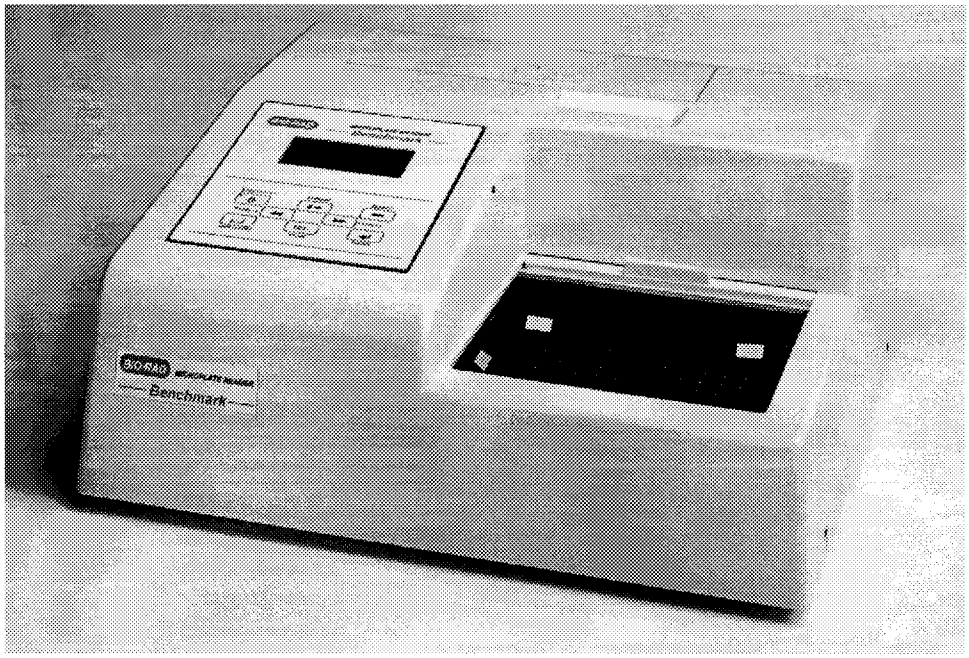


Benchmark Microplate Reader

Instruction Manual

Catalog Number
170-6850

BIO-RAD

Warranty and Regulatory Notices

Warranty Statement

This warranty may vary outside of the continental United States. Contact your local Bio-Rad office for the exact terms of your warranty.

Bio-Rad Laboratories warrants that the Benchmark Microplate Reader (catalog number 170-6850) will be free from defects in material and workmanship, and will meet all performance specifications for the period of 1 year from the date of shipment. This warranty covers all parts and labor.

In the event that the instrument must be returned to the factory for repair under warranty, the instrument must be packed for return in the original packaging.

Bio-Rad shall not be liable for any incidental, special, or consequential loss, damage, or expense directly or indirectly arising from the use of the Benchmark Microplate Reader. Bio-Rad makes no warranty whatsoever in regard to products or parts furnished by third parties, such being subject to the warranty of their respective manufacturers. Service under this warranty shall be requested by contacting your nearest Bio-Rad office.

This warranty does not extend to any instruments or parts thereof that have been subject to misuse, neglect, or accident, or that have been modified by anyone other than Bio-Rad or that have been used in violation of Bio-Rad instructions.

The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability, fitness for a particular purpose or otherwise, expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation, or operation. Bio-Rad will in no event be liable for any special, incidental, or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed.

Regulatory Notices

Important: This Bio-Rad instrument is designed and certified to meet EN55011, EN50082-1, and IEC 1010-1 requirements, which are internationally accepted electrical safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

Void the manufacturer's warranty

Void the regulatory certifications

Create a potential safety hazard

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. 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 harmful interference in which case the user will be required to correct the interference at his own expense.

Table of Contents

Section 1
Introduction

The Benchmark Microplate Reader is a 16-channel vertical pathlength photometer that measures the absorbance of the contents of the wells of 96-well microtitration plates. It performs single or dual wavelength measurements between 340 and 750 nm, and reports absorbance values to three decimal places.

The Benchmark reader is programmed by entering commands through the membrane keypad to define assay blanks, set assay filter(s), set analysis parameters, and select report types. Six different reports may be generated: Raw Data, Absorbance, Limit, Matrix, Cutoff, and Concentration. Hard copy is produced by the instrument's onboard thermal printer.

The Benchmark reader features a built-in RS-232-C serial interface device for convenient computer interfacing. The Microplate Manager® software for the Apple® Macintosh® or IBM® series of personal computers offers a complete analysis program (catalog numbers 170-6800 and 170-6732, respectively).

1.1 Accessories for the Benchmark Microplate Reader

Catalog Number	Product Description
170-6760	Replacement Lamp
170-6761	Printer Paper, 4 rolls/package
170-6906	340 nm Interference Filter
170-6907	405 nm Interference Filter
170-6908	415 nm Interference Filter
170-6909	450 nm Interference Filter
170-6910	490 nm Interference Filter
170-6911	540 nm Interference Filter
170-6912	550 nm Interference Filter
170-6913	570 nm Interference Filter
170-6914	595 nm Interference Filter
170-6915	630 nm Interference Filter
170-6916	655 nm Interference Filter

Custom filters between 340 and 750 nm may be ordered. No special catalog number is required; simply specify the wavelength and the model number of the reader when ordering, *e.g.*, a 560 nm filter for the Benchmark Microplate Reader.

Section 2
Product Description

2.1 Contents of Shipping Carton

The shipping carton should contain the following items.

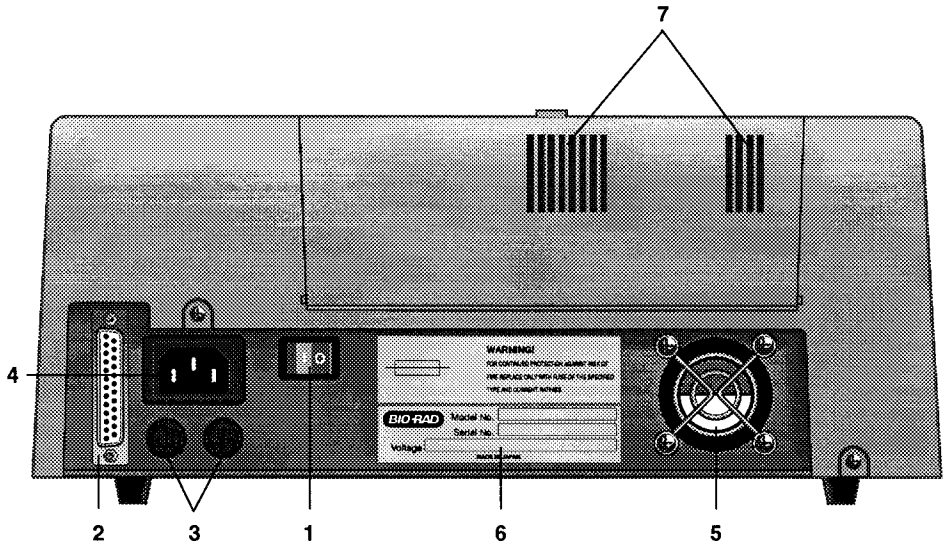
1. Benchmark Microplate Reader
2. 340 nm interference filter (installed in position 1 of the filter wheel)
3. 405 nm interference filter (installed in position 2 of the filter wheel)
4. 415 nm interference filter (installed in position 3 of the filter wheel)
5. 655 nm interference filter (installed in position 4 of the filter wheel)
6. One roll of thermal printer paper
7. Dust Cover
8. 110/220 V Power Cords
9. 220/240 V Power Cords
10. Instruction manual
11. Warranty card

The two additional filters ordered with the instrument are packaged separately and are easily installed by the user. See Section 5.1.

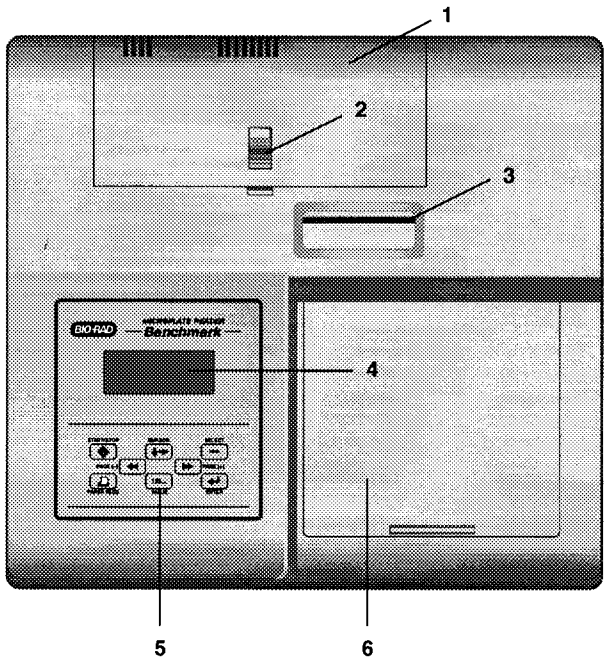
Inspect the outside of the reader for any signs of shipping damage. Contact your local Bio-Rad representative if any of these items are damaged or missing. In the U.S.A., call 1-800-4BIORAD.

Please complete the warranty registration card and return it to Bio-Rad.

2.2 External Features

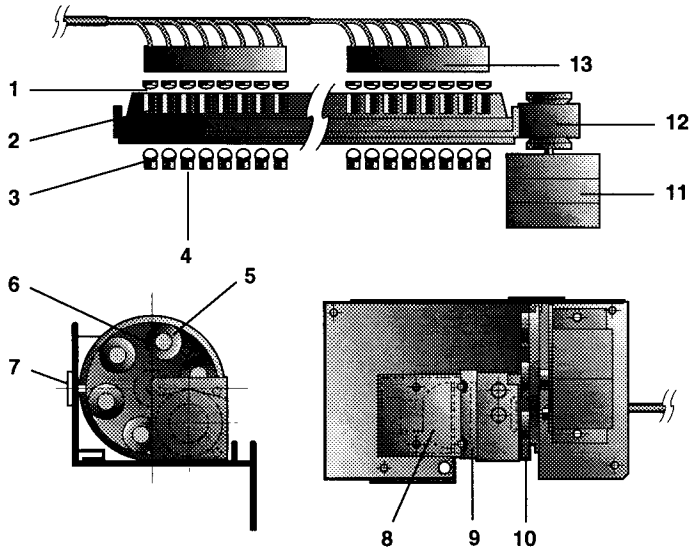


1. ON/OFF switch
2. RS-232-C serial interface
3. Fuses
4. Power cord receptacle
5. Cooling Fan
6. Serial Number Label
7. Cooling Vents



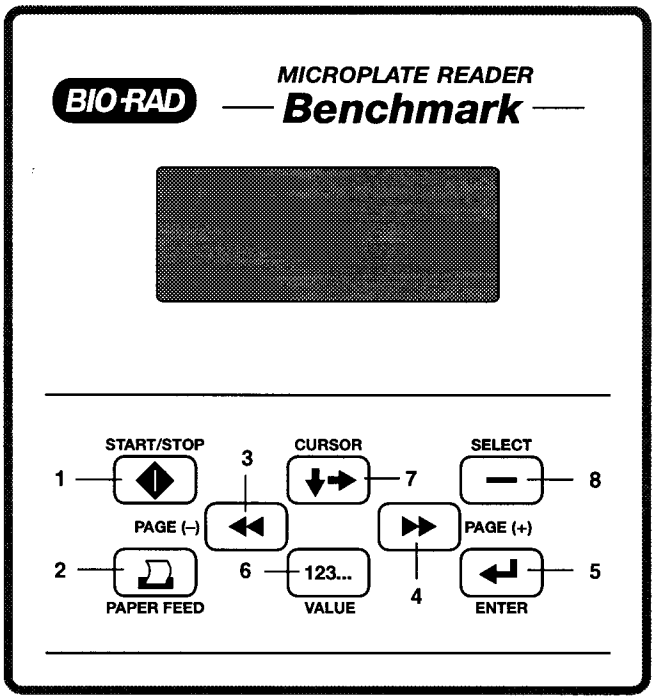
1. Rear cover
2. Release latch for rear cover
3. Printer slot
4. Liquid crystal display (LCD)
5. Membrane keypad
6. Reading chamber door

2.3 Optical System



1. Condensing lenses
2. Microplate wells
3. Silicon photodetectors
4. Spherical lenses
5. Interference filters
6. Filter wheel
7. Wheel positioning sensor
8. Lamp
9. Light balancing filter
10. Interference filter
11. Stepper motor
12. Plate carriage
13. Fiber optic bundle

2.4 Membrane Keypad



1. [START/STOP]: Initiates plate reading using current analysis mode. Stops plate reading.
2. [PAPER FEED]: Paper Feed
3. [PAGE-]: Move up one level in menu hierarchy
4. [PAGE+]: Move down one level in menu hierarchy.
5. [ENTER]: Confirms and completes entry.
6. [VALUE]: Toggles a yes/no switch. Increases numerical entries by increments of one.
7. [CURSOR]: Move the cursor position.
8. [SELECT]: Moves cursor from one line to next.

Section 3
Instrument Set-up

3.1 Initial Start-up

1. Place the instrument on a clean, sturdy table or bench. It is important to keep the instrument in a clean, relatively dust free environment to insure maximum performance.

2. Connect the power cord to the back of the instrument. Before connecting the instrument to the main electrical supply, check that the AC voltage is appropriate for the instrument.

3. Turn on the power switch on the rear panel. The LCD will display the version number of the on-board firmware. After about 3 seconds the instrument will perform an initial self diagnosis that requires about 1 minute. Allow 3 minutes for the instrument to warm up (reach thermal equilibrium) before reading plates. If using the incubator, wait for the instrument to indicate that the incubator has reached set point temperature.

3.2 Installing the printer paper

The thermal printer paper is treated on one side only, and must be properly installed for the printer to function. The side of the paper that faces out from the roll is the treated side.

- Open the rear cover of the instrument.
- Tear off a small piece of the beginning of the roll to form a point.
- Place the roll of paper in the small pan-like holder positioned below the printer.
- While pressing the PAPER FEED key, feed the pointed end of the paper into the paper feed slot in the underside of the printer until the printer grabs the paper and feeds it through the slot in the top of the instrument.
- Securely close the rear compartment.

Section 4
Operation

4.1 Overview

The Benchmark will read microplates in either single- or dual-wavelength mode. Two columns of 16 wells are read simultaneously. There are six positions on the filter wheel and any filter is available for measurement wavelength or reference wavelength.

The Benchmark Microplate Reader has built-in software that allows the user to set the locations of assay blanks, select assay filter(s), set the values of the analysis limits, and select the report types to be printed. The software communicates through the four-line, 16-character LCD and is controlled through the instrument's membrane keypad.

The display shows the current mode of the on-board software. The software has 10 different modes: the PLATE READING mode, in which data are collected, eight modes for setting the reading and reporting parameters, and a REMOTE CONTROL mode. The modes used to set parameters are: PRINT REPORTS, SET ANALYSIS, SET REPORT TYPES, SET BLANKS, SET LIMITS, SET CUTOFF, SET CONCENTRATION, and SET SYSTEM CONFIGURATION. The Remote Control mode is entered when the reader receives an "AQ" command through the RS-232C port. In the Remote Control mode the printer is deactivated. The reader will remain in the Remote Control mode until it receives an "RL" command through the RS-232C port or until START/STOP is pressed on the instrument keypad.

Calculations

The reader uses Beer's Law to calculate the absorbance value of each well. Beer's Law states that absorbance is equal to the log10 of the ratio of the baseline measurement (Io) to the sample measurement (I).

$$\text{Absorbance} = \text{Log10}(\text{Io}/\text{I})$$

Before measuring the plate, the reader takes a reading for all 16 channels. These values are recorded as the baseline measurement (Io) values for each channel, respectively. The reader then records the sample measurement (I) value for each well, and calculates the absorbance using these values. Channel-to-channel error is significantly reduced because the Io value for a given channel is used only in determining the absorbance of the wells of that channel. (The Io values are not averaged.)

Blanking

The blank absorbance value is determined on each plate as it is read (so long as blanks have been defined). The reader calculates the mean and standard deviation for the absorbance values from all the blank wells. The mean absorbance of the blank wells is subtracted from the absorbance of each well to generate the Absorbance, Matrix, Limit, Cutoff, and Concentration reports.

The blank well *locations* defined in the Set Blanks mode will be saved in the memory, but not the blank well *absorbance values* (see Memory).

Memory

The battery back-up provides memory even after the plate reader is turned off. Plate reading parameters and all absorbance values from the last ten plate readings are saved in the memory. The data from the most recent reading are stored in memory 0. The data from the reading immediately preceding the most recent are stored in memory 1. The oldest data set is stored in memory 9. As new data are read and transferred into memory 0, all previously stored data sets move up one position and the data set that was in memory 9 is lost. The following information will be saved in the memory until a new plate is read by the instrument. Note that if a new plate reading is aborted before it is finished, all the previous data remain in memory and none of the data from the new plate are retained.

1. Last 10 plate readings (reading mode and absorbance values for all 96 wells)
2. Reading mode
   a. Single- or dual-wavelength
   b. Measurement filter position and wavelength
   c. Reference filter position and wavelength
   d. Plate mixing ON/OFF and duration
3. Report types selected
4. Blank well locations
5. Upper and Lower limit Values
6. Cutoff report parameters
   a. Report type (Constant or Formula)
   b. Cutoff Constant value
   c. Control well locations for Formula cut-off
7. Concentration report parameters
   a. Number of samples and sample replicates
   b. Standard concentration values
   c. Number of standards and standard replicates
8. Incubator ON/OFF

The first time the instrument is turned on, or after a battery failure, the following default information will be held in the memory.

1. 96 absorbance values of 0.000 for 10 plate readings
2. Dual wavelength reading
3. Measurement filter position is #1 and reference filter position is #2
4. Plate mixing is inactive (Mixing = OFF)
5. Raw data report is selected, all other types are deselected
6. No blank wells are assigned
7. Upper limit is 4.000 and the Lower limit is 0.000
8. The Constant type of cutoff report is selected
9. Constant cutoff value is 0.000

10. Concentration report parameters:
    a. # STDs = 0
    b. # Samples = 0
    c. Replicate STDs = 1
    d. Replicate Samples = 1
    e. All STD concentrations = 0.000
11. All filter wavelength values are set to *** (undefined)
12. Incubator is inactive (Incubator = OFF)

Limits

The microplate reader displays absorbance readings with absolute values as high as 4.000. Out-of-range absorbance values, *i.e.*, those with absolute values greater than 4.000, are displayed as either '*.***' or '-*.***'. For example if the absorbance is 4.500, then the display will read '*.***', and if the absorbance is -4.500, the display will read '-*.***'.

Reports

Six types of reports can be generated by the microplate reader: Raw, Absorbance, Matrix, Limit, Cutoff and Concentration.

The **RAW DATA REPORT** is the uncorrected absorbance values without blank subtraction. In single-wavelength mode, the reported value is the measured absorbance. In dual wavelength mode, the reported value is the difference between the uncorrected readings taken with the measurement filter and with the reference filter.

The **ABSORBANCE REPORT** is the blank-corrected absorbance values. The microprocessor uses the blank information to locate the assay blanks set in Section 4.6. The mean absorbance value of all of the wells designated as assay blanks is calculated and then subtracted from all 96 values of the raw data set to produce the Absorbance Report.

$$\text{Abs} = \text{Raw} - \text{mean}$$

$$\text{mean} = \sum X / n$$

$$\text{S.D.} = [\ \{\sum X^2 - n*(\text{mean})^2\} / \{n - 1\}\ ]^{1/2}$$

where mean = Blank mean
S.D. = Standard deviation
$\sum X$ = Sum total of the raw absorbance values for each blank
$\sum X^2$ = Sum total of the squared raw absorbance values for each blank
n = number of blanks

Notes:

(1) Absorbance values greater than 4.000 or less than -4.000 are out of range.

(2 If an out-of-range absorbance is measured in one of the blank wells, then the report will show

Blank mean *.***
Std. Dev. *.***

If the out-of-range value is below -4.000, then the asterisks will be preceded by a negative sign (-).

(3) If an out of range value is measured for the raw absorbance of any well, it's reported Absorbance (= raw-blank) will be *.*** or -*.*** according to the sign of the value.

(4) If the calculated Absorbance is out of range, then asterisks are displayed as when the raw absorbance is out of range.

(5) If the number of blanks is zero, then the report will show

Blank mean *0.000*
Std. Dev. *0.000*

(6) If the number of blanks is one, the report will show

Blank mean *Raw absorbance value of the one blank well*
Std. Dev. *0.000*

The **MATRIX REPORT** provides a qualitative report of the relative magnitude of the absorbance values on the plate. The absorbance range defined by the upper and lower limits set in Section 4.6 is divided into 10 equal partitions, which are numbered 0 through 9. The blank-subtracted absorbance value of each well is classified according to the partition of the matrix to which it corresponds, and is reported as a single digit. Wells with absorbance values greater then the upper limit are represented by plus signs (+), and wells with absorbance values less then the lower limit by minus signs (-).

The **LIMIT REPORT** provides a qualitative YES/NO report. Wells with blank- subtracted absorbance values between the upper and lower limits are represented with an asterisk (*), wells with absorbance values below the lower limit by minus signs (-), and wells with absorbance values greater then the upper limit by positive signs (+).

The **CUTOFF REPORT** provides a qualitative score for each well compared to a cutoff value. If the absorbance of a well is within 10% (*i.e.*, +/-10%) of the cutoff value, the well is scored '+/-'. If the absorbance of a well is more than 10% greater than the cutoff value, the well is scored '+', and if the absorbance of a well is more than 10% below the cutoff value, the well is scored '-'.

The cutoff value may be assigned by either the CONSTANT method or the FORMULA method. In the constant method, the user inputs an absorbance value to be used as the cutoff, *e.g.* 1.000 O.D. By this example, all wells with absorbance values between 0.9 and 1.1 will be scored '+/-', all wells below 0.900 will be scored '-' and all wells above 1.100 will be scored '+'.

When the formula method is chosen, the user prepares both positive and negative controls. As many as eight sets of controls may be chosen. The mean absorbance values and standard deviations are calculated and reported for both sets of controls, and then a cutoff value is calculated by the formula:

*Cutoff value = Mean of negative controls + 0.10 * Mean of the positive controls*

Consider an example in which the mean absorbance of the negative controls is 0.200 and the mean absorbance of the positive controls is 1.000. The calculated cutoff value will be 0.300 (cutoff = 0.200 + 0.10 * 1.000) and wells with absorbance values between 0.270 and 0.330 (+/- 10% of the cutoff value) will be scored '+/-', wells with absorbance values less than 0.270 or greater than 0.330 will be scored '-' or '+', respectively.

Notes:

(1). If one of the positive or negative control wells has an out-of-range absorbance value, the values reported for positive and negative mean and standard deviation will be asterisks. If the out-of-range value is below -4.000, then the asterisks will be preceded by a minus sign (-). For example, the report may show

Pos. Mean *.***
Pos. Dev. *.***
Neg. Mean *.***
Neg. Dev. *.***
Cutoff *1.234*

(2) If the calculated cutoff value is greater than 4.000 or less than -4.000,then the report will show

Cutoff *.***
or
Cutoff -*.***

(3) If the number of standards is zero for the formula method, then the report will show

Pos. Mean *0.000*
Pos. Dev. *0.000*
Neg. Mean *0.000*
Neg. Dev. *0.000*

(4) If the number of standards is one for the formula method, then the report will show

Pos. Mean *Absorbance value of one positive control*
Pos. Dev. *0.000*
Neg. Mean *Absorbance value of one negative control*
Neg. Dev. *0.000*

(5) If the calculated cutoff value is 0.000, then all readings greater than 0.000 are reported as '+', all negative readings are reported as '-', and all 0.000 readings reported as '+/-'.

The **CONCENTRATION REPORT** lists the absorbance values of the samples and then calculates concentrations of the samples based on the absorbance values of a series of standards. The on-board software calculates the best fit straight line between each set of two consecutive data points in the standard curve of absorbance vs. concentration. When there is only one standard, a line is drawn between the data point and the origin to create a standard curve. When there is more than one standard, the origin is not considered a data point. Because of the way in which standard curves are calculated, standard data must be input in either ascending or descending order.

Consider the case when there are four standards. The software makes a plot of absorbance vs. concentration and then calculates the three equations (Eq1, Eq2 and Eq3) which describe the straight lines that join (1) the first (Conc1, Abs1) and second (Conc2, Abs2) standard data points, (2) the second and third(Conc3, Abs3) standard data points, and (3) the third and fourth (Conc4, Abs4) standard data points. The equation used by the software to calculate the sample concentration depends on the absorbance of the sample. If the sample absorbance is less than Abs1, the software extrapolates Eq1, the line determined by standard data points 1 and 2. The same equation is used to determine the unknown concentration when the unknown's absorbance is between Abs1 and Abs2. However, Eq2 is used to determine the concentration of unknowns with absorbance values between Abs2 and Abs3, and Eq3 is used to determine the concentration of all samples with absorbance values greater than Abs3.

There are six pre-determined microplate formats associated with the concentration report. The assignment of the template is made by the software based on (1) the number of samples which may be set to 0-40, 44, 80 or 88; (2) the number of standards which range from zero to seven; (3) the number of sample replicates, one or two and (4) the number of standard replicates, also one or two. The six different formats are shown in the figure below. Refer to the table below for format information.

Std. Repl	Stds	Sample Repl	Max # samples	Format
1	0–7	1	88	1
		2	44	2
2	0–3	1	88	3
		2	44	4
	4–7	1	80	5
		2	40	6

Notes:

(1) Blank wells that have been defined under the SET BLANKS mode are replaced by those of the templates. In order to set blanks in the SET BLANKS mode, the Concentration report must be de-selected in the REPORT TYPES mode (see Section 4.6).

(2) If the standards are set to zero, then an error message will be printed on the fourth line of the Concentration report, *ERROR: STDs=0.*

(3) If the standards are not input in ascending or descending order, an error message will be printed on the fourth line of the Concentration report, *ERROR: STD* Conc.

(4) Under three different set of conditions, an error may be made in calculation of the calibration curve and an error message will be printed on the Concentration report, *ERROR: Calibration Curve*. Despite the error message, the software does make a calibration curve and uses it to report concentrations. In all three cases, it probably indicates an error was made in preparation of one or more of the standards.

a. If the absorbance of any standard is measured as a negative value.

b. If the sign of the slopes of the different line segments changes. For example, if the standard concentrations are input in decreasing order, then normally the slopes of the individual lines joining consecutive data points would be negative. If any one segment has a positive slope, the error is message is displayed.

c. If the slope of any one line segment is zero, *i.e.*, the same absorbance is measured at two different concentrations.

(5) If a sample absorbance is out of range (one replicate), or if the absorbance of one or more wells of a sample replicate group is out of range, asterisks will be printed for the concentration and absorbance values on the Concentration report. The asterisks will be preceded by a negative sign (-) if the sample absorbance is below -4.000. For example:

Samples = 20, Repl = 2

#	Conc.	Abs.
1	***.*	*.***
2	0.020	2.550

(6) If a sample absorbance is less than zero (*i.e.*, a negative number), the sample concentration will be displayed as asterisks, but the actual absorbance is printed. For example:

Samples = 20, Repl = 2

#	Conc.	Abs.
1	-***.*	-0.123
2	-***.*	-0.234

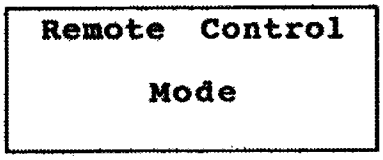
(7) If the calculated concentration is above 999.9 or below 0.000, the reported concentrations will be '***.*' or '-***.*', respectively.
For example:

Samples = 20, Repl = 2

#	Conc.	Abs.
1	***.*	2.567
2	-***.*	0.010

RS 232 Interface

This microplate reader has a built-in bi-directional RS-232 interface. This allows external computers to control the microplate reader through an 'AQ' command. When an external computer is in control of the microplate reader, the printer is automatically deactivated and the display will indicate



The reader will remain in remote control mode until it receives an 'RL' command from the host computer or until START/STOP is pressed.

4.2 Powering up the Benchmark Reader

1. Before turning on the power, open the rear compartment and verify that the correct interference filters are properly installed in the filter wheel. See Section 5.1 for instructions on changing the interference filters. Close the rear cover.
2. Turn on the instrument. The display will show

```
Benchmark  Reader
  Version  1.04
```

After a few seconds the instrument begins a 1 minute self diagnosis during which the display will read

```
Self diagnosis
 in  progress.
```

After a satisfactory self diagnosis, the software switches to the Plate Reading mode. Wait three minutes while the reader warms up before reading a plate. There are several variations of the Plate Reading display. For a single-wavelength reading, the display may show

```
_PLATE  READING
 M=1:405
 Mixing=OFF
 Incu.  =OFF
```

This indicates that neither the mixer nor incubator will be used before measuring absorbance values at 405 nm. Reading will commence as soon as START/STOP is pushed. For a dual-wavelength reading, the display might be

```
_PLATE  READING
 M=1:405,R=4:655
 Mixing=ON(05s)
 Incu.  =ON(23°C)
```

The display indicates that the instrument is heating the incubator toward the 37 °C set point. When the incubator has reached the set point temperature of 37 °C, the display will show

```
_PLATE  READING
 M=1:405,R=4:655
 Mixing=ON(05s)
Warm  up  finished
```

and then

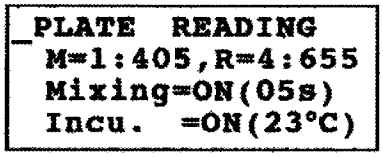
```
_PLATE  READING
 M=1:405,R=4:655
 Mixing=ON(05s)
 Incu.  =ON(37°C)
```

When START/STOP is pressed, the reader will mix the plate for five seconds before taking measurement and reference absorbance data at 405 nm and 655 nm, respectively. Note that the reader will commence data collection when START/STOP is pressed, even if it is pressed before the incubator reaches the set point temperature.

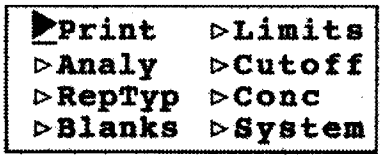
4.3 Setting System Configuration

The assignment of filter wavelengths must be done through the on-board software.

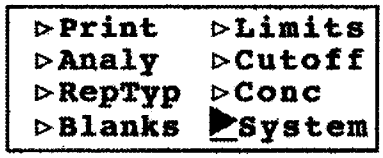
1. Power up the Benchmark reader. After the self diagnosis, the screen will change to the Plate Reading mode



2. Press ENTER or PAGE+ to go to the Main menu

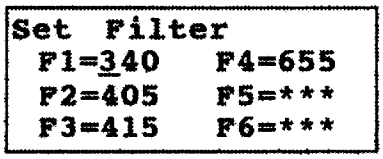


3. Use CURSOR to move down and over to *System*

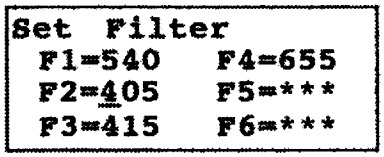


4. Press ENTER or PAGE+

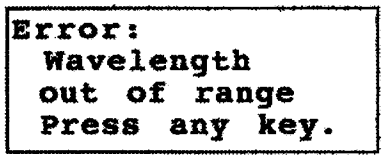
5. **To set the wavelengths of the six filters,** press ENTER or PAGE+



   a. The cursor begins under the first digit of the first filter. Use VALUE to change the first digit. Press CURSOR to move to the second digit and press VALUE to set the digit, then CURSOR to the third digit and set it with VALUE. When all three digits of the first filter are set, press SELECT to move to the second filter



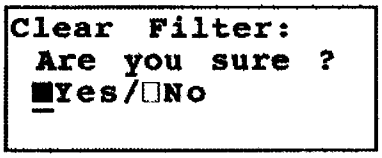
   Note: If an invalid entry is made (<340 or >750), an error message is displayed and the entry reverts to its previous setting



b. Use VALUE and CURSOR to set all three digits of the second filter, then press SELECT to move to the third filter. When all six filters are set, press ENTER or PAGE- to confirm the choices and to go back to the Set System menu

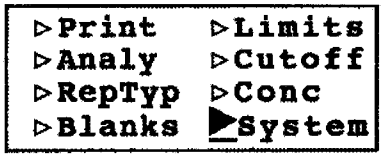
6. To clear the definition of all six filters, CURSOR down to *Clear Filter*

a. Press ENTER or PAGE+



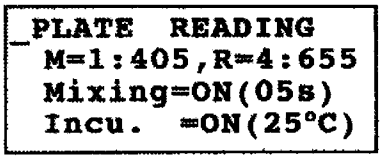
b. Use VALUE to toggle between *Yes* and *No*. Press ENTER or PAGE– to confirm the selection and to return to the Set System menu

c. Press PAGE– to return to the Main menu



4.4 Quick Guide–Reading a plate:

1. Power up the Benchmark reader. After the one-minute self diagnosis, the software goes into the Plate Reading mode and the display will reflect the settings of the last reading



While the instrument is warming up:

2. Enter the SET ANALYSIS mode (Section 4.6) to:

   a. Choose single- or dual-wavelength measurement
   b. Choose the measurement and reference filters
   c. Choose mixing time
   d. Choose whether to use the incubator

3. Enter the SET REPORT TYPES mode (Section 4.6) to choose the type(s) of reports to be generated.
4. All reports other than the Raw report, require blanks. Enter the SET BLANKS mode (Section 4.6) to indicate the position of blanks.
5. The Matrix and Limit reports require assignment of upper and lower limits. If either of these reports is requested, enter the SET LIMITS mode (Section 4.6) to set the upper and lower limits.
6. The Cutoff report requires definition of the cutoff point. If the Cutoff report is requested, enter the SET CUTOFF mode (Section 4.6) to define the cutoff point.
7. The Concentration report requires that standard concentrations and locations be defined. If the Concentration report is requested, enter the SET CONCENTRATION mode (Section 4.6) to define the standards.

After warm-up is complete:

8. Press PAGE– until the software returns to the Plate Reader mode and the display shows

```
_PLATE  READING
 M=1:405,R=4:655
 Mixing=ON(05s)
Warm  up  finished
```

or

```
_PLATE  READING
 M=1:405,R=4:655
 Mixing=ON(05s)
 Incu.  =ON(37°C)
```

9. Place the microplate in the reading chamber, slide the reading chamber door closed and press START/STOP.

4.5 During Plate Reading

When START/STOP is pressed, the display becomes

```
Reading:  Dual
     Start

■□□□□□□□□
```

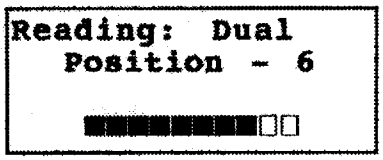
The current instrument status is shown on the second line and the first step indicator on the bottom line is filled. As the reader begins to mix the plate, the display changes to

```
Reading:  Dual
     Mixing

■■□□□□□□□
```

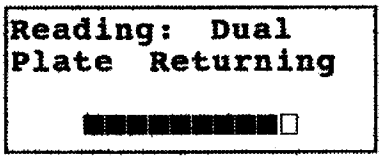
The Benchmark has sixteen photodiodes and reads two columns of eight wells simultaneously. The plate is moved into six different positions in order to read all twelve columns. When the plate is moved into position to read the first two columns, the display becomes

```
Reading:  Dual
  Position - 1

■■■□□□□□□
```

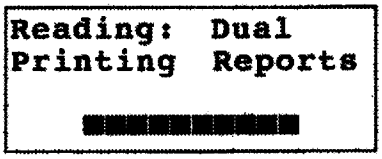
By the time the reader has moved the plate into the sixth position to read the last two columns, the display shows



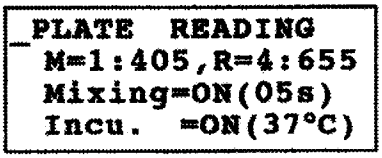
When the last two columns are read, the display changes to show that the plate has begun its return to the home position



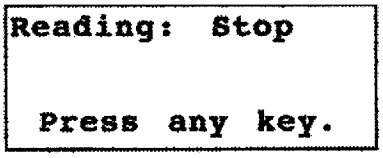
The plate reading may be aborted by pressing START/STOP any time during plate reading up until the plate returns to the home position and the words Plate Returning disappear from the status line. All data will be lost. When the plate is back to the home position, the software will prepare and then print the selected reports. While the reports are being printed, the display will show



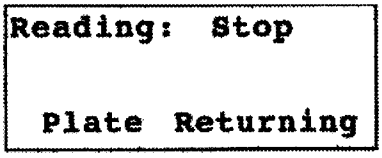
Printing may be interupted by pressing START/STOP. After all reports are printed, the software returns to the Plate Reading mode and the display returns to



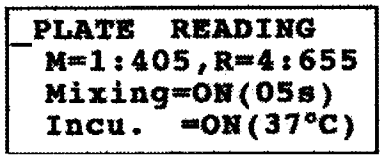
Note: If during the course of a plate reading the reading is aborted by pressing START/STOP, the carriage halts and data collection ceases. The display changes to



After any key is pressed, the plate carriage returns to the home position.



When the plate has been returned to the home position, the software returns to the Plate Reading mode and is ready to begin another plate reading

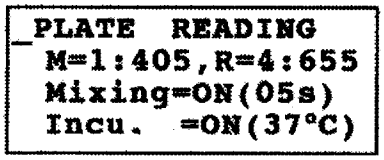


4.6 Detailed Operation

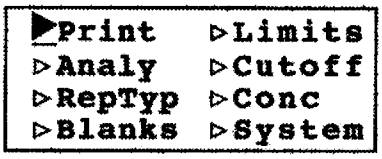
Set Analysis Mode

In this series of screens the user chooses single- or dual-wavelength measurement, assigns the measurement and reference (for dual-wavelength measurement) filters, specifies mixing time and chooses whether or not to use the 37 °C incubator.

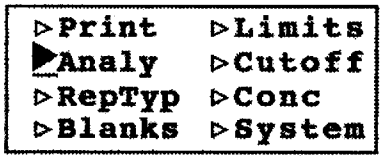
1. If the software is not displaying the Main menu, press PAGE– until it returns to the Plate Reading mode



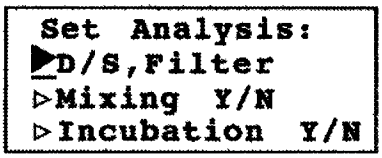
   and then press PAGE+ to get the Main menu



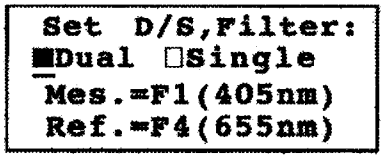
2. CURSOR down to *Analy*



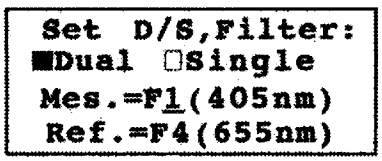
3. Press ENTER or PAGE+



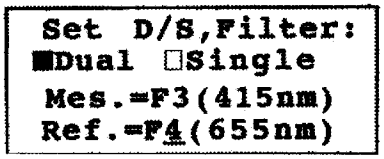
4. **To select single- or dual-wavelength measurement and to specify the filters,** press ENTER or PAGE+. Otherwise skip to step 5.



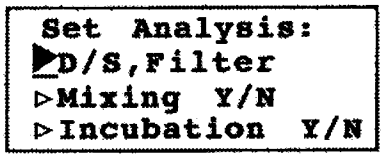
   a. Use VALUE to toggle between single- and dual-wavelength measurement. Press SELECT to seal the choice and the cursor will move to define the measurement filter



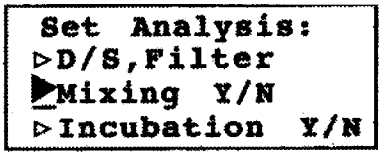
b. Use VALUE to roll through the list of filter choices. Press SELECT to seal the choice and move the cursor to define the reference filter (not necessary for single-wavelength measurements)



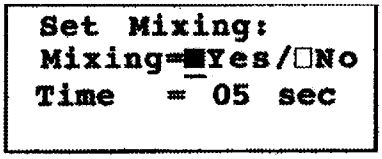
c. Use VALUE to roll through the list of filter choices. Press ENTER or PAGE– to finalize all settings and to return to the Set Analysis menu



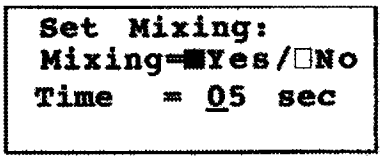
5. **To select mixing,** CURSOR down to *Mixing*. Otherwise skip to step 6.



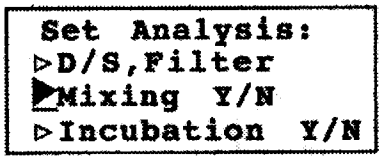
a. Press ENTER or PAGE+



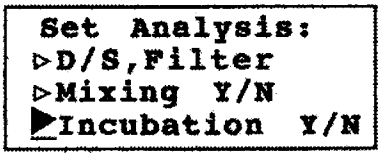
b. Use VALUE to toggle between Yes and No. Press SELECT to seal the choice and to advance the cursor to the tens digit of the mixing time



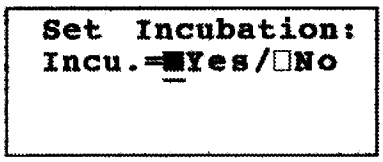
c. Use VALUE to set the tens digit and then CURSOR to the unit digit. Use VALUE to set the unit digit then press ENTER or PAGE– to finalize the selections and to return to the Set Analysis menu



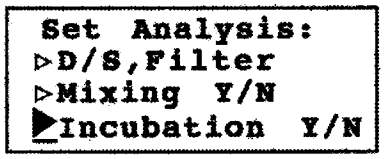
6. **To select the incubator**, CURSOR down to *Incubation*. Otherwise skip to step 7.



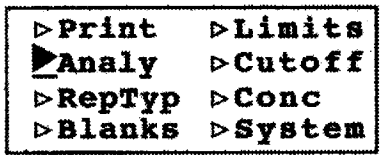
a. Press ENTER or PAGE+



b. Use VALUE to toggle between *Yes* and *No*. Press ENTER or PAGE– to finalize the choice and return to the Set Analysis menu



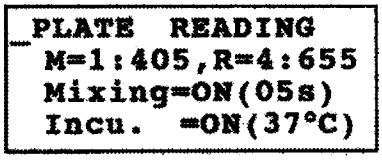
7. Press PAGE– to return to the Main menu



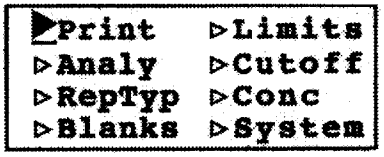
Set Report Types Mode

In this mode, the user chooses the reports to be generated and automatically printed.

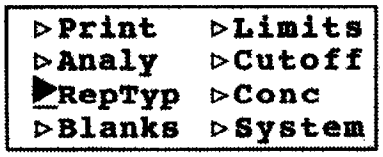
1. If the software is not displaying the Main menu, press PAGE– until it returns to the Plate Reading mode



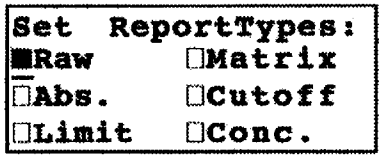
and then press PAGE+ to get the Main menu



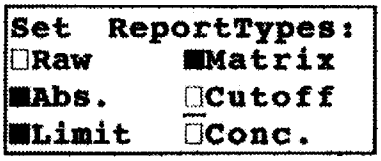
2. CURSOR down to *RepTyp*



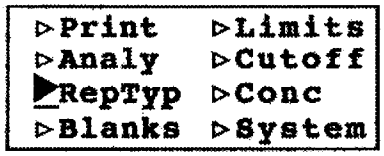
3. Press ENTER or PAGE+



4. Use VALUE to switch on or off the check box next to each report type. If a check box is darkened, the report will be generated and printed immediately following data collection. Use CURSOR to move from one report type to the next.



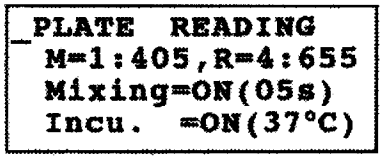
5. When all the desired reports have been selected, press ENTER or PAGE– to finalize the selections and to return to the Main menu



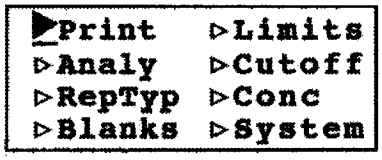
Set Blanks Mode

In this series of screens, the positions of blank wells are defined or edited. Blanks are required for all reports other than Raw. Once blank positions are defined, they are retained in memory until they are edited through the Set Blanks Mode.

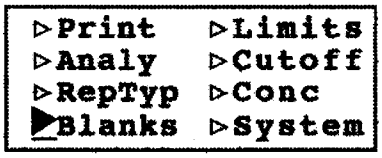
1. If the software is not displaying the Main menu, press PAGE- until it returns to the Plate Reading mode



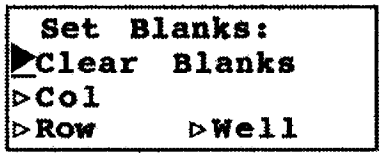
and then press PAGE+ to get the Main menu



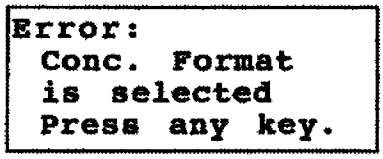
2. CURSOR down to *Blanks*



3. Press ENTER or PAGE+

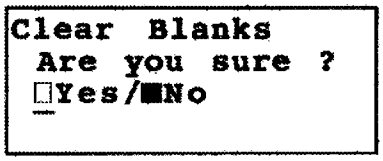


Note: If the display instead changes to

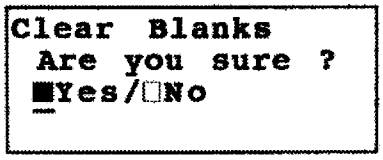


it is because a Concentration report has been included in the list of reports to be generated. Concentration reports require the operator to use one of the six pre-defined plate formats, and the location of the blanks may not be modified. In order to set blanks, first go into the Reports Type mode and de-select the Concentration report.

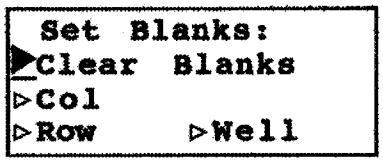
4. **To clear all previously defined blanks simultaneously**, press ENTER or PAGE+. Otherwise skip to step 5.



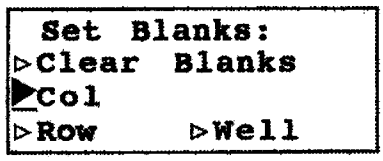
   a. Use VALUE to select the check box next to *Yes*



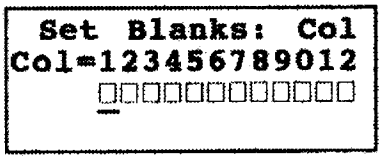
   b. Press ENTER or PAGE– to finalize the choice and return to the Set Blanks menu



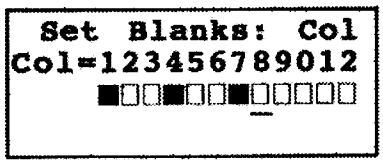
5. **To change the Blank status of one or more entire columns**, CURSOR down to *Col.* Otherwise skip to step 6.



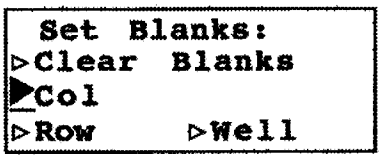
   a. Press ENTER or PAGE+



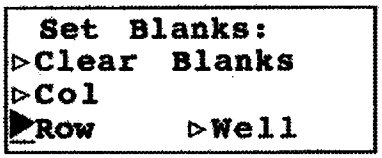
   b. Use VALUE to switch on or off the check box beneath each column. When the check box is selected, each well in the column will be considered a blank. Use CURSOR to move from one column to the next.



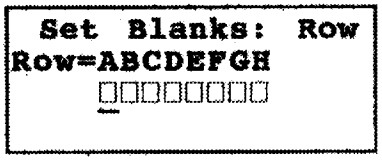
c. When the Blank status of each column is set, press ENTER or PAGE– to finalize the selection and to return to the Set Blanks menu



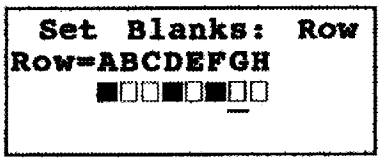
6. **To change the Blank status of one or more entire rows**, CURSOR down to *Row*. Otherwise skip to step 7.



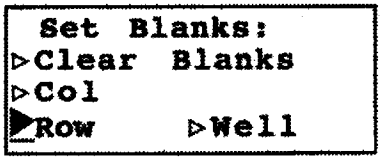
a. Press ENTER or PAGE+



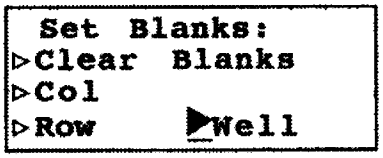
b. Use VALUE to switch on or off the check box beneath each row. When the check box is selected, each well in the row will be considered a blank. Use CURSOR to move from one row to the next.



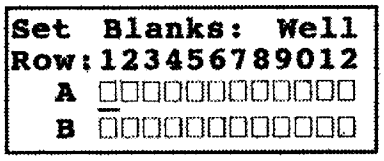
c. When the Blank status of each row is set as desired, press ENTER or PAGE– to finalize the selection and to return to the Set Blanks menu



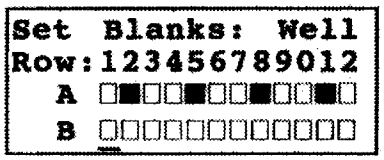
7. **To change the Blank status of one or more individual wells**, CURSOR over to *Well*. Otherwise skip to step 8.



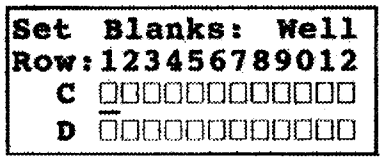
a. Press ENTER or PAGE+



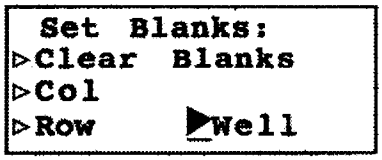
b. Use VALUE to switch on or off the check box beneath each well in Row A. When the check box is darkened, the well will be considered a blank. Use CURSOR to move from one well to the next within the row. Use SELECT to move the cursor down to the next row.



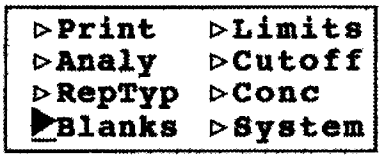
c. Use VALUE to switch on or off the check box beneath each well in Row B. Use CURSOR to move from one well to the next within the row. When the Blank status of each well in Row B is set, press SELECT to bring up a new display for the next two rows



d. Use VALUE, CURSOR and SELECT as described above to set the Blank status of each well. When the Row H is set, press ENTER or PAGE– to finalize the selection and to return to the Set Blanks menu

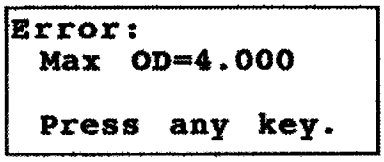


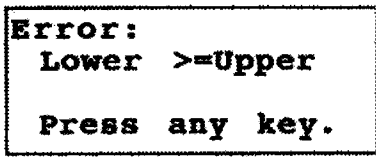
8. Press PAGE– to return to the Main menu



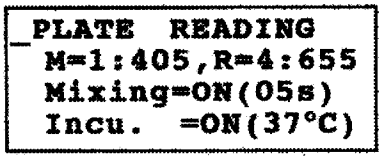
Set Limits Mode

If either the Matrix or Limit report is selected, then Upper and Lower limits must be defined in the Set Limits mode. The Upper limit may not exceed 4.000, nor may it be less than the Lower limit. If either of these situations occurs, an error message will be displayed.

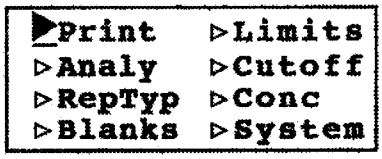




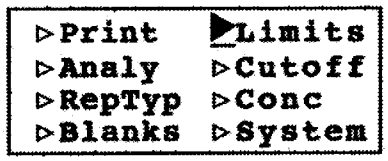
1. If the software is not displaying the Main menu, press PAGE– until it returns to the Plate Reading mode



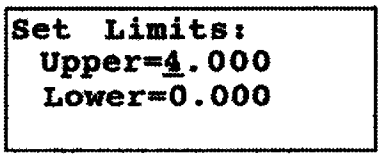
and then press PAGE+ to get the Main menu



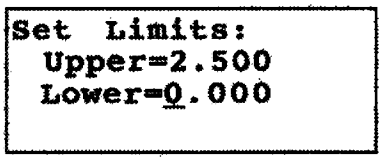
2. CURSOR down and over to *Limits*



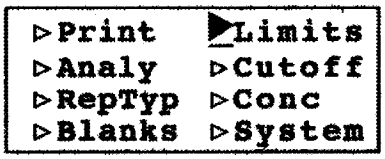
3. Press ENTER or PAGE+



4. The cursor will be below the units digit of the Upper limit. Use VALUE to set the unit digit, then CURSOR over to the next position. Use VALUE to set the tenths digit then CURSOR over to the hundredths digit. Set the last two digits with VALUE, then press SELECT to move the cursor to the unit digit of the Lower limit.



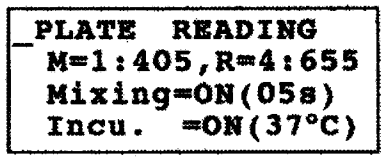
5. Use VALUE to set the unit digit, CURSOR to the next position and set it. When all four digits of the Lower limit have been set, press ENTER or PAGE– to finalize the selection and to return to the Main menu



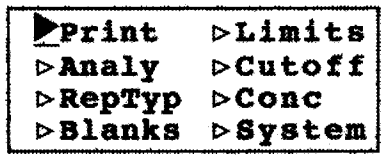
Set Cutoff Mode

This series of screens is used to specify the parameters for a Cutoff report.

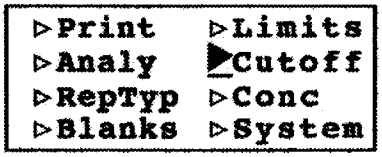
1. If the software is not displaying the Main menu, press PAGE– until it returns to the Plate Reading mode



and then press PAGE+ to get the Main menu

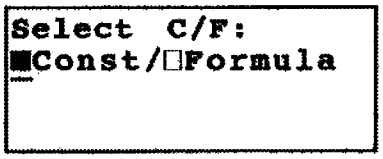


2 CURSOR down and over to *Cutoff*

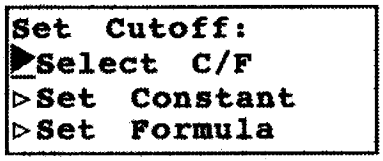


3. Press ENTER or PAGE+

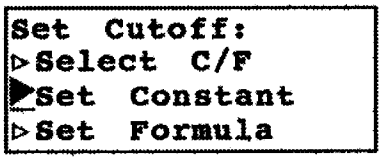
4. Press ENTER or PAGE+



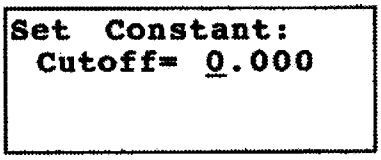
5. There are two methods for determining the cutoff value used in the Cutoff report. In the constant method, the user simply assigns a constant value as the cutoff value. In the formula method, the software calculates the cutoff value based on the mean absorbance values of the positive and negative controls (see discussion on Cutoff report in Section 4.1). Use VALUE to darken the check box next to the desired method of determining the cutoff value. Press ENTER or PAGE– to confirm the choice and to return to the Set Cutoff menu



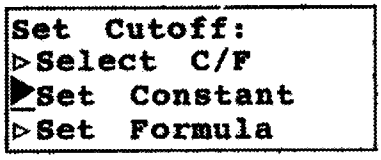
6. If the Formula method was chosen in step 5, then skip to step 7. If Constant was chosen in step 5, CURSOR down to *Set Constant*



a. Press ENTER or PAGE+



b. Use VALUE to set the unit digit of the constant. CURSOR over to the tenths digit and use VALUE to adjust the setting. Use CURSOR and VALUE to set the remaining two digits of the constant. When the last digit is set, press ENTER or PAGE– to confirm the setting and to return to the Set Cutoff menu



c. Skip to step 10.

7. CURSOR down to *Set Formula*

```
Set Cutoff:
▷Select C/F
▷Set Constant
▶Set Formula
```

a. Press ENTER or PAGE+

```
Set Formula: #4
▶Set STDs
▷Set Positives
▷Set Negatives
```

b. In this display, the number on the top line is the presently-defined number of replicates of the positive and negative standards. As many as eight replicates may be specified. To change the number of standards press ENTER or PAGE+

```
Set Formula:
 STDs= 6 (0-8)
```

c. Use VALUE to select the desired number of standards and press ENTER or PAGE– to confirm the choice and to return to the Set Formula menu

```
Set Formula: #6
▶Set STDs
▷Set Positives
▷Set Negatives
```

8. To specify the location of each positive control, CURSOR to *Set Positives*

```
Set Formula: #6
▷Set STDs
▶Set Positives
▷Set Negatives
```

a. Press ENTER or PAGE+

```
Set Pos.: #6
 Pos.1= A-01
 Pos.2= A-01
 Pos.3= A-01
```

b. Use VALUE to specify the row of positive standard #1. CURSOR over to set the column number. Press SELECT to move the cursor down to set the row of the second standard. Continue using VALUE, CURSOR and SELECT to set the location of the first three standards. After the column of the third positive standard is set, press SELECT and a new screen will appear

```
Set Pos.: #6
 Pos.4= A-01
 Pos.5= A-01
 Pos.6= A-01
```

c. Continue setting the positions of the other positive standards. When the column position of the last positive standard is set, press ENTER or PAGE– to confirm all the choices and to return to the Set Formula menu

```
Set Formula: #6
▷Set STDs
▶Set Positives
▷Set Negatives
```

9. To specify the location of each negative control, CURSOR to *Set Negatives*

```
Set Formula: #8
▷Set STDs
▷Set Positives
▶Set Negatives
```

a. Press ENTER or PAGE+

```
Set Neg.: #6
 Neg.1= A-01
 Neg.2= A-01
 Neg.3= A-01
```

b. Use VALUE to specify the row of negative standard #1. CURSOR over to set the column number. Press SELECT to move the cursor down to set the row of the second standard. Continue using VALUE, CURSOR, and SELECT to set the location of the first three standards. After the column of the third negative standard is set, press SELECT and a new screen will appear

```
Set Neg.: #6
 Neg.4= A-01
 Neg.5= A-01
 Neg.6= A-01
```

c. Continue setting the positions of the other negative standards. When the column position of the last negative standard is set, press ENTER or PAGE– to confirm all the choices and to return to the Set Formula menu

```
Set Formula: #8
▷Set STDs
▷Set Positives
▶Set Negatives
```

d. Press PAGE– to return to the Set Cutoff menu

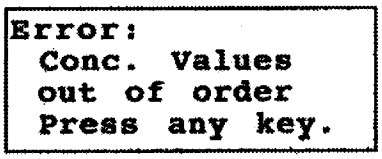
```
Set Cutoff:
▷Select C/F
▷Set Constant
▶Set Formula
```

10. Press PAGE– to return to the Main menu

```
 ▷Print   ▷Limits
 ▷Analy   ▶Cutoff
 ▷RepTyp  ▷Conc
 ▷Blanks  ▷System
```

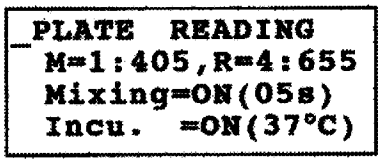
Set Concentration Mode

In this series of screens, the user provides the information necessary for the software to make a calibration curve which is then used to calculate sample concentrations in the Concentration report. The software will assign one of six pre-defined formats to the microplate based on the number of samples, number of standards, and the number of replicates of each. Because of the manner in which the calibration curve is calculated, the concentrations of standards must be input in either increasing or decreasing order, *i.e.*, standard 1 must be the most concentrated standard and standard 2 the next-most concentrated, etc., or standard 1 must be the least concentrated standard, standard 2 the next-least concentrated, etc. (See the description of the Concentration report in Section 4.1 for complete details.) If the concentrations of the standards do not uniformly increase or uniformly decrease, an error message will be displayed.

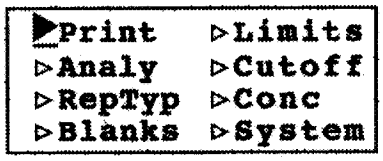


This message will persist until the concentrations are corrected.

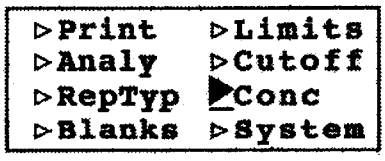
1. If the software is not displaying the Main menu, press PAGE– until it returns to the Plate Reading mode



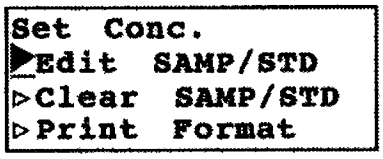
and then press PAGE+ to get the Main menu



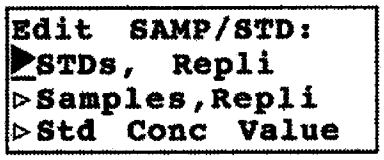
2. CURSOR to *Conc*



3. Press ENTER or PAGE+ to bring up the Set Concentration menu



4. To assign the number of standards and samples, the number of replicates of each, and to assign concentrations of the standards, press ENTER or PAGE+



a. Press ENTER or PAGE+

```
Set  STDs,Repli:
 STDs = 5  (0~7)
 Repli= 2  (1~2)
```

b. Use VALUE to set the number of standards then press SELECT to move the cursor to the next line and use VALUE to set the number of standard replicates. When the number of standard replicates is set, press ENTER or PAGE– to confirm the selections and to return to the Edit Samples/Standards menu.

```
Edit  SAMP/STD:
▶STDs,  Repli
▷Samples,Repli
▷Std Conc Value
```

c. CURSOR down to *Samples, Repli*

```
Edit  SAMP/STD:
▷STDs,  Repli
▶Samples,Repli
▷Std Conc Value
```

d. Press ENTER or PAGE+

```
Set  SAMPs,Repli:
 Samps= 20(0~88)
 Repli= 2  (1~2)
```

e. The cursor will be under the tens digit of the number of samples. Use VALUE to set the tens digit, then CURSOR over to the unit digit and use VALUE to set the unit digit. The software will not allow an entry greater than 88. When the number of samples is set, press SELECT to move the cursor to the next line and use VALUE to set the number of sample replicates. When the number of sample replicates is set, press ENTER or PAGE– to confirm the selections and to return to the Edit Samples/Standards menu.

```
Edit  SAMP/STD:
▷STDs,  Repli
▶Samples,Repli
▷Std Conc Value
```

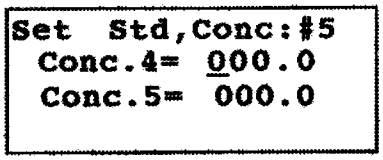
f. CURSOR down to *Std Conc Value*

```
Edit  SAMP/STD:
▷STDs,  Repli
▷Samples,Repli
▶Std Conc Value
```

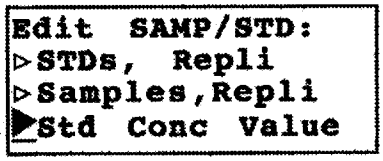
g. Press ENTER or PAGE+ to bring up the Set Standard Concentration screen

```
Set  Std,Conc:#5
 Conc.1= 000.0
 Conc.2= 000.0
 Conc.3= 000.0
```

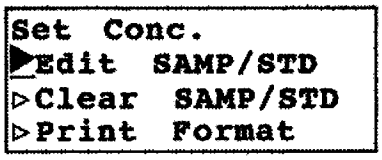
h. The number on the top line indicates the currently-defined number of standards. The cursor begins below the hundreds digit of Standard 1. Use VALUE to set the first digit, then CURSOR to the second digit. Use VALUE and CURSOR to set all four digits of the first standard's concentration. Press SELECT to accept the setting and to move the cursor to define the second standard. After setting the concentration of the third standard, press SELECT and a new screen will appear to allow definition of the remaining standards.



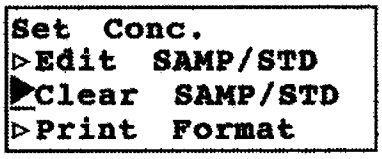
i. Use VALUE, CURSOR, and SELECT to set the concentrations of the last two standards as above. When the last standard concentration has been set, press ENTER or PAGE– to confirm the settings and to return to the Edit Samples/Standards screen



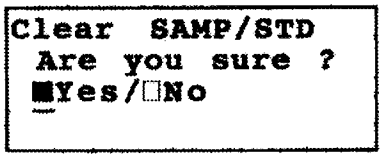
j. Press PAGE– to return to the Set Concentration mode screen



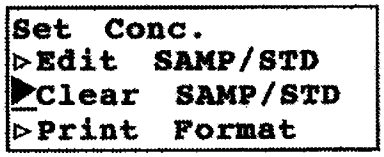
5. To reset the number of samples and standards to zero, CURSOR down to *Clear SAMP/STD*



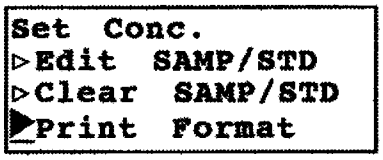
a. Press ENTER or PAGE+



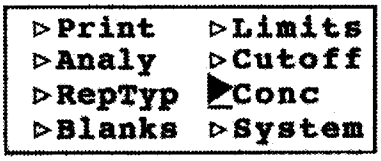
b. Use VALUE to toggle between *Yes* and *No*, press ENTER or PAGE– to confirm the choice and to return to the Set Concentration menu



6. To print the current format, including locations of the blanks and samples and the concentrations of standards, CURSOR down to *Print Format*



and press ENTER or PAGE+ and a complete description of the current format will be printed. After printing is completed, the screen returns to the Main menu



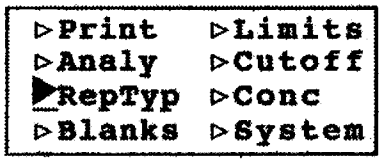
4.6 Printing Reports

All reports selected in the SET REPORT TYPES mode will be printed automatically at the end of plate reading. Since the memory buffer stores plate data from the previous ten readings, other reports may be generated after a plate is read. When a new plate reading is completed, the oldest data set in memory is removed and all other data sets shift up one position. The most recent data set is put into position 0 and the oldest data set is in position 9. When a new plate is read, the data in position 0 shift to position 1, the data in position 8 shift to position 9 and the data in position 9 are lost. If a plate reading is aborted before completion, none of the new data are saved.

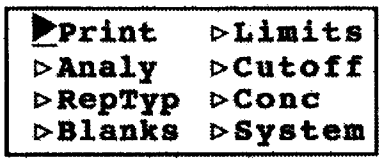
It is also possible to print a report showing system configuration. The data include: the location of blanks, reading mode, limit settings, cutoff settings, concentration settings, and sample positions.

To generate additional reports on saved data sets:

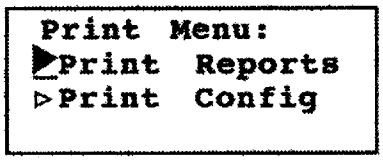
1. Go to Set Reports mode and choose the additional reports to be printed (see details in previous section). When finished, press PAGE– until back at the Main menu



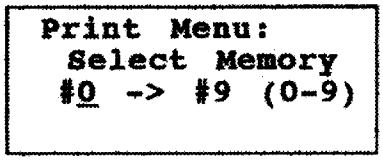
2. CURSOR to *Print*



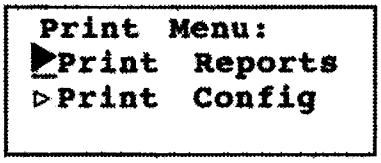
3. Press ENTER or PAGE+ to go to the Print menu



4. Press ENTER or PAGE+

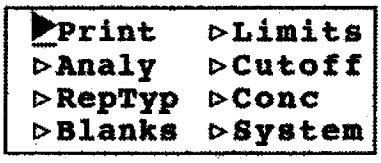


5. It is possible to print the same reports for a range of the stored data sets. Use VALUE to select the first report to print and then CURSOR to the second position and use VALUE to select the last report to print. Press ENTER to begin printing. Printing may be interupted by pressing START/STOP. No other keys respond while the printer is printing. When printing is completed, the screen will return to the Print menu

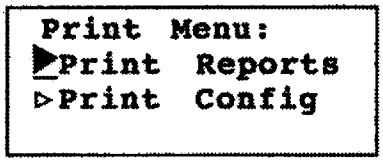


To print configuration data:

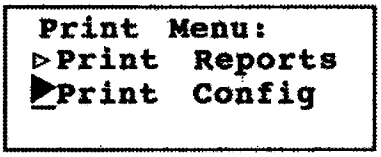
1. From the main menu, CURSOR to *Print*



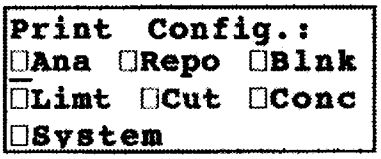
2. Press ENTER or PAGE+



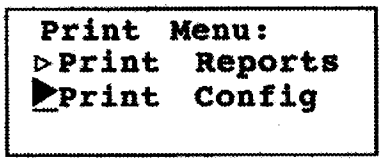
3. CURSOR to *Print Config*



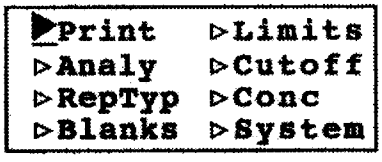
4. Press ENTER or PAGE+ to see the Print Configuration menu



5. Use VALUE to toggle on or off the check box next to each report. If the check box is darkened, the report will be printed. Use CURSOR to move from one report to the next. When all choices are made, press ENTER to begin printing. When printing is completed, the screen reverts to the Print menu



6. Press PAGE– to return to the Main menu



Section 5
Installing Interference Filters and Changing the Lamp

5.1 Changing Interference Filters

1. With the power off, open the rear cover of the instrument by sliding the release latch on the top rear of the unit toward the rear of the unit.
2. Locate the filter wheel between the lamp and the left side of the instrument. Note that the filter wheel has six filter holes. Four filters must be installed in the unit before the Benchmark can complete the self test.
3. Rotate the filter wheel until the desired filter is at about the 2:00 o'clock position. Grasp the filter firmly with the thumb and the index finger of your right hand and rotate the filter counter-clockwise 90° to remove it from the filter wheel.
4. Install the new filter by holding the filter between your index finger and thumb of your right hand. Rotate the desired filter position in the wheel to the 2:00 o'clock position. Line up the two prongs on the filter with the two slots in the filter wheel. Turn the filter 90° clockwise to install.

5.2 Changing the Lamp

Warning: Electrical shock hazard! Always unplug the instrument from the AC power source before changing the lamp.

Warning: The lamp may be very hot! Use necessary caution by allowing the lamp to cool before attempting removal.

1. Open the rear cover of the instrument by sliding the release latch on the top rear of the unit toward the rear of the reader.
2. Remove the two screws that hold the black lamp cover in place. Lift the cover up and away from the instrument.
3. Press down on the spring wire that holds the lamp in place. This will allow the lamp to be pulled free from the lamp housing.
4. Holding the ceramic base in one hand and the lamp housing in the other, carefully pull the lamp free of the base.
5. Carefully insert the new lamp firmly in the ceramic base. Apply even pressure when inserting the lamp so that the bulb does not become misaligned in the reflector housing. Also, be careful not to touch the bulb or the inside of the reflector housing during installation.
6. Position the lamp in the bezel and hold the lamp in this position until it is secured with the spring wire.
7. Replace the lamp cover.
8. Close the rear cover.

Section 6
Troubleshooting

The Benchmark Microplate Reader constantly monitors several vital functions and will display appropriate messages on the LCD when an error condition is encountered.

1. LCD appears blank on power up.	
a. Power switch in not turned on.	Turn on the power switch on the rear panel.
b. Unit is not plugged into AC outlet or power cord is not attached to the instrument.	Check power cord connections at outlet and on the rear panel of the instrument.
c. The AC outlet is dead.	Check circuit breakers or fuses.
d. Instrument has blown a fuse.	Check both fuses on the rear panel of the instrument, and replace if necessary. Replace only with the same type fuse. If the instrument continues to blow fuses, discontinue use and contact your Bio-Rad service representative immediately.
2. Instrument displays "Error: Light Bulb Burnt Out" message indicating that the bulb is burned out.	Check whether lamp is emitting light.
a. Lamp is not emitting light.	Replace lamp as described in Section 5.2.
b. Lamp is emitting light. Check the alignment of bulb in the glass reflector.	Check alignment of the lamp in the bezel. Align or replace lamp if necessary.
3. Printer will not print on command.	
a. Printer paper is not installed properly. Refer to Section 3.2 for details.	Verify that the paper is properly installed.
4. "Error: Printer Hard" displayed	Printer hardware error. Contact your local Bio-Rad representative.
5. Plate carriage jams during reading, and "Error: Plate Carrier" is displayed.	
a. Plate is not seated in carriage properly.	Press the START/STOP button immediately. Position the plate carefully in the carriage, making certain that it is properly seated.
6. "Error: Filter Missing" displayed.	
a. A filter is missing from the wheel.	Install a filter in the empty position.
b. The filters are not properly seated.	Check that all filters are properly seated.

7. Wells have color, but absorbance values seem low.	
a. Incorrect filter used for measurement and or reference wavelength.	Check wavelength used in analysis. Read plate in single wavelength mode at all wavelengths to verify that the proper filter was used. The measurement wavelength should produce the highest absorbance values. The reference wavelength produces the lowest absorbance values.
b. The best measurement wavelength filter for the substrate in use has not been installed in the filter wheel.	Check references for the substrate you are using, or determine the best measurement wavelength by analyzing the substrate product on a scanning spectrophotometer. Custom filters are available from 340 to 750 nm.
c. The filters have been switched in the wheel.	Check that the filters are installed in the correct position.
8. "Error: Filter Wheel Jammed" displayed.	Turn off the instrument, open the rear cover and inspect the filter wheel. Turn by hand to verify that the wheel is moving freely. Make sure that all the filters are properly installed and firmly seated. Contact your local Bio-Rad representative if the filter wheel is jammed or if the problem persists.
9. "Error: SRAM Memory" displayed.	Reader failed SRAM check. Possible battery backup failure or ROM was updated. Contact your local Bio-Rad service center or local representative.
10. "Error Photo Sensor" displayed.	Contact your local Bio-Rad service center or local representative.
11. "Error A/D Hard" displayed.	A/D converter hardware error. Contact your local Bio-Rad service center or local representative.
12. "Error: Incubator Hard" displayed.	Incubator hardware error. Contact your local Bio-Rad service center or local representative.

Section 7
Specifications

7.1 Technical Specifications

Operating panel	Membrane keypad with eight keys
Display	4 lines x 16 characters LCD
Printer	20 column, 5x7 dot matrix, thermal, built-in
Computer Interface	Bi-directional, RS-232C serial communication port
Mixing	0-99 seconds
Incubator	37° +/- 0.5 °C constant or Off
Well-to-well uniformity	+/- 0.5 °C at RT=25 °C
Incubator warm-up time	30 minutes at RT=25 °C
Optical photometric methods	Single or dual wavelength
Spectral range	340 to 750 nm
Indication range	0.000–4.000 A.U.
Resolution	0.001 O.D.
Accuracy	
Single wavelength	< 1.5% at 1.000 O.D.
Dual wavelength	< 2.0% at 1.000 O.D.
Linearity	
340–399 nm	< 2% at 0.000–2.000 O.D.
400–750 nm	< 3.5% at 0.000–4.000 O.D.
Channel-to-channel error	
340–399 nm	< 1.5% at 0.000–2.000 O.D.
400–750 nm	< 1.5% at 0.000–3.000 O.D.
Reproducibility	
340–399 nm	< 2% at 0.000–2.000 O.D.
400–750 nm	< 3.5% at 0.000–4.000 O.D.
Stability and Drift	Automatic calibration at every reading
Instrument reading time	Single wavelength: 7 seconds Dual wavelength: 15 seconds
Light source	20 W Tungsten halogen
Photodetectors	16 silicon photodiode
Filters	8–10 nm bandwidth filters at 340, 405, 490 and 655 nm supplied as standard. Custom filters from 340 to 750 nm are available.
Filter capacity	Six
Compatible microplates	Most standard rigid and flexible 96-well microplates with flat, 'U', or 'V' bottoms
Data reports	Raw, absorbance, matrix, limit, cutoff, and concentration
Assay blanks	Arbitrary
Data buffer	10 sets of data stored in memory
Memory back-up	Five year lithium battery
Self diagnosis	Checks and reports on: lamp, battery, interference filters, incubator and plate transport
Dimensions	315 mm (W) x 285 mm (L) x 130 mm (H)
Weight	5.4 kilograms
Power consumption	100 watts maximum
Line voltage	100–240 VAC at 47–63 Hz
Operating temperature	5° to 35 °C
Storage temperature	-20° to 50 °C
Operating and storage humidity	0 to 95% non-condensing

7.2 RS-232-C Interface Specifications

The following specification describes the syntax and language required for computer control of the reader. In addition, the Benchmark reader will perform passive unidirectional data transmission. The instrument will automatically transmit the data in ASCII code after each reading if it is properly connected to a host computer. See the "RTPLATE" command for an example of ASCII output.

Note: This automatic output will be of Measurement absorbance minus Reference absorbance when in Dual Wavelength mode (*i.e.*, only one set of 96 numbers).

Signals

TXD: transmit data

RXD: receive data

DTR: data terminal ready

DSR: data set ready

CTS: clear to send

RTS: ready to send

SIGNAL GROUND (SHIELD GROUND to hood / chassis ground)

Transmission

Baud rate	9600
Data width	8
Stop width	1
Parity	None

Data

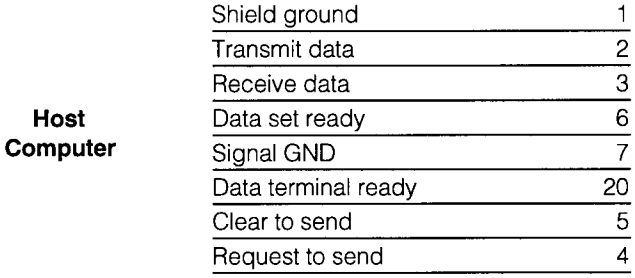
All data must be transmitted in ASCII.

The READER command interpreter must be "case blind" (no distinction between upper or lower case ASCII characters).

All READER commands can be distinguished from each other by the first two characters of the command.

Signal Assignment

Pin #	READER Signal type	Host Signal Pair
1	SHIELD GROUND	SHIELD GROUND
2	TRANSMIT DATA	RECEIVE DATA
3	RECEIVE DATA	TRANSMIT DATA
4	REQUEST TO SEND	CLEAR TO SEND
5	CLEAR TO SEND	REQUEST TO SEND
6	DATA SET READY	DATA TERMINAL READY
7	SIGNAL GROUND	SIGNAL GROUND
20	DATA TERMINAL READY	DATA SET READY



7.3 Command Language for the Benchmark Microplate Reader

Syntax

SYNTAX:<device name><space><command>[<space><command args.>] <cr>

device name: "EIA.READER" -Benchmark Microplate READER

Commands

1. <command>: "ID"-request device ID. Benchmark id; "Benchmark"; no arguments
   <response>: "ERE" <space> <error code> <space> <id> <cr>
   <id>: "Benchmark": Benchmark Microplate READER id code
2. <command>: "AQ"–Acquire remote control of the device and lock front panel key pad; no arguments.
   When acquired, the reader will be in the Remote Control mode. The LCD will display Remote Control mode, and the printer will automatically be deactivated. The reader will stay in the remote control mode until the release (RL) command is received or the START/STOP button on the front panel is pressed.
   <response>: "ERE" <space> <error code> <cr>
3. <command>: "RL"- Release remote control of the device and release front panel key pad; no arguments
   <response>: "ERE" <space> <error code> <cr>
4. <command>: "RS"- Reset device to power up configuration local mode; no arguments
   <response>: "ERE"<space> <error code> <cr>
5. <command>: "MR"- Transmit maintenance report; no arguments
   <response>: "ERE" <space> <error code> <cr> <mrecords> <cr>
   <mrecords>: maintenance records;
   "On/off:" <#on/off> <cr>: Number of times the reader was turned on.
   "Hours:" <#hours> <cr>: Number of hours the reader was on.
   "Plates:" <#plates> <cr>: Number of plates read.
   Note: <#on/off> <#hours> and <#plates> are four ASCII encoded decimal values. This information is kept in battery backup RAM.
6. <command>: "RM"–Reset maintenance report values; no arguments
   <response>: "ERE" <space><error code><cr>
7. <command>: "RWELL"–Read a well; arguments <c1>, <rw>, <wp1>, <wp2>
   <cmd args> c1– Column number ASCII encoded decimal (1–12)
   rw– Row number ASCII encoded decimal (1–8)
   wpl– Measurement filter position ASCII encoded decimal (1–6)
   wp2– Reference filter position ASCII encoded decimal (1–6). Optional: only present for Dual wavelength reading
   <response>: "ERE" <space> <error code> <space> <abs. val1> <space> <optional abs. val2 for reference wavelength> <cr>
   <cmd args> <abs. val1>: Absorbance values at measurement wavelength (ASCII encoded floating point format) *e.g.* "1.234"
   <abs val2>: Absorbance values at reference wavelength (ASCII encoded floating point format)

8. <command>: "RPLATE" Read a plate; arguments <mix>, <wp1>,<wp2>

<cmd args>: mix– Mixing time before reading (in seconds) ; ASCII encoded decimal (0–99)

wp1– Measurement filter position; ASCII encoded decimal (1–6)

wp2– Reference filter position; ASCII encoded decimal (1–6). Optional; only present for Dual wavelength reading

<response>: "ERE" <space> <error code> <space> <abs. data> <cr>

9. <command>: "ISTATUS" Request incubator status from reader; no arguments

<response>: "ERE" <space> <eror code> <space> <incu. status> <cr>

<incu. status>: on/off "0"; incubator OFF

"1"; incubator ON

temp. <temp.> is two ASCII encoded decimal value of the incubator temperature, *e.g.* "37 °C"

10. <command>: "INCU" Incubator ON/OFF control; arguments <on/off>

<cmd args>: on/off "0"; incubator OFF

"1"; incubator ON

<response>: "ERE" <space> <error code> <cr>

11. <command>: "FSTATUS" Request filter wheel status from the reader; no arguments

<response>: "ERE" <space> <error code> <space> <filter statis> <cr>

<filter status>: <pos.1> <space> <pos.2> <space> <pos.3> <space> <pos.4> <space> <pos.5> <space> <pos.6>

<filter status> pos.1 ASCII encoded decimal number of the wavelength filter at position 1 (F1) *e.g.* "405"

pos.2 ASCII encoded decimal number of the wavelength filter at position 2 (F2)

pos.3 ASCII encoded decimal number of the wavelength filter at position 3 (F3)

pos.4 ASCII encoded decimal number of the wavelength filter at position 4 (F4)

pos.5 ASCII encoded decimal number of the wavelength filter at position 5 (F5)

pos.6 ASCII encoded decimal number of the wavelength filter at position 6 (F6)

12. <command>: "RTPLATE" Retransmit the last plate read; no arguments

<response>: "ERE" <space> <error code> <space> <abs. data> <cr>

Absorbance Data

<abs. data>:	<header> <filter info> < Mes.data> <cr> or <header> <filter info> <Mes. data> <cr> <Ref.data> <cr> Ref. data present for dual wavelength readings.
<header>:	"BIO-RAD Benchmark READER"<cr>
<filter info>:	"Mes. filter:" <wp1><cr>
	"Ref. filter:" <wp2><cr> - Present for Dual wavelength reading
<Mes. data>:	".begin" <cr> <8 rows of 12 wells of abs. values> <checksum> <cr>
	".end" <cr>
	Measurement wavelength absorbance values are in ASCII encoded floating point format. Each absorbance value is separated by a space.
	Row1=wells (A1-A12), Row2=wells (B1-B12), ...Row8=wells (H1-H12).
<Ref. data>:	".begin" <cr> <8 rows of 12 wells of abs. values> <checksum> <cr>
	".end" <cr>
	Reference wavelength absorbance values are in ASCII encoded floating point format. Absorbance values are separated by a space.
	Row1=wells (A1-A12), ROW2=wells (B1-B12, ...Row8=wells (H1-H12)
<checksum>:	The checksum is a two byte sum in ASCII encoded decimal (0–255) of all the ASCII characters transmitted in the measurement or reference data block. The checksum does not include the character string ".begin"<cr> in the checksum calculations.

The following is an example of both <Mes. data> and <Ref. data>:

". begin"<cr>

" 0.101 0.102 0.103 0.104 0.105 0.106 0.107 0.108 0.109 0.110 0.111 0.112"<cr>

" 0.201 0.202 0.203 0.204 0.205 0.206 0.207 0.208 0.209 0.210 0.211 0.212"<cr>

" 0.301 0.302 0.303 0.304 0.305 0.306 0.307 0.308 0.309 0.310 0.311 0.312"<cr>

" 0.401 0.402 0.403 0.404 0.405 0.406 0.407 0.408 0.409 0.410 0.411 0.412"<cr>

" 0.501 0.502 0.503 0.504 0.505 0.506 0.507 0.508 0.509 0.510 0.511 0.512"<cr>

" 0.601 0.602 0.603 0.604 0.605 0.606 0.607 0.608 0.609 0.610 0.611 0.612"<cr>

" 0.701 0.702 0.703 0.704 0.705 0.706 0.707 0.708 0.709 0.710 0.711 0.712"<cr>

" 0.801 0.802 0.803 0.804 0.805 0.806 0.807 0.808 0.809 0.810 0.811 0.812"<cr>

<checksum><cr>

" . end"<cr>

Note: Absorbance values greater then 4.000 shall be transmitted as an asterisk.

Error Codes

"0000"–No error
"8071"–Invalid Command
"8072"–Parameter Out of Range
"8073"–Device Not In Remote Mode
"8074"–Device Busy
"8075"–Not assigined
"8076"–Not assigned
"8077"–Light Bulb Burned Out
"8078"–Hardware Error
"8079"–Memory Error
"8080"–Not assigned
"8081"–Not assigned
"8082"–Not assigned
"8083"–Incubator error

Note: Until the Benchmark Reader receives the "AQ" command, the reader will be unable to accept any other command and will only respond with the error code of "8073"- Device Not In Remote Mode.

Once the reader accepts the "AQ" command, the mode will be turned into the "Remote Mode" and then the front key pad will be locked until pressing "START/STOP" key or accepting "RL" command.

Bio-Rad Laboratories

Molecular Bioscience Group

2000 Alfred Nobel Drive
Hercules, California 94547
Telephone (510) 741-1000
Fax: (510) 741-5800

Australia, *Bio-Rad Laboratories Pty Limited, Block Y Unit 1, Regents Park Industrial Estate, 391 Park Road, Regents Park, NSW 2143 • Phone 02-9414-2800 • Fax 02-9914-2889*
Austria, *Bio-Rad Laboratories Ges.m.b.H., Auhofstrasse 78D, 1130 Wien • Phone (1) 877 89 01 • Fax (1) 876 56 29*
Belgium, *Bio-Rad Laboratories S.A./N.V., Begoniastraat 5, 9810 Nazareth Eke • Phone 09-385 55 11 • Fax 09-385 65 54*
Canada, *Bio-Rad Laboratories (Canada) Ltd., 5671 McAdam Road, Mississauga, Ontario L4Z 1N9 • Phone (905) 712-2771 • Fax (905) 712-2990*
China, *Bio-Rad Laboratories, 14, Zhi Chun Road, Hai Dian District, Beijing 100088 • Phone (01) 2046622 • Fax (01) 2051876*
Denmark, *Bio-Rad Laboratories, Symbion Science Park, Fruebjergvej 3, DK-2100 Købebhavn ¥ • Phone 39 17 9947 • Fax 39 27 1698*
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Pacific, *Bio-Rad Laboratories, Unit 1111, 11/F., New Kowloon Plaza, 38, Tai Kok Tsui Road, Tai Kok Tsui, Kowloon, Hong Kong • Phone 7893300 • Fax 7891257*
Singapore, *Bio-Rad Laboratories (Singapore) Ltd., 221 Henderson Rd #05-19, Henderson Building, Singapore 0315 • Phone (65) 272-9877 • Fax (65) 273-4835*
Spain, *Bio-Rad Laboratories, S. A. Avda Valdelaparra 3, Pol. Ind. Alcobendas, E-28100 Alcobendas, Madrid • Phone (91) 661 70 85 • Fax (91) 661 96 98*
Sweden, *Bio-Rad Laboratories AB, Ekensbergsvägen 128, Box 1097, S-172 22 Sundbyberg • Phone 46-(0)8-627 50 00 • Fax 46-(0)8-627 54 00*
Switzerland, *Bio-Rad Laboratories AG, Kanalstrasse 17, Postfach, CH-8152 Glattbrugg • Phone 01-809 55 55 • Fax 01-809 55 00*
United Kingdom, *Bio-Rad Laboratories Ltd., Bio-Rad House, Maylands Avenue, Hemel Hempstead, Herts HP2 7TD • Free Phone 0800 181134 • Fax 01442 259118*

4000091 Rev C *SIG 072296 Printed in USA*