

Model 3550 Microplate Reader

Instruction Manual

Catalog Numbers 170-6601, 170-6602

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Section 1

Introduction

The Model 3550 Microplate Reader is a vertical pathlength photometer which measures the absorbances of the contents of the wells of a 96 well microtitration plate. Single or dual wavelength measurements can be made, and absorbance values are measured to three decimal places.

The Model 3550 Microplate Reader can be programmed by entering commands through the membrane keypad to select analysis parameters, define plate formats, and select report types and print options. Four different reports can be generated: absorbance, limit, matrix, and evaluation (which includes extrapolation of sample concentrations from a linear regression of standard values). Analysis parameters, plate formats, and reports which include an 8 x 12 presentation of data, can be viewed on the liquid crystal display (LCD). The instrument's memory has the capacity to store 9 analysis protocols, 9 formats, and 25 sets of plate data. For hard copy, the Model 3550 Microplate Reader can be interfaced with an optional printer such as the Epson FX 850 (catalog number 125-1155) or Epson EX 800 (catalog number 125-1230).

An optional plate stacker and loading mechanism with 25 plate capacity (catalog number 170-6612) is available for laboratories requiring high throughput. The Model 3550 Microplate Reader includes a built-in RS-232-C serial interface device for convenient computer interfacing with the Microplate Manager[®] and Kinetic Collector[®] software for the IBM PC or Apple Macintosh computer, which offer complete analysis programs.

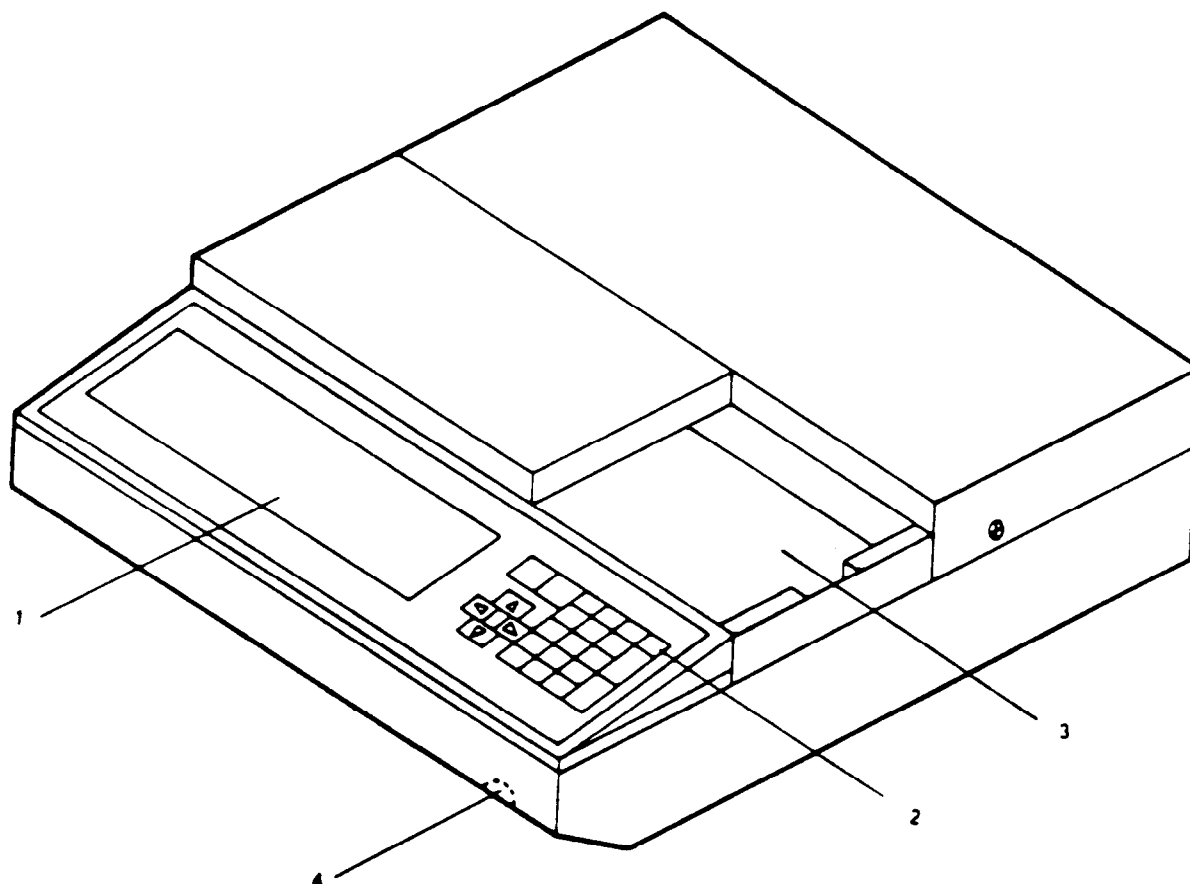
1.1 Accessories for the Model 3550 Microplate Reader

Product	Catalog Number
540 nm Interference Filter	170-6634
550 nm Interference Filter	170-6635
570 nm Interference Filter	170-6636
630 nm Interference Filter	170-6637
Automatic Stack Loading Mechanism	170-6612
Stack Loader Magazines	170-6613
Kinetic Collector [®] /Macintosh Software	170-6615
Kinetic Collector [®] /PC Software	170-6616
Microplate Manager [®] /PC Software	170-6617
Microplate Manager [®] /Macintosh Software	170-6618

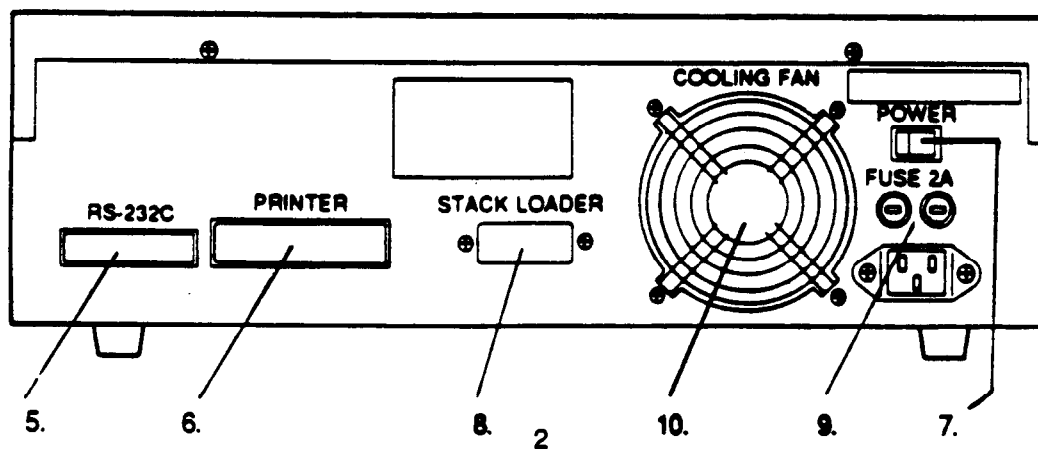
Section 2

Product Description

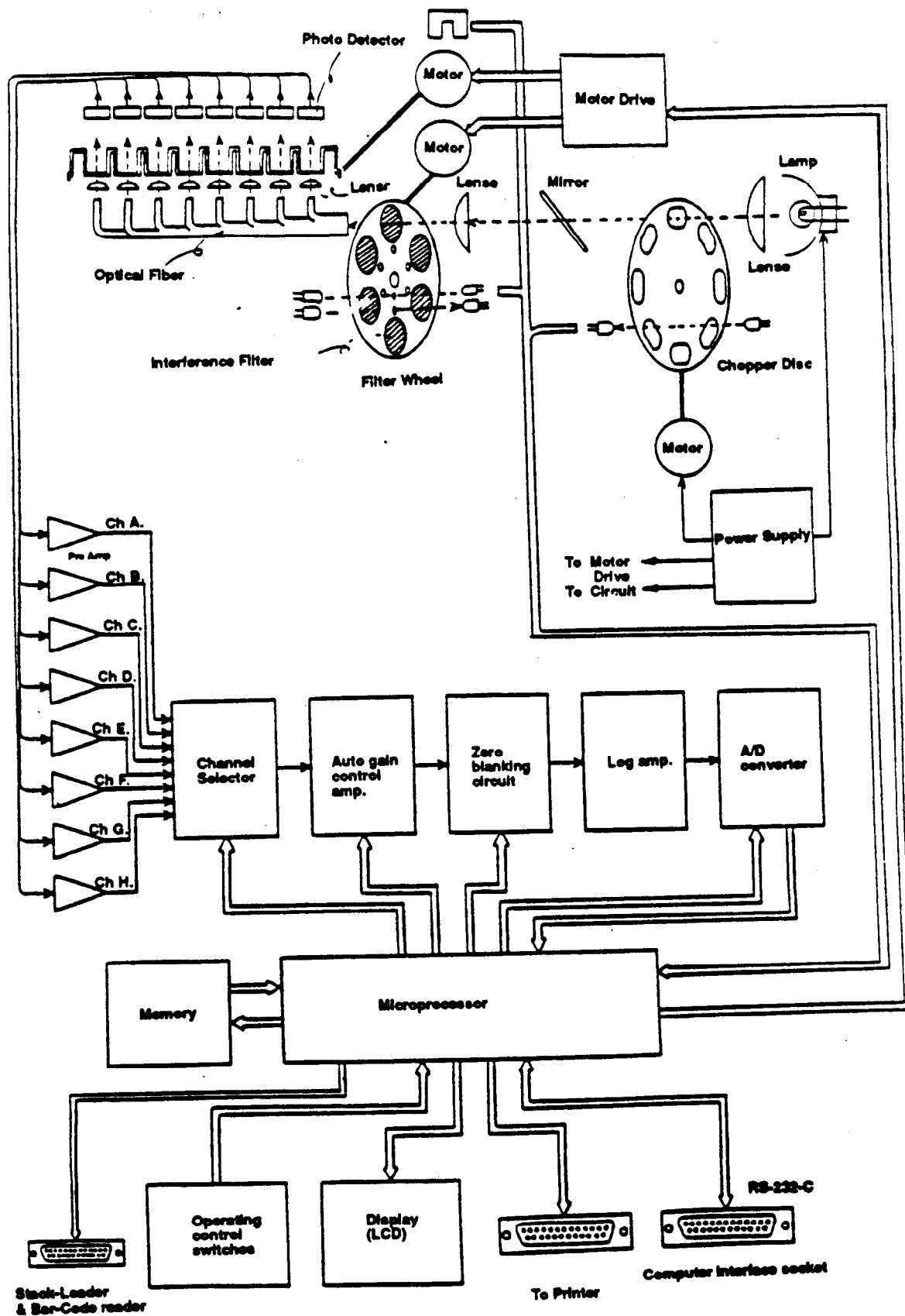
2.1 Model 3550 Microplate Reader, External Features



- | | |
|---------------------------------|-------------------------------------|
| 1. LCD | 6. Centronics parallel printer port |
| 2. Membrane keypad | 7. ON/OFF switch |
| 3. Microtitration plate carrier | 8. Stack loader interface |
| 4. Brightness control for LCD | 9. Fuses |
| 5. RS-232-C serial interface | 10. Cooling fan |

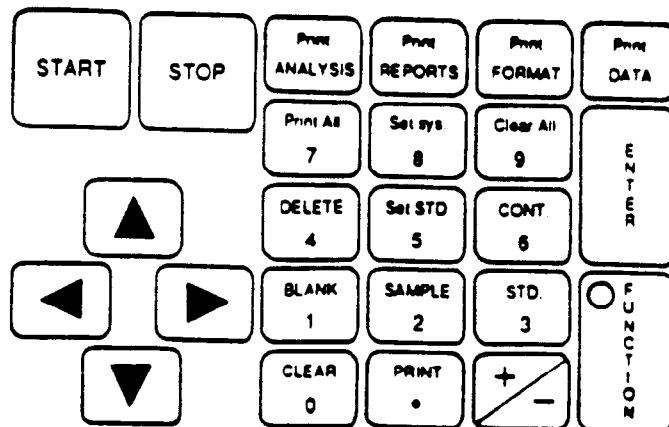


2.2 Block Diagram of Optical System



2.3 Membrane Keypad

Many keys have dual functions, which are printed in blue on the upper case of the key. To access the function denoted in the upper case of a key, first press [FUNCTION], followed by the key. For example, [FUNCTION] [7] will access PRINT ALL.



- [START]
Initiates plate reading using the current analysis.
- [STOP]
Stops a plate reading.
- [ENTER]
Completes or seals a field entry.
- [FUNCTION]
Selects the operation described in the upper case of a key.
- [ANALYSIS]
Displays the current analysis.
- [REPORTS]
Displays the reports selected in the analysis for the current data file.
- [FORMAT]
Displays the current format.
- [DATA]
Allows selection of a data set from memory.
- [FUNCTION] [ANALYSIS] = PRINT ANALYSIS
Prints the current analysis screen in the LCD.
- [FUNCTION] [REPORTS] = PRINT REPORTS
Prints all reports selected in the analysis protocol for the current data set.
- [FUNCTION] [FORMAT] = PRINT FORMAT
Prints the current format.
- [FUNCTION] [DATA] = PRINT DATA
Prints the current raw absorbance data.

- [FUNCTION] [1] = BLANK
Used to define blank wells in a plate format.
- [FUNCTION] [2] = SAMPLE
Used to define sample wells in a plate format.
- [FUNCTION] [3] = STANDARD
Used to define standard wells in a plate format.
- [FUNCTION] [4] = DELETE
Flags data points as outliers or reinstalls data points previously flagged as outliers. Also, changes well designation in format to undesignated state.
- [FUNCTION] [5] = SET STANDARD
Displays the set standards screen.
- [FUNCTION] [6] = CONTINUE
Used to view the remaining portions of reports which do not fit on the LCD. Pressing these keys will scroll to the next portion of the report; if pressed while viewing the end of a report, the LCD scrolls to the beginning of the report.
- [FUNCTION] [7] = PRINT ALL
Prints all the reports selected in an analysis for each data file read and stored in the data buffer.
- [FUNCTION] [8] = SET SYSTEM
Displays the set system parameter screen.
- [FUNCTION] [9] = CLEAR ALL
Clears all data files from memory.
- [FUNCTION] [0] = CLEAR
Clears selected data files, formats, and standard concentration tables from memory, and returns them to the default settings.
- [FUNCTION] [.] = PRINT
Prints the current information in the LCD; like PRINT SCREEN on a computer.
- [+/-]
Plus or minus sign key. Plus is the default; press [FUNCTION] to access the minus sign.
- 0-9
Numeric keys. Additional functions, denoted in the upper case, are accessed by first pressing [FUNCTION].
- .
Decimal point key

Section 3

Operation

3.1 Contents of Shipping Container

The Model 3550 Microplate Reader is supplied with the following items. Contact your local Bio-Rad representative if any of these items are missing.

Model 3550 Microplate Reader
Two 2 amp fuses
Instruction manual
Dust cover
Power cord

3.2 Initial Start-Up

1. Place the instrument on a clean, sturdy table or bench. Maintain the instrument in a clean, relatively dust free environment to insure maximum performance.
2. Inspect the outside of the unit for any signs of damage. If damage is found, contact your Bio-Rad representative. In the USA, call Bio-Rad Technical Service at 1-800-4-BIORAD. Complete the Warranty Registration Card and return it to Bio-Rad Laboratories.
3. Connect the power cord to the back of the instrument. Before connecting the instrument to the main electrical supply, check to make sure that the AC voltage is appropriate for the instrument.
4. Turn on the power switch on the rear panel. An initial self-diagnosis is conducted, which requires about 1 minute. Allow the instrument to warm up for 15 minutes before reading to establish thermal equilibrium. The time remaining in the warm-up period is displayed in the lower right LCD. Programming operations and plate reading may be conducted during the warm-up period. However, the printed reports of any readings taken prior to completion of the warm-up period will note 'the plate was read before completion of warm-up'.
5. Adjust the brightness of the LCD with the wheel under the right side of the front panel.

3.3 Setting the System Parameters

The system parameters are accessed in the set system screen, by pressing Set Sys. ([FUNCTION] [8]). Remember to seal each change by pressing [ENTER].

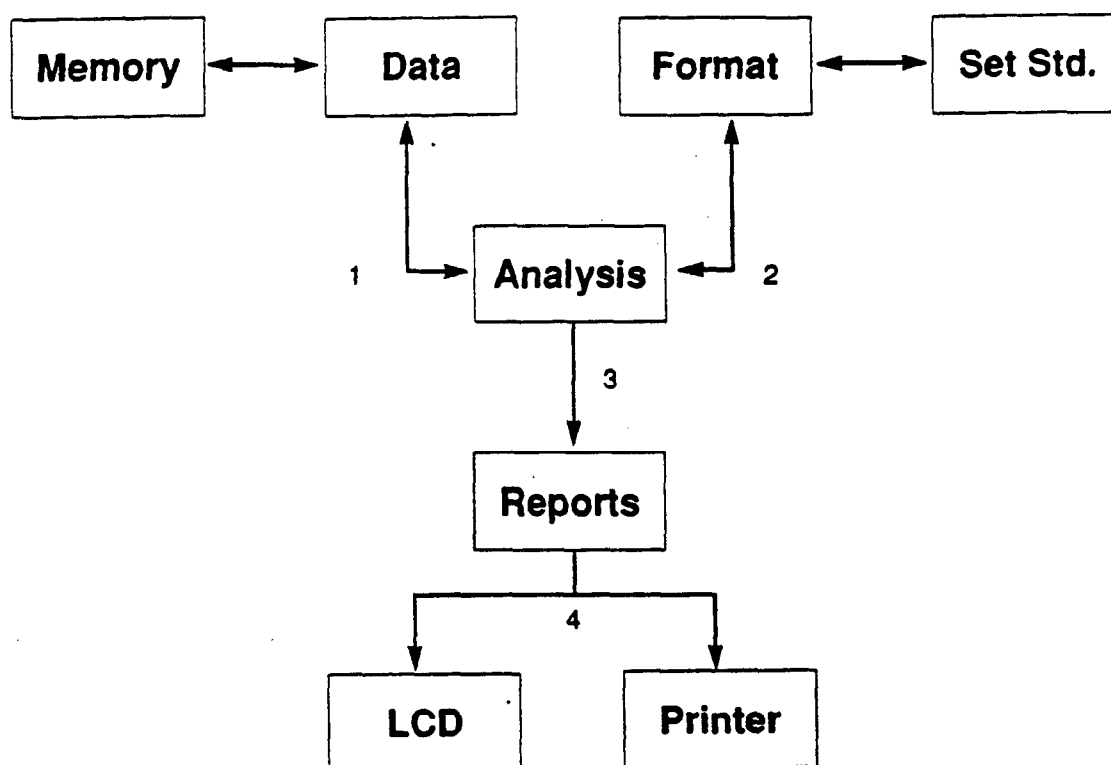
1. Choose the date format to be displayed on the LCD and hard copy by pressing the right or left arrow keys until the preferred format is enclosed in brackets. You may choose either the month/day/year (mm/dd/yy) or the day/month/year (dd/mm/yy) format.
2. Set the date by pressing [ENTER] until the cursor rests on the value to be changed; enter the correct value from the keypad, then press [ENTER] to seal the entry.
3. Set the correct time in the 24 hour format. For example 1:30 PM is entered as 13:30.
4. You may change the default analysis, which is the analysis that appears upon power up.
5. If custom filters are installed, or the position of the filters in the filter wheel is changed, the information should be updated on the set system screen. Press [ENTER] until the cursor rests on the wavelength value of the filter wheel position you wish to change. Enter the correct wavelength, and press [ENTER]. The changes will appear on the analysis screen. Press [ANALYSIS] and verify that the filter wheel positions and corresponding wavelengths are correct. (See Section 9.2 for specific instructions on installing filters.)

3.4 Conceptual Operation of the On-Board Software

The Model 3550 Microplate Reader contains powerful and sophisticated on-board software that can greatly enhance your data analyses. The software combines the information contained in the settings of the analysis, the format, the values of the standards, and the absorbance values to produce the analysis reports (Absorbance, Matrix, Limit, and Evaluation). These four sources of information are interconnected by the software; a change in any one of these will affect the results.

The analysis protocol is the hub that links the various elements together to produce the reports. The analysis protocol specifies the manner in which the data is collected, and the format to be used. The format communicates the function of the wells to the software; it specifies the locations of the blank, standard, and sample wells. If standards are used, the concentrations are communicated via the set standards screen and linked to the format. The data and format information is combined to produce the reports which are displayed on the LCD and printed as specified in the analysis.

Conceptual Diagram of Software Operation



1. The data is collected as specified in the analysis. Each set of data is saved in memory as it is read, and may be recalled and analyzed.
2. The format to be used is specified in the analysis. The concentration values of the standards are linked to the format and are carried with it.
3. The data, the format, and the standard concentrations are combined to produce the reports.
4. The reports are presented on the LCD and printed as specified in the analysis.

Section 4

Analysis Protocols

The parameters of the analysis screen may be saved as an analysis protocol. The Model 3550 Microplate Reader will save nine analysis protocols, which may be easily recalled and used.

```

ANALYSIS NUMBER 1
=====
Format Number (1-9) 3 Blank[Each]/ First Plate Time Delay Yes/[No]
Time 0:00 (min:sec)
Print Reports[Yes]/ No Single/[Dual]Wavelength Absorbance Delay Yes/[No]
Absorbance [Yes]/ No Absorbance Value 0.000
Matrix [Yes]/ No Measurement Wavelength [Above]/ Below abs. value
Limit [Yes]/ No [405]415 450 490 595 655 Well: [A]B C D E F G H
Evaluation [Yes]/ No [1]2 3 4 5 6
Reference Wavelength 7 8 9 10 11 12
Matrix Maximum 2.000 405 415 450 490 595[655]
Upper limit 1.500
Lower limit 0.050 Auto Mixing [Yes]/ No Multiple Readings Yes/[No]
Number of readings 1
Interval 0:00 (min:sec)

```

4.1 Selecting an Analysis Protocol

An analysis protocol may be set when the analysis screen first appears after warm up or whenever [ANALYSIS] is pressed. The analysis screen displays a blinking cursor prompting for the analysis number. Enter the number (1-9) of the analysis protocol you wish to access, and press [ENTER]. The protocol number that you selected will become the current protocol, and the parameter settings will be displayed as they were the last time the protocol was modified. The cursor will move to the parameter field of Format Number.

4.2 Setting the Analysis Parameters

Parameters are set by pressing [ENTER], or the Up and Down arrow keys to move the flashing cursor to the parameter you wish to change. The fixed parameters, e.g. Print Reports, are changed by pressing the right or left arrow keys until the brackets surround the desired option. The variable parameters, e.g. the Matrix Maximum, require numbers to be entered from the keypad. In all cases, parameter changes must be sealed by pressing [ENTER] or the up and down arrow keys to move to another parameter. The change will not be accepted if it is not sealed.

1. Format number sets the format to be used for the analysis. The format number is set by pressing the number of the format you wish to use (1-9). The Model 3550 Microplate Reader will store nine formats that define the locations of assay blanks, standards, and samples. Formatting is described in Section 5.
2. Print Reports will cause the reports to be printed immediately after the plate is read. Only the reports that are selected "yes" will be printed. To obtain a hard copy, the Model 3550 Microplate Reader must be connected via its parallel interface to an Epson or compatible dot matrix printer.
3. The reports that are selected "yes" on the analysis protocol will be printed when the print reports option is used, and when [PRINT REPORTS] and [PRINT ALL] are pressed. The selected reports may also be viewed on the LCD by pressing the [REPORTS] key. Reports that are not selected on the analysis screen will not be printed or displayed.
4. Matrix Maximum is the value used to create the matrix partitions. The absorbance range between zero and the matrix maximum is divided into ten equal partitions which are numbered 0 through 9. The absorbance values of the data are classified and reported according to the partition to which they correspond. Any value from 0 to 3.000 may be set as the matrix maximum.

5. Upper and Lower Limits define the boundaries of the Limit Report. Absorbance data that fall within the boundaries are displayed as asterisks; data below or above the limits are represented by minus or plus signs, respectively. Limits from 0 to 3.000 may be set. However, the lower limit may not exceed the upper limit.
6. Blank Each/First Plate. Blank Each Plate specifies that the blank value will be determined for each plate independently. Blank First Plate specifies that the blank value of the first plate of a series will be used for all the plates in the series. The First Plate is the plate to be read after the analysis is selected and set. The blank value may be negated by deleting the blank wells from the data set of the first plate. (See Section 6.3 for information on editing data.) When the blanking mode is changed from Blank First Plate to Blank Each Plate, the blank value of the first plate is deleted from memory so that it will not be used for subsequent plates. The strategy for using this mode is as follows:

Set the Analysis screen for the assay:

Set the format number to the format of the first plate (which contains the defined blanks).

Set the blanking mode to Blank First plate.

Read the first plate.

Return to the Analysis screen and change the format number to the format of the subsequent plates.

Read all subsequent plates.

5. Set the blanking mode to Blank Each Plate after all of the plates have been read. Doing so will discontinue the blank value of the first plate. (Turning off the power to the instrument will also discontinue the blank value of the first plate.)
7. Single/Dual Wavelength specifies the optical mode used for the analysis. Single wavelength mode reads the absorbance of the wells at the measurement wavelength, which ideally is the wavelength of maximum absorbance of the substrate. Dual wavelength mode reads the absorbance at the measurement wavelength and the reference wavelength. The reference wavelength should be the minimum absorbance for the substrate, essentially the background absorbance. The dual wavelength mode subtracts the reference absorbances from the measurement absorbances to reduce artificial well to well variation.
8. The values listed for the measurement and reference wavelengths are entered in the Set System screen. If custom filters are installed or if the filter positions are changed, the information in Set System should be updated as described in Section 3.3.
9. Auto Mix will agitate the plate for 3 seconds before reading to disperse the substrate and product, and to promote even wetting of the wells.
10. Time Delay specifies that the reading of the plate will be delayed by the specified time after pressing [START]. To use the time delay:

Select "yes" for Time Delay.

Enter the desired minutes of delay, from 0 to 99 minutes.

Enter the desired seconds of delay, from 0 to 59 seconds.

Press [START] when you are ready to read the plate. The LCD will display the time remaining until the plate is read.
11. Absorbance Delay will delay data collection until the specified well reaches a specified absorbance. To use the absorbance delay:

Select "yes" for Absorbance Delay.

Set the threshold absorbance value.

Set "above" if you want to read the plate when the absorbance of the well exceeds the threshold absorbance. Select "below" if you want to read when the absorbance of the well falls below the threshold value. For most conventional ELISAs using alkaline phosphatase and horseradish peroxidase, "above" is the proper selection. Set the row and column co-ordinates of the target well.

Press [START] when you are ready to begin monitoring the plate. The instrument will repeatedly read the plate until the absorbance delay conditions are met, then the absorbance data will be collected and displayed.

Time and Absorbance Delays may be set simultaneously. Time Delay will count down first, then Absorbance Delay will be activated.

12. Multiple Plate Readings may be used to record sequential readings of a single plate for kinetic or other analyses.

Select "yes" for multiple plate readings.

Set the number of times the plate will be read, from 1 to 25 times. The maximum number of repetitions will depend on the memory available. The LCD will instruct you to delete a file(s) or clear the buffer if necessary. See Section 8 for more information on the memory buffer.

Set the interval between readings by entering the minutes and seconds desired. The instrument defaults to the minimum reading time for the given reading parameters, and will not accept intervals less than the minimum reading time.

<u>Reading Parameter</u>	<u>Reading Time</u>
Single wavelength	12 seconds
Single wavelength with mixing	15 seconds
Dual wavelength	22 seconds
Dual wavelength with mixing	25 seconds

The time that you set will be the actual time between sequential readings, including the time required to read the plate.

Note: When using the multiple reading mode, it is possible to set reading conditions that cause the actual time required to read to be greater than the multiple reading time interval. For example, setting the multiple reading interval for 12 seconds, and changing the reading mode from single wavelength to dual wavelength creates a conflict with the multiple reading interval. When a conflict occurs, the multiple reading interval will be replaced with the lowest possible default value, and the LCD will display the following message: "Warning! The multiple reading time interval has been replaced with the default by a related field entry." Press any key to clear the warning message. This message will only appear when the multiple reading mode is in use, that is when YES is selected for the multiple reading mode.

Section 5 Formatting

Assay formats communicate the function of each well to the instrument's microprocessor, which combines the format information with the absorbance data to produce the four different analysis reports. You can create assay formats of single or replicate blanks, standards, and samples. Up to nine formats can be saved in memory, and may be recalled at any time.

FORMAT NUMBER 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	S01	S02	S03	S04	S05	S06	S07	S08
B	B	S01	S02	S03	S04	S05	S06	S07	S08
C	B	S01	S02	S03	S04	S05	S06	S07	S08
D	B	S01	S02	S03	S04	S05	S06	S07	S08
E	B	X01	X02	X03	X04	X05	X06	X07	X08	X09	X10	X11
F	B	X01	X02	X03	X04	X05	X06	X07	X08	X09	X10	X11
G	B	X01	X02	X03	X04	X05	X06	X07	X08	X09	X10	X11
H	B	X01	X02	X03	X04	X05	X06	X07	X08	X09	X10	X11

[BLANK] SAMPLE NO. X01 STANDARD NO. S01

5.1 Opening a Format

To begin formatting, press [FORMAT], enter the format number, and press [ENTER]. The selected format will be displayed. Blanks are represented by "B", standards by "S", and samples by "X". The samples and standards are followed by a two digit identification number. The well types, (blank, sample, and standard) are listed at the bottom of the screen. The active well type is enclosed in brackets.

5.2 Assigning Blanks

Up to 96 blanks maybe assigned in any location or combination on the format matrix.

1. Press [FUNCTION] [BLANK] to make it the active well type. The brackets will enclose the word "BLANK" at the bottom of the screen.
2. Use the direction keys to move the cursor to the well location you wish to assign as assay blank.
3. Press [ENTER]. The location will be marked "B", indicating that it has been designated a blank. Repeat steps 5.2.2 and 5.2.3 to assign additional blanks.

5.3 Assigning Standards

Up to 40 single or replicate standards may be assigned in a single format.

1. Press [FUNCTION] [STANDARD] to make it the active well type. The brackets will enclose "STANDARD NO. 01" at the bottom of the LCD.
2. Use the direction keys to move the cursor to the well location you wish to assign as the first standard.
3. Press [ENTER]. The location will be marked "S01", indicating that it has been designated the first standard. Repeat steps 2 and 3 to assign replicates of the first standard. Replicates may be assigned in any combination and location, and need not be continuous.
4. Press the [+/-] key to progress to the next standard. The LCD will display: "[STANDARD NO. 02]". Pressing [FUNCTION] and [+/-] will lower the standard number. Each press of [+/-] or [FUNCTION] [+/-] will increase or reduce the standard number by one, respectively.
5. Use the direction keys to move the cursor to the well location you wish to assign as the second standard.
6. Press [ENTER]. The location will be marked "S02," indicating that it has been designated the second standard. Repeat steps 5.3.3 through 5.3.6 to assign additional standards.

5.4 *Setting the Values of the Standards*

The value of the assay standards must be communicated to the microprocessor in order for it to perform the Evaluation Report. This is achieved by entering the standard values in the set standard screen.

1. Verify that the analysis and format numbers are set properly:
Press [ANALYSIS], enter the analysis number if necessary, and press [ENTER]. Enter the correct format number if necessary.
2. Press [FUNCTION] [SET STANDARDS]. The cursor will be located at the entry field for the value of the first standard.
3. The concentration values of standards are set in scientific notation.
First enter the mantissa value, including the decimal point, from the keypad.
Press the [+/-] key or the [FUNCTION] [+/-] to enter the sign of the exponent, and enter the value of the exponent. Press ENTER to seal the transaction and advance to the next standard. Repeat this step until all of the standard values are set.
4. The LCD will display entry fields for 20 standards. If your format has more than 20 standards, press [CONTINUE] to view and set the remaining entry fields.

5.5 *Assigning Samples*

Up to 96 single or replicate samples may be assigned in a single format. Press [FUNCTION] [SAMPLE] to make it the active well type. The remaining procedures for assigning samples are analogous to those for assigning standards. See Section 5.3.

5.6 *Editing Formats*

Select the proper well type and number, move the cursor to the well location that you want to change, and press [ENTER]. For example, if you want to change a well designation to Sample 5, you would:

1. Move the cursor to the well location to be edited.
2. Press [FUNCTION] [SAMPLE].
3. Adjust the value of the sample to 5 by pressing the [+/-] key.
4. Press [ENTER].

5.7 *Removing Well Designations*

Any well designation may be changed to an undesignated state to indicate that the well has no function in the assay. For example, all empty wells would be set as undesignated as follows:

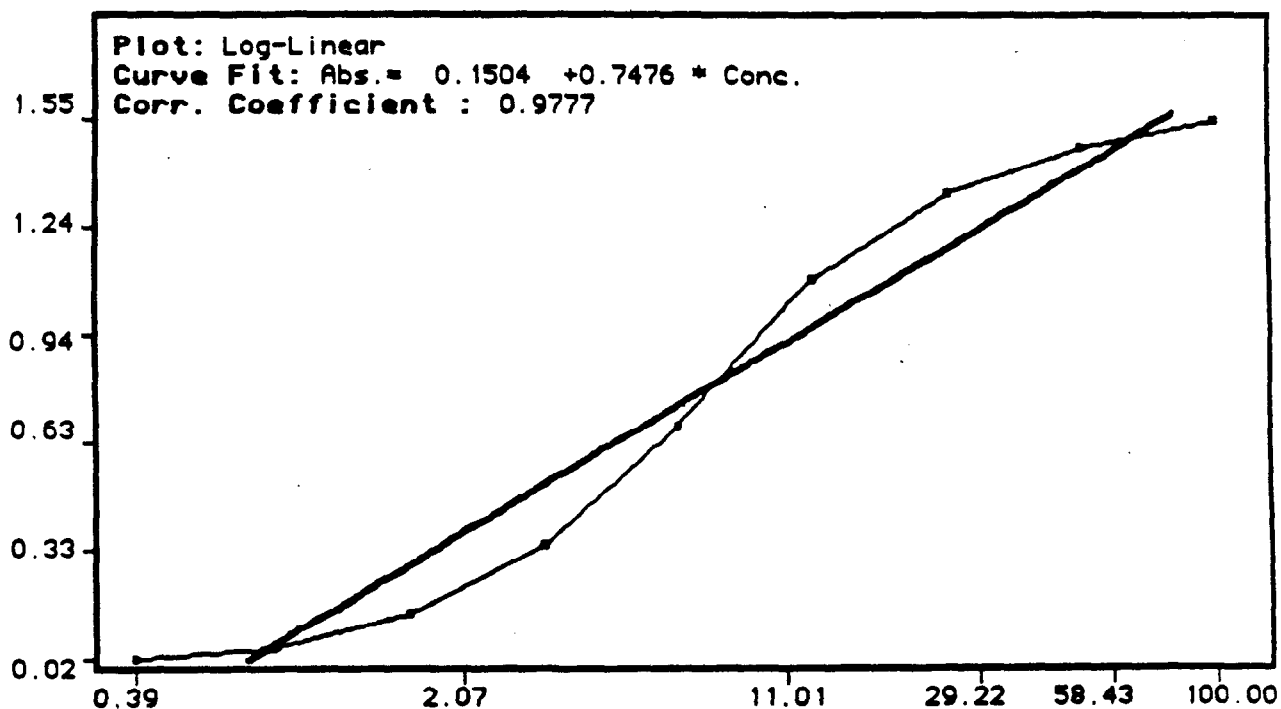
1. Move the cursor to the well location.
2. Press [FUNCTION] [DELETE]. The well location will display three dots to indicate that it is undesignated. The results of undesignated wells are displayed in the raw data set but not in the reports.

Section 6 Viewing and Editing Reports

6.1 Types of Reports

1. The Absorbance Report displays the blank subtracted absorbance values. The mean blank value is subtracted from all 96 raw absorbance values. If the Blank Each Plate option is used, the blank value is the mean absorbance value of the blank wells assigned in the format. If the Blank First Plate option is used, the blank value is the mean absorbance value of the blank wells of the first plate in the series.
2. The Matrix Report divides the absorbance range between zero and the matrix maximum into ten equal partitions, which are numbered 0 through 9. The absorbance values are classified and reported according to these partitions. The matrix maximum may be set on the analysis protocol and when the report is viewed on the display.
3. The Limit Report classifies the absorbance data according to a single partition defined by the upper and lower limits. Absorbances within the limits are displayed as asterisks. Absorbances above or below the limits are displayed as plus signs or minus signs, respectively. The limits may be set on the analysis protocol, and when the report is viewed on the display.
4. The Evaluation Report performs a linear regression of the standards and calculates the concentrations of the samples using the regression formula. The mean, standard deviation, % coefficient of variation, and the concentration value are tabulated for all the wells defined in the format. The equation of the regression is displayed with its correlation coefficient or "r" value. The "r" value of the regression can often be improved by deleting outliers and non-linear tails. The procedure for deleting outliers is described in Section 6.3.

Note: A relatively high correlation coefficient may mask an undesirable fit of the data. The data plotted below is fit with a linear regression, which has a correlation coefficient of 0.9777. However, the regression may not be suitable for some quantitative purposes. For new and unfamiliar assays, we recommend that you plot the results the first few times to verify that regression results are optimal. (This graph was generated with the Microplate Manager software).



6.2 Viewing the Reports on the LCD Display

1. Only the reports that are selected "yes" on the analysis protocol may be viewed on the display. Check the analysis protocol to verify that the reports you wish to view are selected.
2. Press [REPORTS] to view the first report selected "yes" in the analysis. Press [REPORTS] again to view the second selected report, etc. The reports sequence is a loop, which will show each selected report in turn as you to press [REPORTS].

6.3 Editing Reports

The raw data may be edited to remove errant data or outliers. Non-linear portions of the standard curve may also be removed to improve the curve fit. The raw data may be edited when it appears on the screen, immediately after reading the plate, or when the data is recalled from the memory.

After each plate is read, or when data is recalled from memory, the raw data is displayed with the cursor flashing on well A-1. Move the cursor to the well location you wish to remove, then press [FUNCTION] [DELETE]. The value of flagged data will be replaced by a dot on all reports, and the data will not be used in the reports or calculations. Flagged data may be reinstated by repeating the editing procedures. Move the cursor to the value you wish to reinstate and press [FUNCTION] [DELETE]. The original absorbance value will reappear and will be used in all reports.

Note: The data cannot be edited immediately after reading when the Multiple Plate Reading option is used. Data from multiple plate readings may be recalled from memory and edited. See Section 8 for more information on using the memory buffer.

Section 7

Printing Reports

The Model 3550 Microplate Reader offers many convenient methods for printing your results. See Appendix A for a sample of the hard copy produced by the Model 3550 Microplate Reader.

7.1 Automatic Report Printout

Selecting Print Reports in the analysis protocol will automatically print all the reports selected "yes" after the plate reading is completed. When the Multiple Plate Reading option is used, printing will commence after the series of readings is completed. See Section 4 for more information on setting the analysis protocol parameters.

7.2 PRINT REPORTS

Prints all reports selected in the analysis protocol for the current data set.

7.3 PRINT ALL

Prints all selected reports for all the data sets read and stored in the memory, using the current analysis protocol. Make sure that the desired reports are selected.

7.4 PRINT ANALYSIS

Prints the current analysis protocol.

7.5 PRINT FORMAT

Prints the current format.

7.6 PRINT DATA

Prints the current raw data.

7.7 PRINT

Prints the the current information on the LCD display.

7.8 Printing the Raw Data of Plates Read by Remote Control

The Model 3550 reader will print the raw data of a plate read via remote computer control using the following procedure:

1. Verify that the printer is properly connected to the reader.
2. Press the [FUNCTION][.] or Print key immediately after each plate is read via remote computer control.

Note: Occasionally, the keyboard may be locked because the "AQ" command has been issued by the host computer. In this event, the keyboard may be released by pressing the STOP key. (Note: It is not advisable to perform this operation when using the Kinetic Collector software or Multiple Plate Reading Mode of the Microplate Manager software, as pressing the STOP key will discontinue the kinetic or multiple plate reading.)

Section 8

Using the Data Buffer

The Model 3550 Microplate Reader is equipped with battery backed-up memory, which will store 25 sets of data. Each plate is issued a number (1-25) as it is read. This number appears at the top of the display, and is printed in the header of every report. This number corresponds to the position of the data set in the memory buffer. The memory can be thought of as a rack of 25 slots with the capacity for one data set per slot. The data can be thought of as cards that are inserted in the slots as they are saved. Every plate is saved in the memory as it is read, and is assigned the first available slot in the rack until all the slots are full.

When the memory is full, the display will present a message instructing you to delete a plate(s) from the memory or clear the memory completely. A similar message will appear when using the Multiple Plate Reading option if the available memory is insufficient to store all the data sets in the series. When a file is deleted, it cannot be retrieved. Data sets can be deleted from the any position in the rack. For example, if data set 12 is deleted, the next plate to be read will be saved in slot 12 in the memory. This method allows you to read another plate without disturbing the order of the remaining data sets in the memory.

When reading a series of plates, you may prefer to save them as a continuous series in the buffer. If sufficient space is not available, you may delete the required number of continuous data sets from the memory. For example, if the memory is full, and you wish to save five files continuously, you must delete five continuous data sets (plates 6-10, or 20-25, etc.). Often, it will be more convenient to clear the memory entirely. You may find it desirable to document your results as soon as possible, so that you can clear the memory and not worry about losing data. However, keep in mind that when a data set is deleted, you cannot get it back.

8.1 Recalling Data from Memory

The raw data of any plate may be recalled by pressing DATA, entering the plate number, and pressing ENTER. The raw data set will be displayed with the cursor flashing on A-1. The data may be edited as described in Section 6.3.

8.2 Deleting Individual Data Sets

1. Recall the data set you wish to delete using the procedure described above.
2. Press [FUNCTION] [0] or CLEAR. The data will change to zeros.

8.3 Clearing the Memory

[FUNCTION] [9] or CLEAR ALL clears the memory. The display will ask you to confirm your decision by pressing [[FUNCTION] [0] or CLEAR. If you change your mind, press any other key to escape the Clear All function.

8.4 Transmitting Raw Data

Data from the reader's data buffer may be transferred through the RS-232-C port via direct, one way data transmission. Files may be transferred individually, or all 25 files in the data buffer may be transferred at once. The data may be collected by a variety of commercially available communications packages such as XTALK or Red Ryder. (Note: the data is transmitted as ASCII characters, not as numbers.)

For single file transmission:

1. Recall the desired data set from memory by pressing the DATA key, entering the number of the desired data set, and pressing the ENTER key.
2. Press the Function key, then the +/- key to transmit the current set of raw data with header information. (See examples below.)

For complete data buffer transmission:

1. Press the DATA key. Do not select a data set, and do not press ENTER.
2. Press the Function key, then the down arrow key to transmit all of the data sets in the data buffer with header information for each file. Empty data sets will be transmitted as data sets with zeros for absorbance values. (See examples below.)

Single wavelength result format:

```
"RAW DATA REPORT"<cr>
"PLATE NUMBER 01"<cr>
"DATE 05/16/89"<cr>
"TIME 10:36:57"<cr>
"Measurement filter 405nm."<cr>
<cr> or "PLATE ID NUMBER 12345" <cr> if bar code reader is installed
".begin"<cr>
*0.069 0.071 0.060 0.098 0.059 0.064 0.074 0.062 0.060 0.061 0.095 0.064"<cr>
*0.060 0.064 0.067 0.061 0.060 0.061 0.076 0.060 0.068 0.063 0.062 0.058"<cr>
*0.061 0.062 0.061 0.073 0.061 0.065 0.061 0.064 0.064 0.062 0.065 0.067"<cr>
*0.057 0.060 0.060 0.073 0.061 0.066 0.063 0.065 0.057 0.062 0.063 0.062"<cr>
*0.065 0.057 0.064 0.068 0.064 0.061 0.065 0.063 0.063 0.057 0.061 0.060"<cr>
*0.061 0.061 0.075 0.065 0.084 0.063 0.064 0.062 0.058 0.061 0.061 0.087"<cr>
*0.056 0.061 0.071 0.069 0.064 0.060 0.059 0.069 0.069 0.069 0.066 0.061"<cr>
*0.065 0.058 0.057 0.065 0.062 0.066 0.063 0.076 0.066 0.061 0.071 0.058"<cr>
".end"<cr>
<cr>
```

Dual wavelength results format:

```
"RAW DATA REPORT"<cr>
"PLATE NUMBER 02"<cr>
"DATE 05/16/89"<cr>
"TIME 10:43:05"<cr>
"Measurement filter 405nm."<cr>
"Reference filter 655nm."<cr>
<cr> or "PLATE ID NUMBER 12345" <cr> if bar code reader is installed
".begin"<cr>
*0.014 0.016 0.013 0.022 0.012 0.013 0.019 0.016 0.015 0.014 0.021 0.014"<cr>
*0.015 0.017 0.018 0.015 0.016 0.017 0.022 0.016 0.018 0.017 0.015 0.013"<cr>
*0.014 0.015 0.015 0.019 0.015 0.017 0.014 0.016 0.016 0.015 0.015 0.016"<cr>
*0.013 0.014 0.015 0.021 0.016 0.018 0.018 0.016 0.014 0.017 0.016 0.014"<cr>
*0.017 0.015 0.016 0.019 0.018 0.017 0.016 0.017 0.017 0.013 0.015 0.014"<cr>
*0.016 0.014 0.018 0.018 0.019 0.017 0.017 0.015 0.015 0.016 0.016 0.025"<cr>
*0.013 0.017 0.020 0.018 0.015 0.016 0.016 0.018 0.019 0.016 0.017 0.016"<cr>
*0.016 0.016 0.015 0.016 0.020 0.018 0.016 0.020 0.017 0.017 0.020 0.016"<cr>
".end"<cr>
<cr>
```

Section 9

Replacing the Lamp and Installing Interference Filters

The Model 3550 Microplate Reader requires very little maintenance. Two important maintenance procedures are changing the lamp and installing interference filters.

Warning! Electrical Shock Hazard! Always unplug the instrument from the AC power source before opening the case.

9.1 Changing the Lamp

1. **Warning!** The lamp may be very hot. Use caution and allow the lamp to cool before attempting removal. Open the rear case of the instrument by removing the four anchor screws; two on the back panel and one each on the sides. Lift off the rear cover and locate the lamp housing on the left rear near the cooling fan.
2. Loosen the two screws that hold the lamp housing cover. Then slide the cover and lift it off.
3. Release the spring clamp that secures the lamp.
4. Carefully pull the lamp assembly away from the bezel.
5. Holding the ceramic base in one hand and the lamp housing in the other, carefully pull the lamp free of the base.
6. 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. **Do not touch the bulb or the inside of the reflector housing during installation.**
7. Position the lamp in the bezel so that the tab on the lip of the reflector housing fits in the notch at the 12 o'clock position on the bezel. Hold the lamp in this position until it is secured with the spring clamp.
8. Replace the lamp housing cover and the rear compartment cover.

9.2 Installing an Interference Filter

1. Open the rear compartment cover as described in Section 9.1, Step 1.
2. Locate the filter wheel and rotate it until the filter you wish to remove is in the 12 o'clock position.
3. The filters are held in position by small spring clips, and can be removed from the wheel without tools. Firmly grasp the filter housing, being careful not to touch the glass element, and pull straight up.
4. Insert the new filter with the spring clip indentations facing the front of the instrument. Be sure that the filter is firmly seated.
5. Update the filter information in the set system screen. See Section 3.3 for specific instructions.

Section 11

Troubleshooting

1. LCD appears blank on power up.
 - a. LCD brightness is turned too low.
 - b. Power switch is not turned on.
 - c. Unit is not plugged into AC outlet or power cord is not attached to the instrument.
 - d. The AC outlet is dead.
 - e. Outlet is not the proper voltage for the instrument.
rear panel.
 - f. Instrument has blown a fuse.

Adjust the screen brightness using the knob beneath the right side of the front panel.

Turn on the power switch on the rear panel.

Check power cord connections at the outlet and on the rear panel of the instrument.

Check circuit breakers or fuses.

Check the voltage of the AC line and the voltage requirements of the instrument as stated on the

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 message indicating that the bulb is burned out or the light is insufficient.
 - a. Lamp is not emitting light.
 - b. Lamp is emitting light.

Check lamp to see if it is emitting light.

Replace lamp as described in Section 9.1.

Check alignment of lamp in the bezel. Check alignment of bulb in glass reflector. Replace lamp if necessary.

3. Printer will not print on command.
 - a. Printer is not properly connected.
 - b. Printer is off line.
 - c. Printer is not turned on, or is not plugged in.

Check the printer interface connections on the printer and the instrument.

Check printer status.

Check printer power switch and cord.

4. Plate carriage jams during reading.
 - a. Plate is not seated in carriage properly.

Turn instrument off immediately. Remove plate from carriage. (This may require removal of the reading chamber cover.) Turn instrument on: the carriage will return to the load position. Place the plate carefully in the carriage, making certain that it is properly seated.

5. LCD displays message that an interference filter is missing.
 - a. A filter is missing from the wheel.
 - b. The filter is not properly seated.

Install a filter in the empty position.

Check that all filters are properly seated.

- c. Filter is installed backward.
- 6. Wells have color, but the absorbance values seem very low:
 - a. Wavelength selection in the analysis was not sealed.
 - b. Incorrect filter used for measurement and/or reference wavelength.
 - c. The best measurement wavelength for the substrate in use has not been installed in the filter wheel.
 - d. The filters have been switched in the wheel, but the set system screen was not updated to reflect the changes.
- 7. LCD displays error message that the filter wheel is jammed.

Check that all filters are installed with the spring clip notch facing forward.

Any change of filter selection in an analysis must be sealed by pressing [ENTER] or the Up or Down arrow keys, otherwise the change will not be recognized or carried out.

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 produce the highest O.D. values. The reference wavelength produces the lowest O.D. values.

Check references for the substrate you are using, or determine the measurement wavelength by analyzing the substrate product on a scanning spectrophotometer. Custom filters are available from 380 to 750 nm.

Check that the filter positions and their wavelengths are as listed in the set system screen.

Turn off the instrument, remove the rear cover panel, and inspect the filter wheel. Turn the wheel by hand and determine whether the wheel is hanging up. Make sure that all filters are properly installed and firmly seated.

Section 11 Specifications

11.1 Technical Specifications

How intern.
consistent
is Machine

- | | |
|------------------------------------|--|
| 1. Optical photometric methods | Single or dual wavelength |
| 2. Spectral range | 380 to 750 nm |
| 3. Indication range | 0.000 to 3.000 O.D. |
| 4. Resolution | 0.001 O.D. |
| 5. Accuracy | Single wavelength: $\pm 1.5\%$ at 1.000 O.D.
Dual wavelength: $\pm 2.0\%$ at 1.000 O.D. |
| ★ 6. Linearity | $\pm 2.0\%$ of full scale 0.000 to 2.000 O.D. |
| 7. Channel-to-channel error | $\pm 1.5\%$ or 0.010 O.D. from 0.000 to 2.000 O.D. |
| 8. Stability | Less than 0.005 O.D. per hour
Less than 0.010 O.D. per 5 hours
(both at 405 nm after 15 minute warm-up) |
| 9. Instrument reading time | Single wavelength: 12.0 seconds
Dual wavelength: 22.0 seconds |
| 10. Light source | Tungsten halogen, 6000 hour lifetime |
| 11. Photodetectors | Silicon photodiode 2600? |
| 12. Filters | 8-10 nm bandwidth filters at 405, 415, 450, 490, 595, and 655 nm supplied as standard.
Custom filters from 380 to 750 nm available. |
| 13. Filter capacity | Six |
| 14. Operating panel | Membrane key pad |
| 15. Display | 80 x 16 character LCD |
| 16. Computer interface | RS-232-C, built-in |
| 17. Compatible microplates | Most standard rigid and flexible 96 well microplates with flat, 'U', or 'V' bottoms |
| 18. Data reports | Raw, Absorbance, Matrix, Limit, Evaluation |
| 19. Plate mixing duration | 3 seconds |
| 20. Time delay | 1 second to 99 minutes and 59 seconds |
| 21. Multiple reading option | Up to 25 sequential readings |
| 22. Analysis protocols | Nine user programmable protocols stored in memory |
| 23. Plate formats | Nine user programmable plate formats stored in memory |
| 24. Data buffer | 25 sets of data stored in memory |
| 25. Memory back-up | Stored protocols, formats, and data backed-up by a Ni-Cd battery with a two year life. |
| 26. Self diagnosis | Checks and reports on: lamp, battery, interference filters, plate transport, stack loader transport |
| 27. Dimensions | 45 cm x 41 cm x 14 cm, 17.7" x 16.1" x 5.5" (L x W x H) |
| 28. Weight | 15 Kg, 33.2 lbs |
| 29. Power consumption | 150 watts maximum |
| 30. Line voltage | 90-132 VAC or 198-264 VAC, at 47-63 Hz |
| 31. Operating temperature | 5° to 37°C |
| 32. Storage temperature | 0° to 30° C |
| 33. Operating and storage humidity | 0 to 95% non-condensing |
| 34. Safety standards | Generally complies with UL 1262, IEC 348, IEC601, VDE |
| 35. Supplied accessories | Power cord, two 2A fuses, dust cover |

Precision/Reproducibility

$\pm 0.005\%$

or 21

$\pm 0.05\%$ or 0.01000 whichever greater

N=7

Section 12

Computer Specifications

12.1 RS-232-C Interface Specifications

1. Signals

TXD: transit 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/chasis ground)

2. Transmission

Baud rate 9,600
 Data width 8
 Stop width 1
 Parity
 NONE

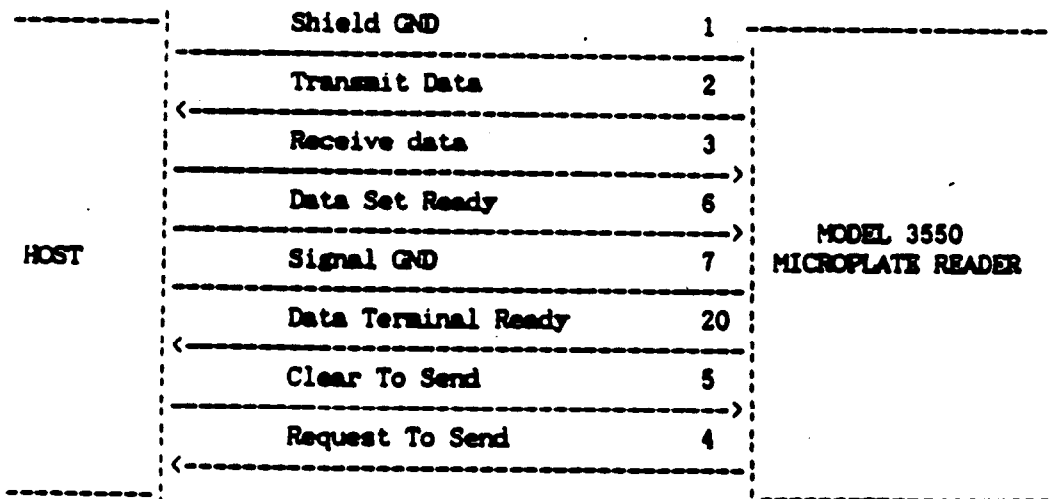
3. Data

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.

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



12.2 COMMAND LANGUAGE

eia.reader Rplate 50012<cr>

SYNTAX:

<device name><space><commands><space><command args.><cr>

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

<command>:	"ID" - request device ID.EIA READER ID; no arguments
<response>:	"ERE" <space><error code><space><ID><cr>
<ID>:	"0770": Model 3550 ID code]
<command>:	"AQ" - Acquire remote control of the device and lock front panel key pad; no arguments
<response>:	"ERE" <space><error code><cr>
<command>:	"RL" - Release remote control of the device and release front panel key pad; no arguments
<response>:	"ERE" <space><error code><cr>
<command>:	"RS" - reset device to power sup configuration local mode; noarguments
<response>:	"ERE" <space><error code><cr>
<command>:	"FSTATUS" - Request filter wheel status from the reader.
<response>:	"ERE" <space><error code> <space><filter status><cr>
<filter status>:	<pos.1><space><pos.2><space><pos.3><space><pos.4> <space><pos.5><space><pos.6>
<pos.1>:	ASCII encoded decimal number of the wavelength filter at position 1 (example: "405").
<pos.2>:	wavelength filter at position 2
<pos.3>:	wavelength filter at position 3
<pos.4>:	wavelength filter at position 4
<pos.5>:	wavelength filter at position 5
<pos.6>:	wavelength filter at position 6
<command>:	"RWELL" - Read a plate well: argument cl,rw,wp1,wp2
<cmd args>:	cl - column number ASCII encoded decimal (1-12) rw - row number ASCII encoded decimal (1-8) wp1 - filter position (1-6) for measurement wavelength - reader: ASCII encoded decimal wp2 - filter position (1-6) for reference wavelength reading;ASCII encoded decimal
<response>	"ERE" <space><error code><space><abs. val1><space> <abs. val2><cr>
<abs. val1>:	absorbance values at measurement wavelength (ASCII encoded floating point format) example "1.987"
<abs. val2>:	absorbance values at reference wavelength (ASCII encoded floating point format) example "1.987"
<command>:	"MR" - Transmit maintenance report; no arguments
<response>:	"ERE" <space><error code><space><mrecords><cr>
<mrecords>:	maintenance record; "On/off:" <#on/off><cr> :Number of times the instrument was turned on. "Hours" <#hours><cr> "Number of hours the instrument was on. "Plates" <#plates><cr> :Number of plates read. Note: <#on/off><#hours>, and <#plates> are ASCII encoded decimal values. This information is kept in battery backed-up RAM.

<command>: "RM" - Reset maintenance Report values; no arguments
 <response>: "ERE" <space><error code><cr>

<command>: "RPLATE" - Read plate; argument mix, load, stack, wp1, wp2
 <cmd args>: mix - mixing time before reading (in seconds); ASCII encoded decimal
 load - "0"; do not load plate from stack loader before reading.
 "1"; load plate from stack loader before reading.
 Note: load argument is ignored if the stack loader is not installed.
 stack - "0"; do not stack plate (remove plate from plate carriage) after plate reading.
 "1"; stack plate (remove plate from plate carriage) after plate reading. Note: stack argument is ignored if the stack loader is not installed.
 wp1 - position on filter wheel (1-6) for measurement filter; ASCII encoded decimal
 wp2 - (optional; only present for dual wavelength readings) position on filter wheel (1-6) for reference filter, ASCII encoded decimal.

<response>: "ERE" <space><error code><space><abs. data><optional abs. data for second wavelength><cr>

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

<response>: "ERE" <space><error code><space><abs. data><optional abs. data for second wavelength><cr>

<abs. data>: <header><time><date><filter info><bar code><Meas. data><Ref. data><cr>

<header>: "BIO-RAD MODEL 3550 EIA READER" <cr>

<time>: "Time: 10:00:00" <cr>: Time the reading was taken

<date>: "Date: 12-25-86" <cr>: Date the reading was taken

<filter info>: "Measurement filter xxx nm." <cr>
 "Reference filter xxx nm." <cr> (present for dual wavelength readings)

<bar code>: Bar code information; <cr> if no bar code.

<Meas. data>: ".begin" <cr><8 rows of 12 wells of abs. values><checksum><"end"> <cr>
 Measurement wavelength absorbance values are in ASCII encoded floating point format. Each absorbance value is separated by a space. Row 1 = wells (A1-A12), Row 2 = wells (B1-B12),....Row 8 = wells (H1-H12). Each absorbance value is separated by a space.

<Ref. data>: ".begin" <cr><8 rows of 12 wells of abs. values><checksum><"end"> <cr>
 Reference wavelength absorbance values are in ASCII encoded floating point format. Each absorbance value is separated by a space. Row 1 = wells (A1-A12), Row 2 = wells (B1-B12),.... Row 8 = wells H1-H12). Each absorbance value is separated by a space.

<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 <Meas. 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 value greater than 2.999 shall be transmitted as asterisk.

EIA READER ERROR CODES

"0000" - No error
 "8071" - Invalid command
 "8072" - Parameter out of range
 "8073" - Device not in remote mode
 "8074" - Device busy
 "8075" - Device filter wheel jammed
 "8076" - Plate stacker empty
 "8077" - Light bulb burned out
 "8078" - Hardware error
 "8079" - Memory error
 "8080" - Warm-up in progress

12.3 Helpful Hints for the Command Language

1. Data Buffer
 The buffer receiving data should contain at least 1000 bytes available for single wavelength readings and 1500 bytes available for dual wavelength readings.
2. Error Message
 An error code will be transmitted for every incorrect command received. This can cause confusion in two circumstances.
 1) If the reader is being acquired, the error code will be 8073 or "Device not in Remote Mode" even though the acquire command is placing the reader into remote mode. the acquire is successful despite the error code information received. For acquire commands only, treat 8073 as "No error"
 2) If a true hardware error occurs, the reader will continue to send the error code until a read plate command is sent. This can cause confusion especially when using the Stack Loader and the error 8076 or "Loading Magazine Empty" occurs. This error code may continue to appear if the release command, acquire command or other commands than read plate (or read well) are sent. This will happen even if you have re-filled the loading magazine or corrected the hardware error. This error occurs because the reader does not check for errors unless trying to read a plate.
3. Arguments
 Arguments are the additional features after the read plate command; for instance the mixing time, presence of the Stack Loader, 1st and 2nd wavelength. Arguments must be separated by spaces.

Arguments for the Stack Loader are either	0<space>0	No loading/No stacking
	1<space>1	Loading and Stacking
	0<space>1 or 1<space>0	Not accepted

Appendix A

Sample Report Documentation

BIO-RAD Model 550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

SYSTEM CONFIGURATION PARAMETERS

=====

DATE FORMAT: [MM/DD/YY]/ DD/MM/YY
 DATE: Month 04 Day 20 Year 1988
 TIME: Hour 15 Minute 43

DEFAULT ANALYSIS NUMBER: 1

FILTER WHEEL: Position #1 405 nm.
 Position #2 415 nm.
 Position #3 450 nm.
 Position #4 490 nm.
 Position #5 595 nm.
 Position #6 655 nm.

BIO-RAD Model 550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

CONCENTRATIONS OF STANDARDS (Format Number 3)

=====

Standard Number	Concentration	Standard Number	Concentration
STD # 1	1.00xE 02	STD # --	
STD # 2	5.00xE 01	STD # --	
STD # 3	2.50xE 01	STD # --	
STD # 4	1.25xE 01	STD # --	
STD # 5	6.25xE 00	STD # --	
STD # 6	3.31xE 00	STD # --	
STD # 7	1.56xE 00	STD # --	
STD # 8	7.80xE-01	STD # --	
STD # --		STD # --	
STD # --		STD # --	

BIO-RAD Model 3550 MICROPLATE READER

ASSAY ----- OPERATOR -----

PLATE ID ----- NOTES :

FORMAT NUMBER 3

```
=====
      1      2      3      4      5      6      7      8      9      10     11     12
A      B      S01     S02     S03     S04     S05     S06     S07     S08     ...     ...     ...
B      B      S01     S02     S03     S04     S05     S06     S07     S08     ...     ...     ...
C      B      S01     S02     S03     S04     S05     S06     S07     S08     ...     ...     ...
D      B      S01     S02     S03     S04     S05     S06     S07     S08     ...     ...     ...
E      B      X01     X02     X02     X04     X05     X06     X07     X08     X09     X10     X11
F      B      X01     X02     X02     X04     X05     X06     X07     X08     X09     X10     X11
G      B      X01     X02     X02     X04     X05     X06     X07     X08     X09     X10     X11
H      B      X01     X02     X03     X04     X05     X06     X07     X08     X09     X10     X11

```

[BLANK]

SAMPLE NO. X01

STANDARD NO. S01

BIO-RAD Model 3550 MICROPLATE READER

ASSAY ----- OPERATOR -----

PLATE ID ----- NOTES :

RAW DATA REPORT PLATE NUMBER 8

DATE 04/20/88 TIME 15:40

ANALYSIS 1 FORMAT 3

Measurement 405nm. Reference 655nm.

```
=====
      1      2      3      4      5      6      7      8      9      10     11     12
A      0.013 1.828 1.034 0.716 0.450 0.269 0.157 0.084 0.051 0.030 0.021 0.023
B      0.009 1.852 1.034 0.707 0.454 0.271 0.153 0.087 0.051 0.032 0.022 0.023
C      0.006 1.819 1.007 0.702 0.455 0.262 0.150 0.082 0.056 0.045 0.017 0.018
D      0.011 1.823 1.037 0.714 0.459 0.270 0.152 0.085 0.050 0.033 0.020 0.024
E      0.011 1.802 1.033 0.718 0.461 0.268 0.153 0.086 0.049 0.029 0.018 0.020
F      0.009 1.798 1.023 0.717 0.464 0.268 0.152 0.084 0.047 0.029 0.019 0.020
G      0.012 1.806 1.023 0.706 0.456 0.270 0.152 0.085 0.049 0.033 0.022 0.026
H      0.010 1.795 1.001 0.699 0.450 0.261 0.147 0.080 0.046 0.030 0.019 0.021

```

BIO-RAD Model 5550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

ANALYSIS NUMBER 1

DATE 04/20/88 TIME 15:45

```
=====
Format Number (1-9) 3 Blank[Each]/ First Plate Time Delay Yes/[No]
Time 0:00 (min:sec)
Print Reports[Yes]/ No Single/[Dual]Wavelength Absorbance Delay Yes/[No]
Absorbance [Yes]/ No Absorbance Value 0.000
Matrix [Yes]/ No Measurement Wavelength [Above]/ Below abs. value
Limit [Yes]/ No [405]415 450 490 595 655 Well: [A]B C D E F G H
Evaluation [Yes]/ No [1]2 3 4 5 6
Reference Wavelength 7 8 9 10 11 12
Matrix Maximum 2.000 405 415 450 490 595[655]
Upper limit 1.500 Multiple Readings Yes/[No]
Lower limit 0.050 Auto Mixing [Yes]/ No Number of readings 1
Interval 0:00 (min:sec)
```

BIO-RAD Model 5550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

ABSORBANCE REPORT PLATE NUMBER 8

DATE 04/20/88 TIME 15:40

```
=====
ANALYSIS 1 FORMAT 3 Measurement 405nm. Reference 655nm.
Blank 0.010 S.D. 0.002
```

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	1.818	1.024	0.706	0.440	0.259	0.147	0.074	0.041
B	-0.001	1.842	1.024	0.697	0.444	0.261	0.143	0.077	0.041
C	-0.004	1.809	0.997	0.692	0.445	0.252	0.140	0.072	0.046
D	0.001	1.813	1.027	0.704	0.449	0.260	0.142	0.075	0.040
E	0.001	1.792	1.023	0.708	0.451	0.258	0.143	0.076	0.039	0.019	0.008	0.010
F	-0.001	1.788	1.013	0.707	0.454	0.258	0.142	0.074	0.037	0.019	0.009	0.010
G	0.002	1.796	1.013	0.696	0.446	0.260	0.142	0.075	0.039	0.023	0.012	0.016
H	0.000	1.785	0.991	0.689	0.440	0.251	0.137	0.070	0.036	0.020	0.009	0.011

BIO-RAD Model 3550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

MATRIX REPORT PLATE NUMBER 8 DATE 04/20/88 TIME 15:40

=====

ANALYSIS 1 FORMAT 3 Measurement 405nm. Reference 655nm.
Blank 0.010 S.D. 0.002

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	9	3	3	2	1	0	0	0	.	.	.
B	-	9	3	3	2	1	0	0	0	.	.	.
C	-	9	4	3	2	1	0	0	0	.	.	.
D	0	9	3	3	2	1	0	0	0	.	.	.
E	0	8	3	3	2	1	0	0	0	0	0	0
F	-	8	3	3	2	1	0	0	0	0	0	0
G	0	8	3	3	2	1	0	0	0	0	0	0
H	0	8	4	3	2	1	0	0	0	0	0	0

MATRIX MAXIMUM
ABSORBANCE 2.000

BIO-RAD Model 3550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

LIMIT REPORT PLATE NUMBER 8 DATE 04/20/88 TIME 15:40

=====

ANALYSIS 1 FORMAT 3 Measurement 405nm. Reference 655nm.
Blank 0.010 S.D. 0.002

	1	2	3	4	5	6	7	8	9	10	11	12
UPPER LIMIT	-	+	*	*	*	*	*	*	-	.	.	.
ABSORBANCE 1.500	-	+	*	*	*	*	*	*	-	.	.	.
LOWER LIMIT	-	+	*	*	*	*	*	*	-	.	.	.
ABSORBANCE 0.050	-	+	*	*	*	*	*	*	-	-	-	-
	-	+	*	*	*	*	*	*	-	-	-	-
	-	+	*	*	*	*	*	*	-	-	-	-

BIO-RAD Model 550 MICROPLATE READER

ASSAY _____ OPERATOR _____

PLATE ID _____ NOTES :

EVALUATION REPORT PLATE NUMBER 8 DATE 04/20/88 TIME 15:40

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ANALYSIS 1 FORMAT 3 Measurement 405nm. Reference 655nm.

Linear Regression Analysis: Slope 1.74xE-02 Intercept 1.28xE-01 "r" 0.990

		MEAN	S.D.	%C.V.	CONC.
Blank		0.000	0.002	***	***E **
STD	1	1.821	0.015	00.81	1.00xE 02
STD	2	1.018	0.014	01.38	5.00xE 01
STD	3	0.700	0.006	00.92	2.50xE 01
STD	4	0.445	0.004	00.83	1.25xE 01
STD	5	0.258	0.004	01.58	6.25xE 00
STD	6	0.143	0.003	02.06	3.31xE 00
STD	7	0.075	0.002	02.79	1.56xE 00
STD	8	0.042	0.002	06.45	7.80xE-01
SAMPL	1	1.790	0.005	00.27	9.51xE 01
SAMPL	2	0.879	0.164	18.67	4.30xE 01
SAMPL	3	0.689	0.000	00.00	3.21xE 01
SAMPL	4	0.448	0.006	01.37	1.83xE 01
SAMPL	5	0.257	0.004	01.54	7.38xE 00
SAMPL	6	0.141	0.003	01.92	7.41xE-01
SAMPL	7	0.074	0.003	03.57	***xE **
SAMPL	8	0.038	0.002	03.97	***xE **
SAMPL	9	0.020	0.002	09.35	***xE **
SAMPL	10	0.010	0.002	18.23	***xE **
SAMPL	11	0.012	0.003	24.44	***xE **