

Repeatability – Test B

1. Calculate the mean and standard deviation for the five readings taken above at each concentration. Only one 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 Abs, calculate the allowed deviation using the repeatability specification for a 96-well format of ±1.0% ± 0.005 Abs. If above 2.000 Abs, apply the ±3% ± 0.005 specification.
3. The standard deviation for each set of readings should be less than the allowed deviation.

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For example:

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

Repeatability Specification:

±1% ± 0.005 Abs from 0.000 to 2.000 Abs

±3% ± 0.005 Abs from 2.000 to 3.000 Abs

Alignment – Test C

1. Using the plate prepared for Test A above, conduct a turnaround test by reading the plate with the A1 well in the H12 position five times. This test results in values for the four corner wells that can be used to determine alignment.
2. Calculate the means of the wells A1 and H1 in the normal plate position (data is from Test A) and in the turnaround position (from Step 1 above). Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the accuracy specification for the instrument.

   For example:

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

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Liquid Test 3

To verify operation of the *µQuant* at 340 nm, perform the Liquid Test 3 Procedure described on page 4-23.

Required Materials:

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

Stock Buffer Solution Formulation:

Table 4-5
Phosphate Buffered Saline 10X Concentrate Solution

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

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Procedure A:

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

Procedure B:

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

Liquid Test 3 Procedure:

1. Check the absorbance of a sample of either high-level test solution created in Procedure A or B at 340 nm on the microplate reader. This solution will have an optical density (absorbance) of approximately 0.7 to 1.0. This value is not critical, but it should be within this absorbance range. Adjust up by adding β-NADH powder, if low, until the high-level test solution is at least at the lower end of this range. Do not adjust if slightly high.

2. Carefully prepare a mid-level test solution by diluting 15 ml of the high-level test solution with 5 ml of the Sigma PBS solution. (If using the 10X-concentrate PBS solution, you should mix one part of the concentrate with nine parts of deionized water to obtain a low-level buffer similar to the Sigma PBS. Then, use 5 ml of this solution as the diluent.) This will be the mid-level solution.

3. Carefully prepare a low-level test solution by diluting 10 ml of the high-level test solution with 10 ml of the Sigma PBS solution. (If using the 10X-concentrate PBS solution, you should mix one part of the concentrate with nine parts of deionized water to obtain a low-level buffer similar to the Sigma PBS. Then use 10 ml of this solution as the diluent.) This will be the low-level solution.

4. Pipette 150 µl of the concentrated solution into each well of the first two columns, A1 to H1 and A2 to H2, of a flat-bottom microplate (Costar® #3590 is recommended). Next, pipette 150 µl from the mid-level solution into the wells of columns 3 and 4 of the microplate. Finally, pipette 150 µl of the low-level solution into the wells of column 5 and 6 of the microplate.

5. Read the microplate using High Precision read mode, single wavelength at 340 nm, no blanking (or blank on air). Repeat the read four times for a total of five reads.

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Repeatability – Test A

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

   For example:

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

Linearity – Test B

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

   For example:

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

   Note: The expected values column should be started with a value very near the observed value for the high-level solution as it is difficult to accurately make up the dye solution. The next two values are 75% and 50% of the high-level solution's assigned expected value respectively.

   #### Expected results:

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

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Appendix A
Decontamination

This appendix contains the procedure for decontaminating the μQuant Scanning Spectrophotometer.

Decontamination Procedure

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

Purpose

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

General Considerations

Any laboratory instrument that has been used for clinical analysis is considered a biohazard and should be decontaminated prior to handling. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not be always be visible. Prophylactic gloves must be worn when handling instruments that have not been decontaminated. Gloved hands should be considered contaminated at all times and must be kept away from eyes, mouth and nose at all times.

Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosols.

Eating and drinking while decontaminating instruments is not advisable.

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Procedure

- Prepare an an aqueous solution of 0.5% Sodium Hypochlorite (bleach). Be sure to check the % NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, use a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case use a 1:10 dilution. This is a caustic solution. It is important to wear gloves and eye protection when handling it.
- Wipe down the carrier and all exposed surfaces of the unit with the bleach solution. Remove the top shroud of the instrument and wipe down the top surface of the instrument base, as well as the inside of the top shroud.
- Reassemble the unit and discard the used gloves and towels.

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Appendix B
Computer Control

The µQuant can be controlled either from the reader's front panel or from a computer connected to the reader via the computer's serial port. This appendix describes the features of computer control, and explains the information necessary to program the computer to control the reader.

Overview

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

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

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

Controlling the Reader with KC*junior*™ *for Windows*®

The *µQuant* can be operated using a computer running KC*junior* software. Follow the steps below:

- Power off the computer and the reader. Connect the appropriate serial cable between the two machines. A Null is required to connect the reader to most computers. If a 25-pin (female) to 9-pin connector is used, the Null is built into the cable. Bio-Tek's serial cable (PN 75053) may be used with no additional Null.
- Power up both machines.
- Install KC*junior* on the computer's hard drive.

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- Once installed, start KC*junior*. Select **Setup**, then **Reader**. To select the *µQuant* reader and define the communications parameters. Choose the following setup variables:

  ⇒ Instrument type : MicroQuant

  ⇒ Serial Port: COM1 or COM2 (select the COM (serial) port used for the RS-232 cable connection.

  ⇒ Baud Rate: 9600

  ⇒ Parity: None

  ⇒ Data Bits: 8

  ⇒ Stop Bits: 2

- Choose the **Test Communications** button to attempt to establish communications with the reader, using the currently defined communication parameters.

- Click **OK.**

Problems

If *KCjunior* fails to run and displays a serial communications ERROR, check the cable plug-in location to ensure it matches the setup choices and is not a NULL cable. If this is suspected, add another NULL and try again. Also, ensure that the *µQuant* is set up for the same baud rate as *KCjunior*.

Controlling the Reader Using Serial Protocol

At baud rates of 1200, 2400, and 9600, the *µQuant* is capable of sending and receiving data through its serial port (RS-232C). The baud rate used for transmission is held in nonvolatile memory and can be changed by the user. Other serial port parameters, No Parity, 8 Data Bits, and 2 Stop Bits are fixed and cannot be changed.

The RS-232C serial port on the *µQuant* is configured as a DTE (see the section Setting Up the Serial Port for Communications in *Chapter 2*, page 2-9); that is, the unit is wired to resemble a modem. Data is received on Pin 3 (the RX Pin), and transmitted on Pin 2 (the TX pin).

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Computer Control Command Set

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

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

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

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

Some commands described here may not be available with older reader basecode versions (prior to v2.00), indicated if the Check Feature command returns a <NAK>. These commands are marked with a '#'.

The following section describes the supported computer control command set.

Table B-1

ASCII Control Characters Used in Computer Control Protocol

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

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STORE PLATE CARRIER ('A')

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

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

RUN AREA SCAN ('c')

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

host:	valid limits:	response:
'c'		<ACK/NAK>
data bytes:		
1 - 5	Y start point (0.01 millimeters, "00914" – "07666")	
6 – 10	X start point ("01201" – "11549")	
11 – 15	X finish point ("01201" – "11549")	
16 – 18	number of points to read ("001" – "255")	
19	position in scan series	
	'0': single scan	
	'1': first scan in series	
	'2': next scan in series	
	'3': final scan in series	
<ETX>		<ACK> if data valid
reader response protocol:		
initial status string (5)		
for each wavelength:		
data start code (1)		<CR>
for each point indicated:		
comma separator (1)		','
sign (1)		'+' or '-'
data (4)		"1234"
row terminator (2)		<CR>, <LF>
wavelength terminator (1)		<^Z>
checksum (1)		0 - 255
final terminator (1)		<^Z>

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Points for data collection are measured from the outer edge at the base of the upper left corner of a microplate. Units are 0.01 millimeters; i.e. "12345" represents 123.45 millimeters. An X start point greater than the X finish point will cause the plate to be processed from right to left. For example, such a pass across a 96-well plate would start with well A12 and finish with A1. However, data is always returned as if the pass were performed from left to right.

Only endpoint reads may be specified.

See the Read Plate command for details concerning results data format, and error handling and status string information. This command always returns data using the **ELx format**.

READ WELL SET ('d')

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

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

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reader response protocol:	
initial status string (5)	
for each kinetic interval (one if endpoint read):	
for each wavelength:	
data start code (1)	<CR>
for each of eight wells:	
comma separator (1)	','
sign (1)	'+' or '-'
data (4)	"1234"
row terminator (2)	<CR>, <LF>
wavelength terminator (1)	<^Z>
checksum (1)	0 - 255
final terminator (1)	<^Z>

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

See the Read Plate command for details concerning results data format, and error handling and status string information. This command always returns data using the **ELx format**.

GET BASECODE VERSION ('e')

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

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

reader response protocol:	
part number (7)	"0000000" – "9999999"
intermediate string (10)	" Version "
version code (7)	"x.xxyyy"
status string (5)	

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

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PRESENT PLATE CARRIER ('J')

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

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

SET READ MODE ('k')

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

host:	valid limits:	response:
'k'	no arguments	<ACK>
data bytes:	mode	
	'0': slow (enhanced) mode	
	'1': fast mode	
	'2': sweep mode	
		status string (5)

CONFIGURE WAVELENGTHS ('M')

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

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

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

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After wavelength configuration, a self-test ('*' command) sequence must be run to calibrate gains and generate self-check information for the new wavelength configuration. It is not absolutely necessary to configure and calibrate wavelengths for use, but performance may be compromised slightly if this is not done. The wavelengths stored are automatically calibrated during the instrument power-up self-test.

READ PLATE ('S')

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

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

reader response protocol:
status string (5)
for each kinetic interval (one if endpoint read):

	for each wavelength expected:	**(312 format: one, ELx format: up to six)**	
	wavelength start code (1)		<CR>
	for each row within specified row range:		
	for each column (well) within specified column range:		
	comma separator (1)		','
	sign (1)		'+' or '-'
	data (4)		"1234"
	row terminator (2)		<CR>, <LF>
	assay name (8)	**(312 format only)**	"ABCDEF", <CR>, <LF>
	unused extra characters (49)	**(312 format only)**	
	wavelength terminator (1)		<^Z>
	checksum (1)	**(ELx format only)**	0 - 255
final terminator (1)		**(ELx format only)**	<^Z>

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

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

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

312 Format:

This format is selected by default at reader power-up, and every time a new assay definition is loaded, either via serial control 'V' command, or by the user from the front panel. The reader always returns data as an 8 x 12 matrix, regardless of the selected plate geometry, along with the assay name and extra characters. There are no checksums or final terminator.

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

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

ELx Format:

If any of the following occurs, the ELx format is selected and remains enabled until a new definition is loaded, either via serial control 'V' command, or by the user from the front panel:

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

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

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

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

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

See Status String Format for error descriptions.

SET ASSAY DEFINITION ('V')

This command downloads to the reader an assay definition table to be used in subsequent plate reads, area scans, or wellset reads ('S', 'c', or 'd' commands) until a new definition replaces it, either via serial control or by the user from the front panel.

host:		valid limits:	response:
'V'			<ACK/NAK>
data bytes:			
1		don't care	
2 - 7		assay name (ASCII characters)	
8 - 29		don't care	
30		subtraction (0x00: none, 0x40: dual wavelength)	
31		encoded byte:	
	bits 0 - 6 (0x3F)	0	
	bit 7 (0x80)	1: use total kinetic time, 0: use kinetic read count	
32 - 49		don't care	
50 – 52		measurement wavelength ("000", "200" - "999")	
53 - 55		reference wavelength ("000", "200" - "999")	
56		read type (0: endpoint, 2: kinetic)	
57 - 59		don't care	
60 - 61		kinetic interval (seconds, 0 - 9999)	
62 - 63		kinetic read count (2 - 9999)	
64 - 66		don't care	
67 - 69		first wavelength ("000", "200" - "999")	
70 - 84		remaining wavelengths ("000", "200" - "999")	
85 - 165		don't care	
166 - 167		total kinetic read time (minutes, 1 - 9999)	
168		delay before first read (0: no, 1: yes)	
169 - 170		delay (seconds, 0 - 999)	
			status string (5)

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

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

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

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

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

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

GET WAVELENGTH TABLE ('W')

This command uploads the wavelength setup table from the reader.

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

HALT ('X')

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

host:	valid limits:	response:
'X'	no arguments	none (see below)

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The following events occur when this command is invoked:

1. The scan or read process is halted, and all axes are returned to their home positions (the plate is moved back outside where it can be accessed).
2. The reader transmits a <DLE> character to the computer when the above process is completed.
3. No more data is transmitted by the read or scan in progress.

SET PLATE GEOMETRY ('{')

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

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

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

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

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SET ROW RANGE ('r')

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

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

By default the instrument is initialized to perform full plate reads, and automatically reverts to this default when a new assay definition table is loaded, either from the front panel or by computer control. The well range is also reset when the Set Geometry command is sent.

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

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

SET COLUMN RANGE ('%')

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

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

By default the instrument is initialized to perform full plate reads, and automatically reverts to this default when a new assay definition table is loaded, either from the front panel or by computer control. The well range is also reset when the Set Geometry command is sent.

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

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

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GET MINIMUM KINETIC INTERVAL ('$')

This command provides a means for the user to determine the minimum kinetic read interval, as dictated by the current assay definition table, plate geometry, well range, and even baud rate selected.

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

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

Minimum kinetic intervals are not available for wellset reads.

SCAN PLATE ('&')

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

host:	valid limits:	response:
'&'		<ACK/NAK>
data bytes:		
1 - 2	row ("01" - "16")	
3 - 4	column ("01" - "24")	
5 - 7	start wave ("200" - "999")	
8 - 10	stop wave ("201" - "999")	
11 - 13	wave step ("001" - "799")	
14	series option ('0' - '3')	
	'0': single scan	
	'1': first scan in series	
	'2': next scan	
	'3': last scan	
15	calibrate option ('0' - '1')	
	'0': calibrate only if necessary	
	'1': calibrate before scanning	
16 - 18	don't care	
<ETX>		<ACK> if data valid

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

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

If an error is detected during the plate read, data transmission is halted, indicated by a single <DLE> character (0x10).

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

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

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

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

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

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

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

SELF-TEST ('*')

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

host:	valid limits:	response:
'*'		<ACK/NAK>
		data stream
		status string (5)

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

GET INSTRUMENT CONFIGURATION ('}')

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

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

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

code:	description:
0x0076	µQuant

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CHECK FEATURE ('z')

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

host:		**valid limits:**	**response:**
'z'		no arguments	<ACK/NAK>
data bytes:			
1 - 4	updated feature:		associated commands:
	"1001": well set reads		'd'
	"1002": reads using two wavelength sets		(legacy - 'V')
	"1003": area scanning		'c'
	"1004": serial wavelength configuration		(legacy – 'M')
	"1005": shaking		(not available)
	"1006": absorbance reads		(legacy)
	"1007": high-speed sweep reads		'S', 'c', '&', 'k'
	"1008": partial plate reads		'r' (legacy - '%','$')
	"1009": incubation		(not available)
	"1010": sensitivity response		(not available)
	"1011": multi-wavelength read format		(legacy – 'V')
<ETX>			
reader response protocol:			
feature available (1)			'0' / '1'
miscellaneous additional data (16)			
status string (5)			

If the 'z' is acknowledged (ACK), the instrument shall then return '1' if the feature is available, '0' if not.

If the 'z' command is not recognized (indicated by a NAK response to the 'z'), it means the instrument basecode version was released prior to implementation of this command (along with the other commands marked as such with a '#'). In this case, if the feature is marked in the table above as "legacy", the indicated commands and functions are and always have been available anyway.

Miscellaneous data TBD.

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Using the Stop Key to Halt Plate Scans and Reads

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

1. The scan or read process is halted, and all axes are returned to their home positions (the plate is moved back outside where it can be accessed).
2. The reader transmits a <DLE> character to the computer when the above process is completed.
3. No more data is transmitted by the read or scan in progress.

Status String Format

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

The status string consists of five successive ASCII characters -- RS, S1, S2, S3, and ETX:

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

⇒ **S1:** Always ASCII zero ('0')

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

ERROR CODES

Error codes indicate the following:

'0': no fault or error

'8': instrument failure - perform self-test

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

'A': error in well range selection

'B': incubator setpoint error

'C': incubator temperature error

'E': barcode error

⇒ **S3:** Always ASCII zero ('0')

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

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

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Appendix C
Report Format

This appendix contains the various types of reports available on the µQuant

The following are examples of reports that can be generated and/or printed from the *µQuant*. Refer to *Chapter 2*, *Output Options*, 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.

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Bio-Tek Instruments

Assay:ASSAY 01 Date:07/23/96 Lot: _____

Time:03:05:35AM Operator:

Wavelength:405 Temp: Plate ID:SONTA

COMMENTS

OUT indicates an out of range value was read.

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

Figure C-1: *Samples with calls on Matrix Report*

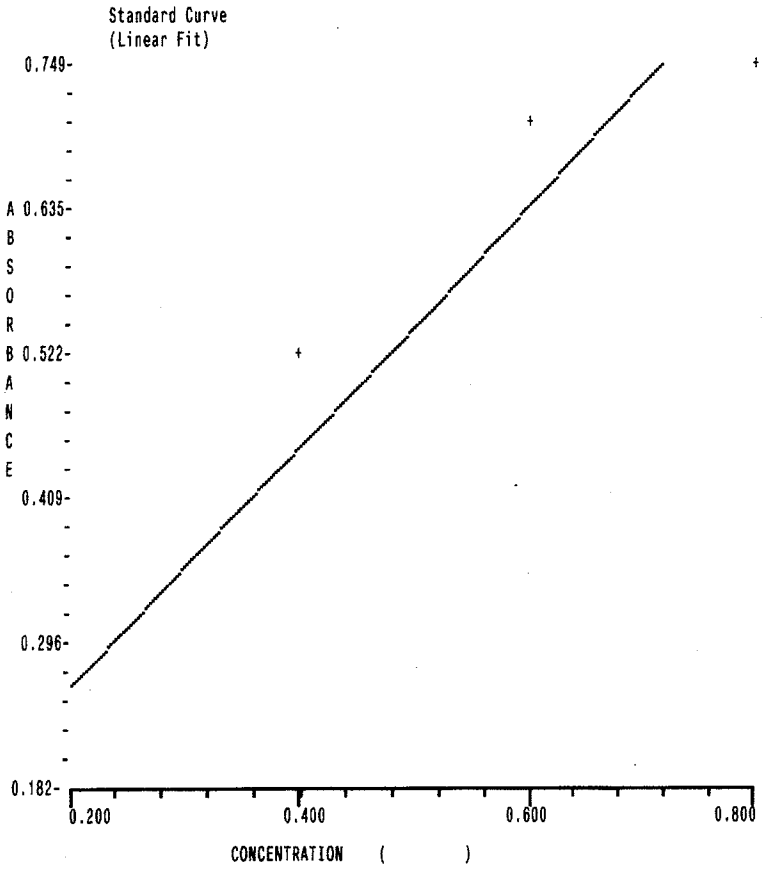
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Bio-Tek Instruments

Assay:ASSAY 01	Date:07/23/96	Lot: _____
	Time:03:05:35AM	Operator:
Wavelength:405	Temp:	Plate ID:SONTA

COMMENTS

OUT indicates an out of range value was read.



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

Figure C-2: *Curve Fit Report*

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Bio-Tek Instruments

Assay:ASSAY 01 | Date:07/23/96 | Time:03:05:35AM | Lot: _____ | Operator: | Wavelength:405 | Temp: | Plate ID:SONTA

COMMENTS

OUT indicates an out of range value was read.

Interpretation of Results

GREYZONE=0.00 =0.000
CUTOFF=.2 =0.200

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

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

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Figure C-3: *Samples with calls on Column Report*

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Bio-Tek Instruments

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

COMMENTS
OUT indicates an out of range value was read.

Interpretation of Results

GREYZONE=0.25 =0.250
CUTOFF=.1 =0.100

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

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

End of Report

Figure C-4: *Column Report without samples*

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Bio-Tek Instruments

Panel

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

COMMENTS

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

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***Figure C-5:** Panel Report*

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```
ASSAY DEFINITION
Assay Name:   ASSAY3013A3B1DB (Endpoint)
02:06AM       11/02/96
```

Well Geometry:	96 wells	
Dual Wavelength:	405\630	
Blanking:	AIR	
Average Standards:	NO	
Std.Concentrations:	STD1	10.0
	STD2	20.0
	STD3	30.0
	STD4	40.0
	STD5	50.0
	STD6	60.0
Curve Fit:	PT-PT	Axis = LIN
Edit Standards:	NONE	
Extrapolate:	NO	

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

Figure C-6a: *Assay Detail Report (Sheet 1 of 2)*

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```
Assay List
Version: 1.01
```

0	SPECTRAL SCAN
1	ASSAY 01
2	ASSAY 02
3	ASSAY 03
4	ASSAY 04
5	ASSAY 05
6	ASSAY 06
7	ASSAY 07
8	ASSAY 08
9	ASSAY 09
10	ASSAY 10
11	ASSAY 11
12	ASSAY 12
13	ASSAY 13
14	ASSAY 14
15	ASSAY 15
16	ASSAY 16
17	ASSAY 17
18	ASSAY 18
19	ASSAY 19
20	ASSAY 20
21	ASSAY 21
22	ASSAY 22
23	ASSAY 23
24	ASSAY 24
25	ASSAY 25
26	ASSAY 26
27	ASSAY 27
28	ASSAY 28
29	ASSAY 29
30	ASSAY 30
31	ASSAY 31
32	ASSAY 32
33	ASSAY 33
34	ASSAY 34
35	ASSAY 35
36	ASSAY 36
37	ASSAY 37
38	ASSAY 38
39	ASSAY 39
40	ASSAY 40
41	ASSAY 41
42	ASSAY 42
43	ASSAY 43
44	ASSAY 44
45	ASSAY 45
46	ASSAY 46
47	ASSAY 47
48	ASSAY 48
49	ASSAY 49
50	ASSAY 50
51	ASSAY 51
52	ASSAY 52
53	ASSAY 53
54	ASSAY 54
55	ASSAY 55

Figure C-6b: *Assay Detail Report (Sheet 2 of 2)*

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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

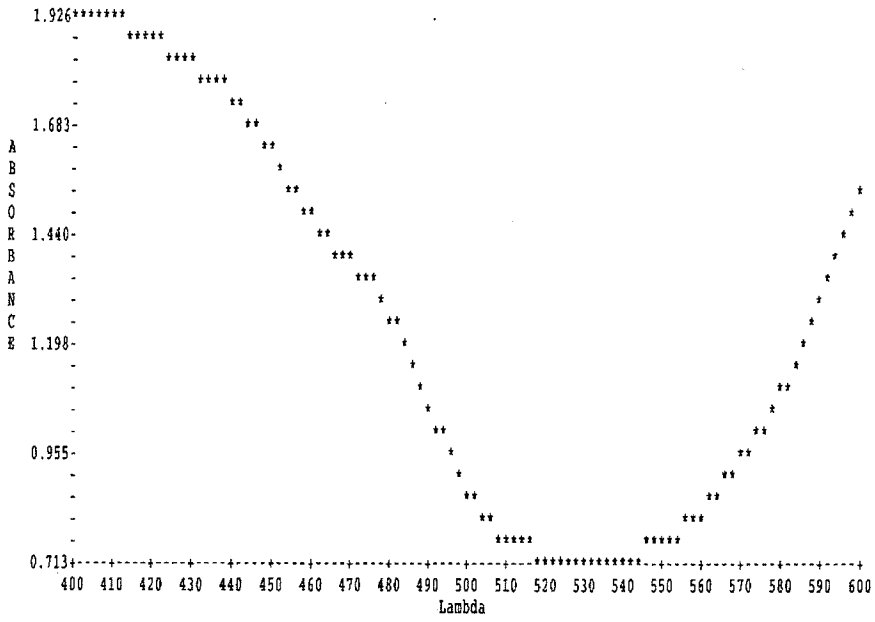


***Figure C-7:** Spectral Scan*

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Appendix D
Using 384-Well Geometry

The following is a way of manipulating data received from a 384-well geometry.

In order to capture and manipulate the raw data using a 384-well geometry, you must use KC*junior*™ for Windows® or KC4™. The Optical Density values will be downloaded, via the RS232 port, and logged into the data logging program.

KC*junior*

KC*junior*, Bio-Tek's data reduction package for Windows®, can control the *µQuant* in 6-, 12-, 24-, 48-, 96-, and 384-well formats, as well as in 72- and 96-well Terasaki plate formats.

1. Start KC*junior*.

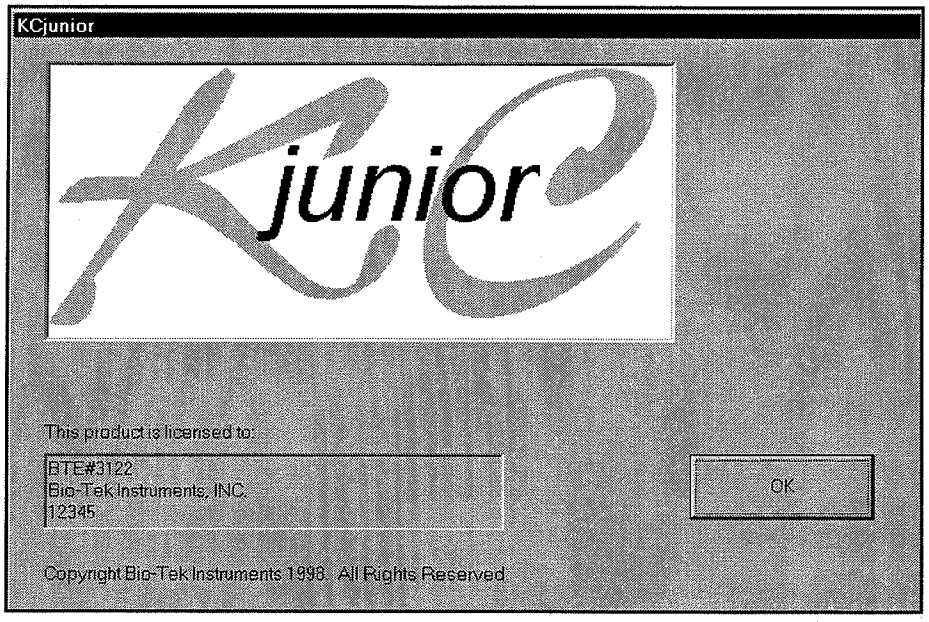


***Figure D-1**: Main Menu screen*

2. Select **OK**.

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3. Choose **Setup**, then **Reader** to select the *µQuant* reader and define the communication parameters.

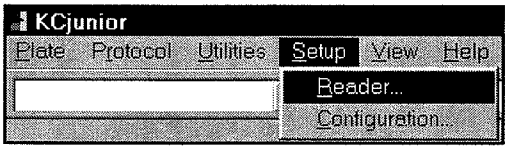


***Figure D-2**: The Setup screen*

4. Select **READ PLATE**.

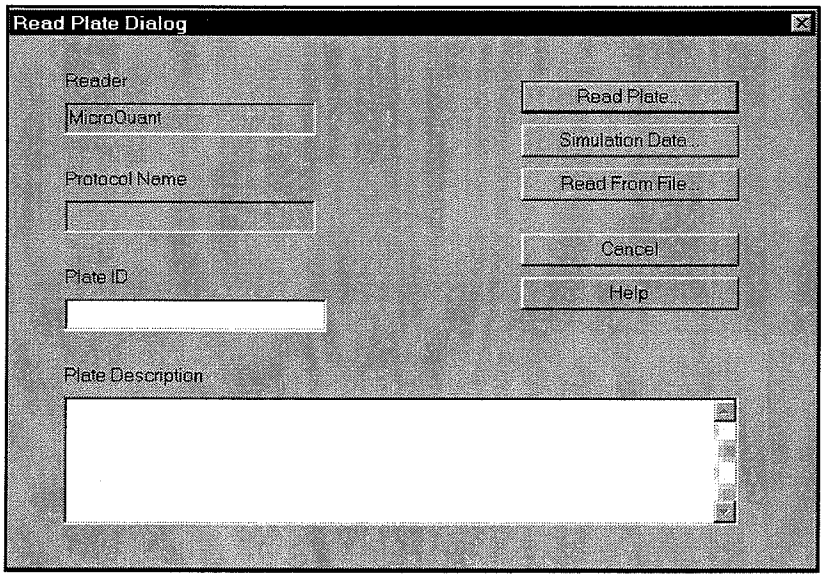


***Figure D-3:** Enter Plate ID*

5. Enter the **Plate ID**.
6. Choose **Read Plate.**
7. Define the **Read Method** parameters.
8. Set the **Plate Geometry**, for instance, to **8 X 12**.

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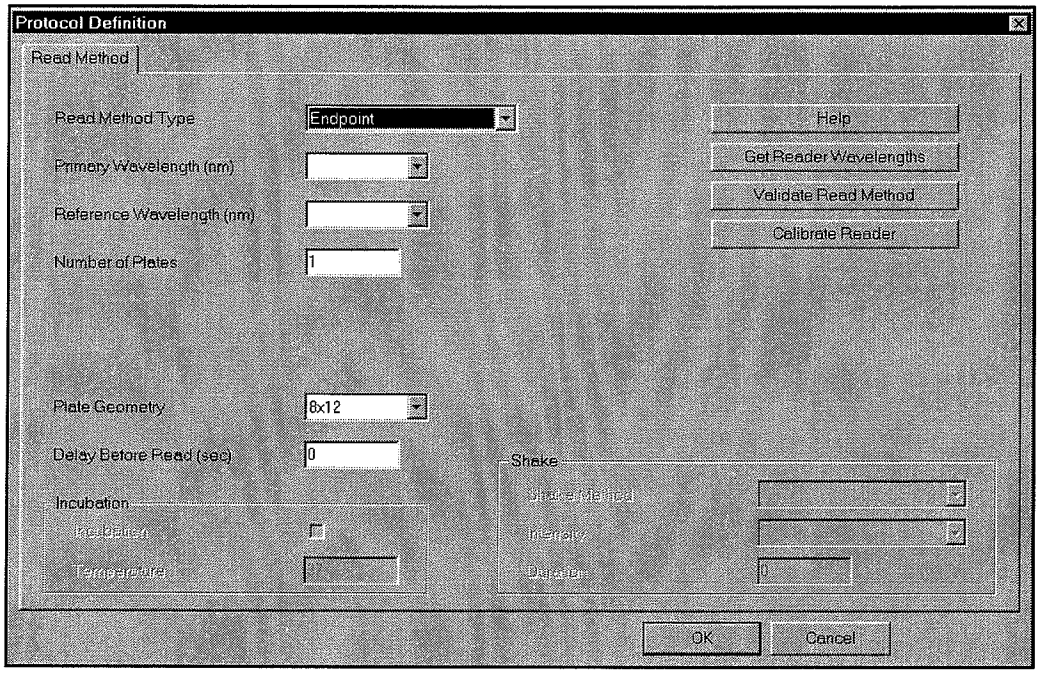


Figure D-4: *Defining the Read Method*

9. Click **OK**.

The plate will be read and the results displayed.

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Appendix E
Error Codes

This is a list of Error Codes that may appear on the reader.

If an error is displayed, 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 Bio-Tek's Technical Assistance Center (refer to *Chapter 1* for contact information).

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.

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Error Codes

Displayed Error	Potential Cause
ERROR 0200	Plate carrier did not find the home sensor
ERROR 0201	Order sorting filter wheel did not find the home sensor (not user serviceable)

Errors 200 and 201 indicate that a motor was not able to move to its "home" position as registered by feedback from an optical sensor.

PROBABLE CAUSE:

- Carrier movement may be limited so that the optical sensor cannot be interrupted. Check the carrier path for obstructions.
- Order sorting filter wheel not rotating. Optical sensor defective.

Displayed Error	Potential Cause
ERROR 0300	Carrier failed to find light beam
ERROR 0301	Filter wheel did not find home
ERROR 0302	Monochromator failed to find the white light

Errors 300 and 301 indicate that a particular axis has moved to a point where the light beam from the optics is no longer detectable by the measurement electronics.

PROBABLE CAUSE:

- Carrier - Lamp may be out or blocked by a faulty filter wheel. A loose belt or a loose motor pulley may cause the carrier to ignore movement instructions.
- Filter Wheel Motor - The filter wheel is missing or loose, or a loose filter may be impeding filter wheel movement.

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Displayed Error	Potential Cause
ERROR 0400	Carrier axis failed position verify
ERROR 0401	Order sorting filter wheel failed position verify (not user serviceable)
ERROR 0402	Monochromator position verify failure

Errors 400 and 401 indicate that an axis failed its Position Verify test. After moving a predefined number of steps from a home position, the motor should return to the home position in the proper amount of time and steps. If the axis moves back to its home position in the wrong amount of time or in too few or too many steps, the test fails.

PROBABLE CAUSE:

- Mechanical obstruction.
- Carrier belt or pulley slipping.
- Loose filter wheel hub.
- Bad motor drive circuit or 12-volt power problem.
- Lamp flashing intermittently.
- Monochromator defective.
- Light detection circuit failure.

Displayed Error	Potential Cause
ERROR 0500	A/D signal is saturated

Error 500 indicates that the circuit detected light saturation where there shouldn't be any.

PROBABLE CAUSE:

- Door open during the read.
- Defective light detection circuit.

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

Errors 601-606 indicate that the gain for a specific filter is out of the range. Gain out of range indicates that the lamp is out, or that a particular filter absorbs or transmits entirely incorrect light levels.

PROBABLE CAUSE :

- Flash lamp not flashing or missing flashes.
- Defective order sorting filter.
- Dirty optics.

Displayed Error	Potential Cause
ERROR 0700	Reader failed noise test

Error 700 indicates significant variations in background electronic noise were detected when blocking the light and increasing the Gain to maximum.

PROBABLE CAUSE:

- Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened.
- Ambient light leak. Check that the plate chamber door is closed to keep out ambient light.
- Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.

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Displayed Error	Potential Cause
ERROR 0800	Reader failed offset test

Error 800 indicates background electronic signal detected is outside of acceptable limits at maximum gain when blocking the light.

PROBABLE CAUSE:

- Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened.
- Ambient light leak. Check that the plate chamber door is closed to keep out ambient light.
- Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.

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

Error 900 indicates that the dark current value taken during the current read, is significantly different than the same reading taken during the power up self-check.

PROBABLE CAUSE:

- Read chamber door has opened or closed relative to when the unit was powered ON. Close door and reboot reader to correct.
- Electrical noise penetrating the measurement chamber. The bottom and top shrouds are part of the electrical shielding. Check that the shrouds are installed and properly fastened.
- Internal electronic noise caused by a faulty Analog PCB or faulty internal grounding.

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

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

PROBABLE CAUSE:

- Lamp has failed since power-up. Check that the lamp is lit and replace if necessary.
- Analog PCB failure.

Displayed Error	Potential Cause
ERROR 0C00	Printer time out error

Error C00 indicates that the time allotted for the instrument to make a valid connection to a printer has expired.

PROBABLE CAUSE:

- Printer out of paper, not connected, or not on-line.
- A printer that has a long initialization time.

Displayed Error	Potential Cause
ERROR 0D00	Reader failed calibration checksum test. Internal software corruption.
ERROR 0E00	Wavelength not detected in reader's Lambda table. Compare filters wavelengths to the filter table.
ERROR 0F00	Reader's wavelength signal is out of specified range. Check for lamp failure. Run a system test to get a report.

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Displayed Error	Potential Cause
ERROR 1000	Required reader configuration data missing. Re-download software basecode and assays.
ERROR 1100	Failed configuration checksum test. Re-download software basecode and assays.
ERROR 1200	Calibration data missing.
ERROR 1300	Motor not homed. Autocal error, see error 0200.
ERROR 1400	Assay incubation Error. Assay requires incubation but the instrument does not appear to have an incubator.
ERROR 1700	Kinetic interval too short for selected options.
ERROR 1800	Too many kinetic intervals selected.
ERROR 1900	Memory allocation failure. This error is used only for software development.
ERROR 1A00	Store curve failure.
ERROR 1B00	Get curve failure.
ERROR 1C00	A/D reset/calibration STBY signal transition not detected.
ERROR 1D00	Results data error.

Contact Bio-Tek's Technical Assistance Center for further assistance if any of the above errors occur.

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