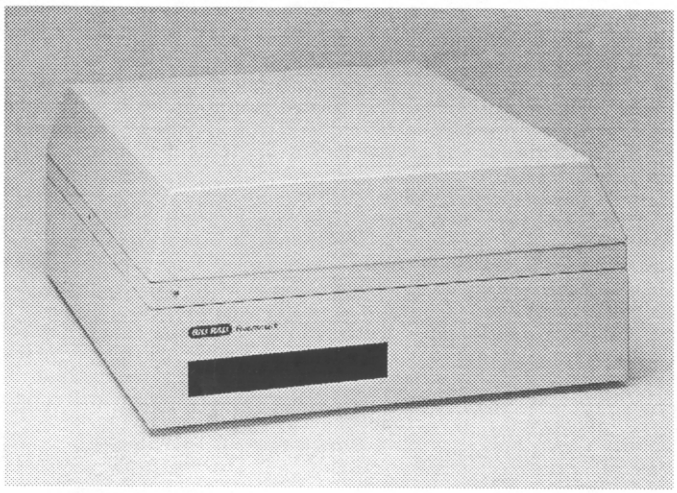


Fluoromark™ Microplate Fluorometer and Fluoromark Software

Instruction Manual

Catalog Numbers
170-6941, 170-6966, 170-6970, 170-6942, 170-6967, 170-6971, 170-6943, 170-6968, 170-6972, 170-6944, 170-6969 and 170-6973

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 Fluoromark system 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 Fluoromark system. 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
General Information

1.1 Introduction

The Fluoromark microplate fluorometer provides all the flexibility you need for virtually any fluorescent assay. You can use it to perform top and bottom epifluorescence for detection in solution assays or cell monolayers bound to well bottoms, and use 6, 12, 24, 48, 96, and 384 well plate formats. With the widest working wavelength range of any microplate fluorometer (250–900 nm), the Fluoromark fluorometer lets you employ fluorophores such as dansylamide for protein quantitation or Cy® dyes for DNA quantitation. You can run both time-resolved studies using lanthanide chelates and standard fluorescent determinations. For assays requiring temperature control, the optional incubator features a range from ambient to 45 °C. Features of the Fluoromark system include

- Perform both standard and time-resolved fluorescent measurements
- Read either from the top or the bottom of the plate
- Use any microplate format including 6, 12, 24, 96, and 384 well plates
- Optional incubation capabilities
- Extended ultraviolet read capability down to 250 cm
- Cleaner, economical, more sensitive alternative to radioactive assays

1.2 Specifications

Light source	High energy Xenon flash lamp	
Detector	Side window, current type photomultiplier tube	
Filters	Excitation filter wheel for 8 interference filters Emission filter wheel for 8 interference filters	
Excitation wavelength range	290–900 nm (250 nm optional)	
Emission wavelength range	300–900 nm	
Raw result range	0–9999	
Sensitivity	5 pg/well sodium fluorescein	
Incubation system	Ambient to 42 °C (optional)	
Interface	RS232 bi-directional	
Power	150 VA 100/120 V and 220/250 V	
	Fuses: T2.5 A 250 V for main power 230 V T1.6 A 250 V for main power 115 V	
Physical	20 x 44 x 48 cm (h x w x d)	
Weight	23 Kg	
Operating Conditions	Operating temperature	15–30 °C
	Storage temperature	-10–50 °C
	Humidity	20%–80%

This instrument conforms to Overvoltage Category II and Contamination Class II

Section 2
Equipment Set-up and Installation

2.1 Unpacking the Fluoromark Microplate Fluorometer

1. Before opening the box, check it for any outer damage. Make a note of any damages, if present.
2. Place the box upright with the TOP label facing up, and open the container. Remove the instrument from the box in this position only.

   Warning: Grasp the instrument firmly under the right and left sides before attempting to lift or carry it.
3. Check the instrument for damaged or broken parts. Note specifically any damages, if they are present.
4. Each Fluoromark instrument includes the following accessories. Verify that they are present.
   1 power cord
   1 RS232 computer cable
   1 instruction manual
   3 Software diskettes
   1 box containing 2 spare fuses
   3 Allen wrenches
   Excel Software
5. Verify that the serial number on the back of the instrument is the same as that on the outside of the box. Check also that the main voltage shown on the back of the instrument corresponds to the local voltage in your country.

2.2 RS232 Cable Connection

The RS232 port is on the back of the instrument. The included interface cable will connect the Fluoromark microplate fluorometer to COM1 of your PC.

Note: Communication parameters of the PC interface are automatically configured by the software.

2.3 Power Connection

1. Plug the included power cord into the main power connector port. Do not switch power on yet. Make sure that the instrument is connected to a grounded conductor of the main power outlet.
2. Before powering the instrument up for the first time, allow it to warm to room temperature for 6 hours, to prevent any condensation problems.

2.4 Location of the Instrument

The Fluoromark microplate fluorometer is a precise optical measuring instrument. The following conditions must be met to insure maximum sensitivity.

1. Place the instrument on a flat and vibration free surface.
2. Do not place the instrument in direct sunlight.
3. Insure that the location is dust free.

Section 3
Maintenance

3.1 Disinfection Procedure

All parts of this instrument that can come in contact with patient sera or positive samples must be handled as hazardous items. Bio-Rad recommends using gloves when performing maintenance or working with the instrument.

Note: It is very important that the instrument undergo thorough disinfection before performing maintenance or before physical removal of the instrument from the laboratory. The instrument must be disinfected before you send it back to Bio-Rad for service. For safety reasons, you must fill out a Disinfection Certificate and include it with the instrument. Without a Disinfection Certificate, Bio-Rad will not accept any returned instruments.

Bio-Rad recommends using a solution of 10% formaldehyde, 70% alcohol, and 20% deionized water. Make sure to observe all national regulations for safe handling of formaldehyde. The disinfection procedure must be performed by authorized persons wearing one way gloves and protective clothes. The location must possess adequate ventilation.

To disinfect Fluoromark, use the following procedure.

Note: Be sure that you wear disposable gloves.

1. Disconnect the instrument from the main power supply.
2. Remove the RS232 cable from its port.
3. Clean all the outside surfaces of the instrument carefully with a wad of cotton which has been soaked in the formaldehyde solution.
4. Place the instrument in a sealed plastic bag.
5. Repeat the procedure for disinfection on any accessories which are also being returned with the instrument.
6. Complete the Certificate of Disinfection and send it in with the instrument

Section 4
Software Installation

The Fluoromark Excel software runs on IBM compatible PC processors under Microsoft Windows® Version 3.1, 3.11 and '95. The CPU must be a 486 or higher with minimum 4 Mb RAM. The data reduction is done in the Microsoft spread sheet program Excel version 5.0c. Before installing Fluoromark software, verify proper installation of Excel 5.0c.

4.1 Installing Excel 5.0 Software

The data base engine, Microsoft Query, must be installed during Excel installation.

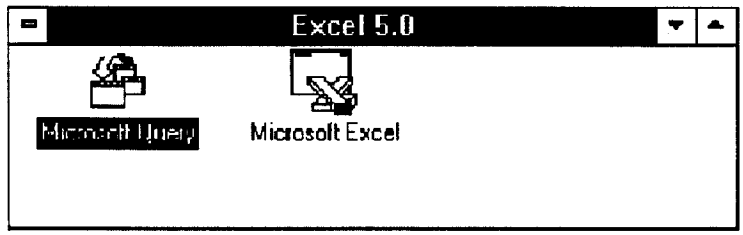


Fig. 4.1. Required components of Excel software.

If Microsoft Query is not installed on your computer, you must reinstall Excel specifying Query, or Data Access during custom installation.

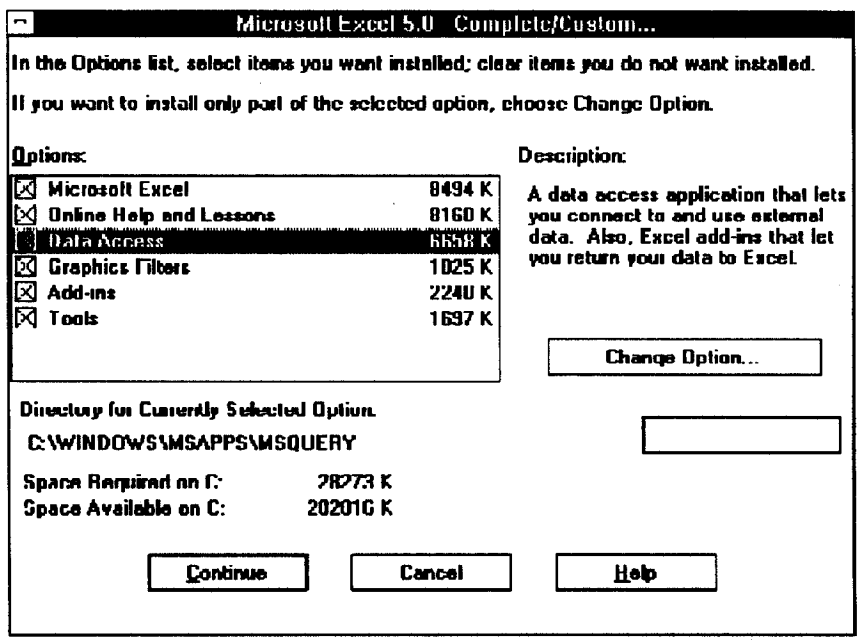


Fig. 4.2. Reinstallation of the data base handler Microsoft Query.

After proper installation of Excel; the Microsoft Query icon should appear in the Excel program group, and the data base driver ODBC should appear in the System Control program group.

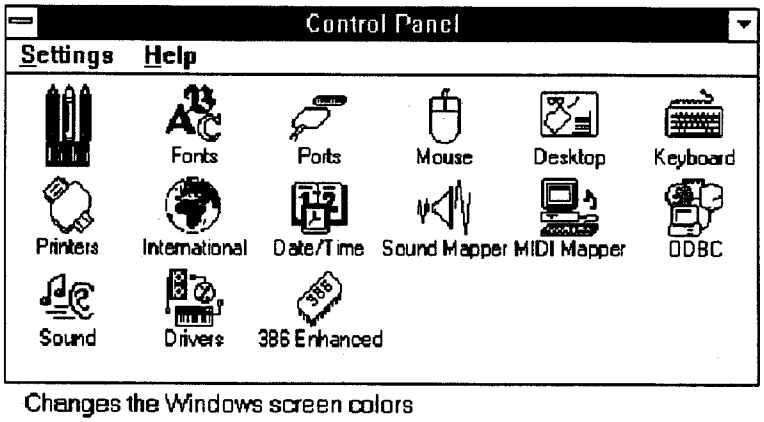


Fig. 4.3. ODBC driver in system control.

4.2 Installing the Fluoromark Software

After verifying installation of Excel 5.0c, install the Fluoromark software by placing the installation diskette in the drive, and running the Setup program from the Program Manager.

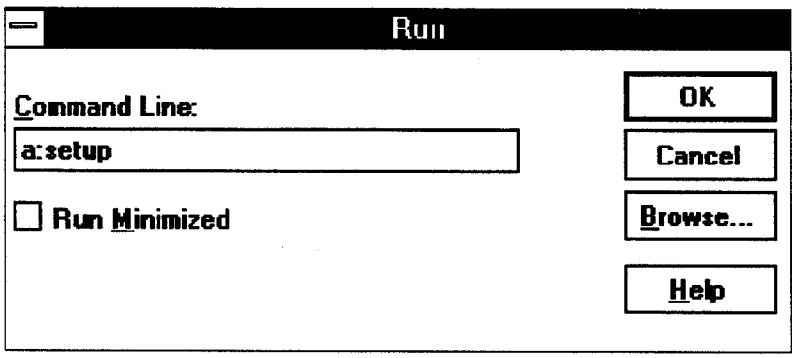


Fig. 4.4. Starting the Fluoromark installation.

The installation program will prompt for the directory in which to install the Borland database tools.

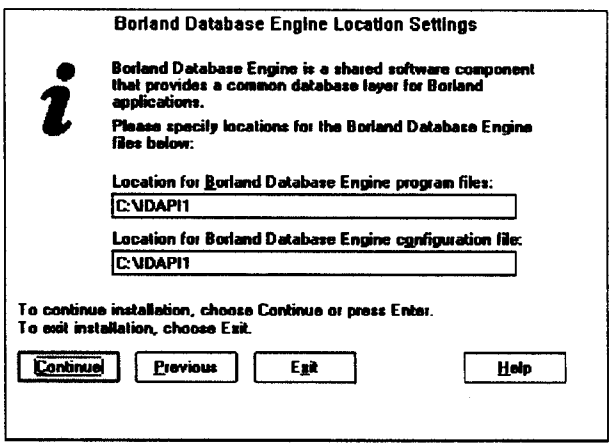


Fig. 4.5. Directory for the database.

The database of the Fluoromark software will be installed in the selected directory, from diskettes 1 and 2. Next install the Fluoromark reader software by running the Setup program on diskette 3.

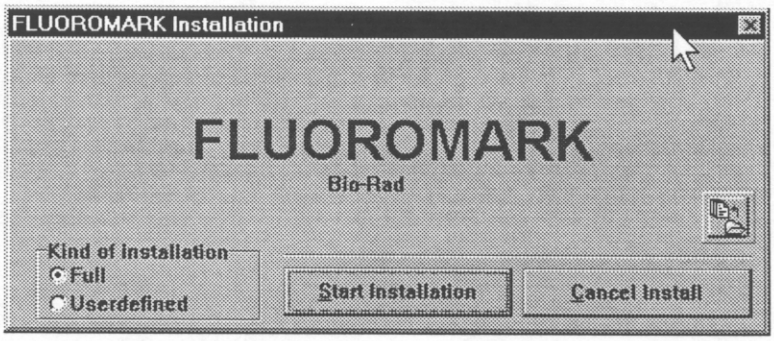


Fig. 4.6. Fluoromark installation screen.

After selecting the Start Installation option, the setup program prompts for the directory in which to install the Fluoromark program files.

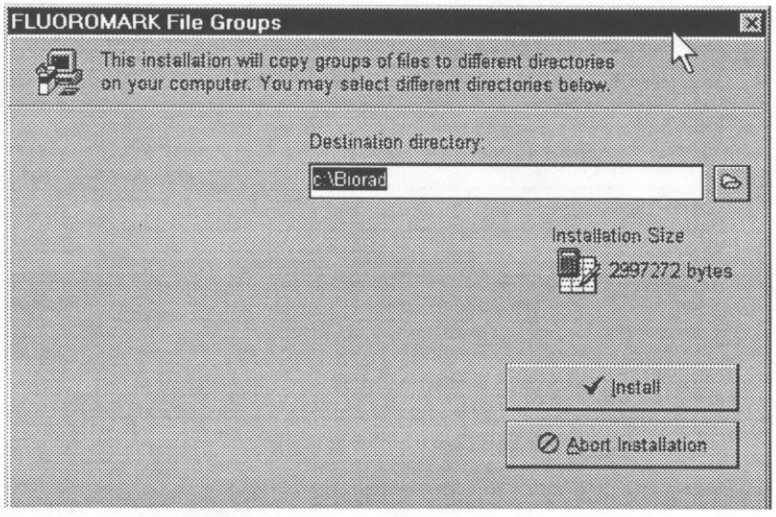


Fig. 4.7. Directory for the Fluoromark software.

After installation of the Fluoromark software, a Fluoromark icon will be created in a Program Group named Fluoromark.

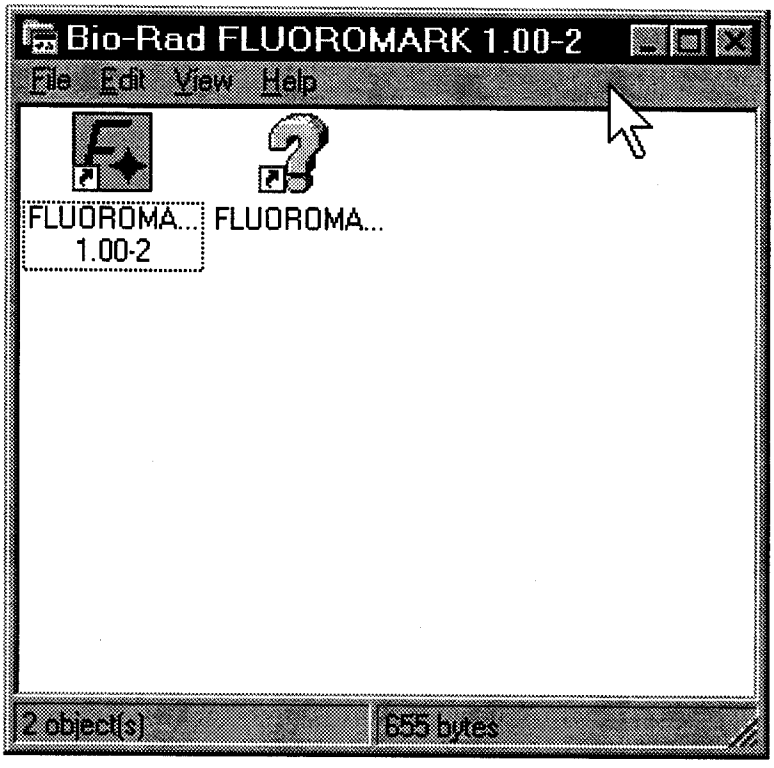


Fig. 4.8. Screen to start Fluoromark software.

You are now ready to begin using the Fluoromark package.

Section 5
Using the Fluoromark Windows PC Software

5.1 Hints for Operation

The Fluoromark software runs under Microsoft Windows version 3.1, 3.11 (Workgroups), or '95. While any computer capable of running Windows will run the Fluoromark software, a Pentium processor with a minimum of 16 Mbytes of RAM is recommended.

The red transport locking pin must be removed to free up the plate carrier after the instrument is installed.

The Offset values from the back of the instrument must be entered on the Instrument Setup, Offset, screen before a measurement can be made.

All screen savers must be disabled while using the Fluoromark PC program, as the system can lose data across the serial line from Fluoromark to the PC when screen savers are in effect.

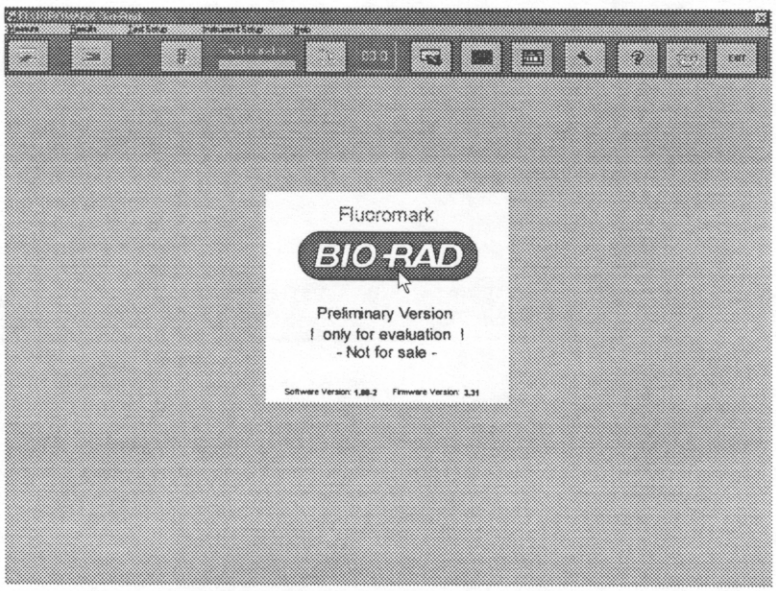


Fig. 5.1. Startup screen with menus and symbols.

5.2. General View of the Menus and the Corresponding Icons

Frequently used menu selections can be accessed directly from the Tool Bar. Tool Bar icons perform the same functions as the menu items listed below.

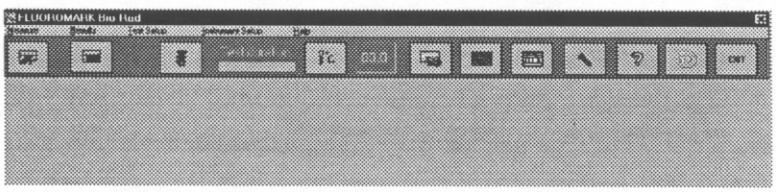
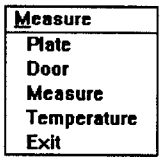
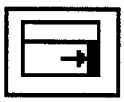
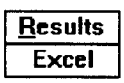
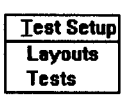
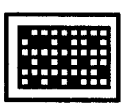
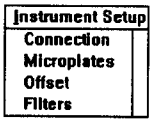
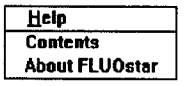


Fig. 5.2. Menus and symbols.

Icon	Description	Action
	Measure	Menu for Fluoromark hardware control and for starting test runs. The Fluoromark microplate fluorometer has to be switched on and connected to the PC for this menu to be active.
	Plate out	Opens the Fluoromark microplate fluorometer door and ejects plate carrier. If the plate carrier is already out, no action is taken.
	Plate in	Brings the plate carrier in. If the plate carrier is already in, no action is taken.
	Door open	Open the door
	Door close	Close the door
	Measure	Performs a measurement on a microplate, using a pre-defined test procedure
	Real-time graphic	During a test run the measure icon will change to the real-time graphics icon. This opens the real-time graphic screen.
	Temperature	Sets the target temperature of the incubator 25 °C to 45 °C in 0.1 °C steps, and begins incubating
	Results	Transfers control to Excel data reduction template for measurement results
	Results	Transfers control to Excel data reduction template for measurement results
	Test setup	Menu to define microplate sample positions and test protocols
	Layouts	Menu to define the positions of Standards, Blanks, Unknowns, Empty wells, and concentrations
	Tests	Menu to define measurement timing, shaking, and filters for excitation and emission

Icon	Description	Action
	Instrument setup	Defines hardware configurations and plate types
	Connection	Menu to define com port (1 to 4) for connecting Fluoromark microplate fluorometer to PC
	Microplates	Menu to define the dimensions of microplate
	Offset	Menu to set the "home position" for each Fluoromark instrument
	Filters	Menu to define excitation and emission filters
	Help	Menu for the integrated help function of the program with information about the version number of the software
	About Fluoromark	Software version, version number software of the PC software. Firmware version, version number of the Fluoromark software.
	Stop button	Stops an active test run, saving any data collected
	End of program	Ends the program, sends init command to the Fluoromark fluorometer

5.3 Performing a Measurement

Test procedures are performed by selecting the  symbol or by selecting Measure in the measurement menu. After initiating the test run, a message is displayed prompting for insertion of a microplate.

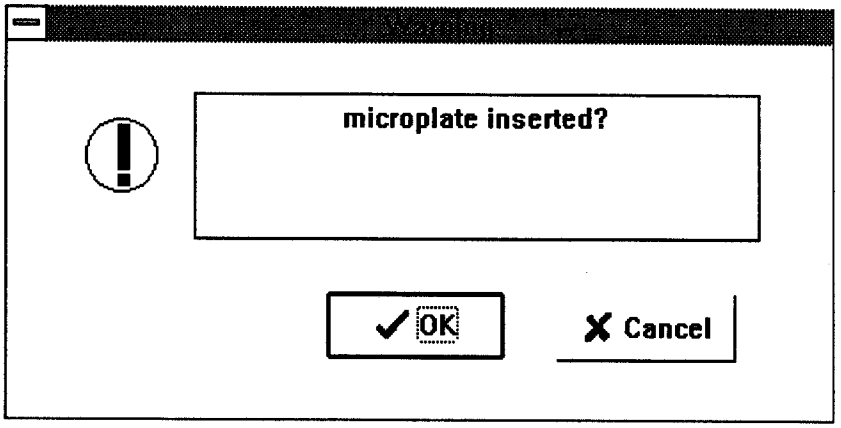


Fig. 5.3. Warning before starting the test run.

The warning screen (Figure 5.3) prompts for insertion of a microplate before a test run.

Select buttons

OK proceeds to the next menu for selecting a pre-defined test

Cancel aborts measurement, returns to main menu

After pressing OK button, all pre-defined tests will be listed.

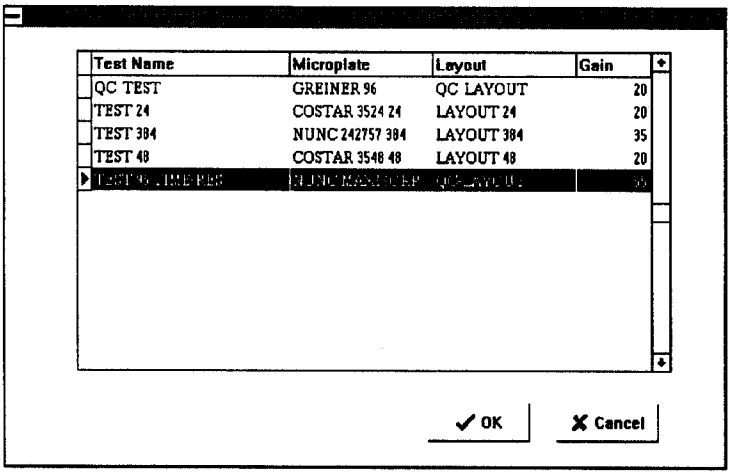


Fig. 5.4. Listing of all pre-defined tests for selection.

The test screen (Figure 5.4) lists all pre-defined tests, showing the following information.

Test Name previously defined test procedures

Microplate microplate type for the corresponding test

Layout layout name for the corresponding test

Gain defined sensitivity (gain value) for the corresponding test

Select a test procedure with a mouse click or the cursor keys, and start the selected test by pressing the OK button.

Select buttons

OK proceeds to the next screen, for selection of the plate ID

Cancel aborts measurement, returns to main menu

After specifying a test procedure, an alpha numeric identifier for the experiment may be entered. This name will be saved with the results of the measurement, and may be used to recall, calculate, and export the data.

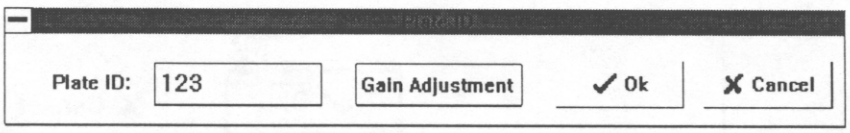


Fig. 5.5. Enter an identification number before test start.

The enter an identifier for the test run screen (Figure 5.5) lets you enter an identification number. The name or number will not be used in calculations.

Select buttons

Gain Adjustment proceeds to the next screen, see Figure 5.8, for running Gain adjustment

OK begin measuring the selected test

Cancel aborts measurement, returns to main menu

After starting a test, a time gauge will be displayed in the tool bar, indicating the elapsed time and time left for completion of the measurement. This gauge is updated dynamically during the test run. If temperature control has been selected, the actual temperature will also be displayed.

The measure icon will change to the real-time graphics icon in the Tool Bar.

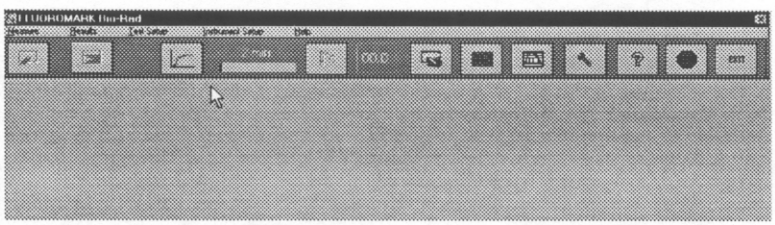


Fig. 5.6. Menu and symbols during test run.

After selecting the real-time graphics icon, the following screen will appear.

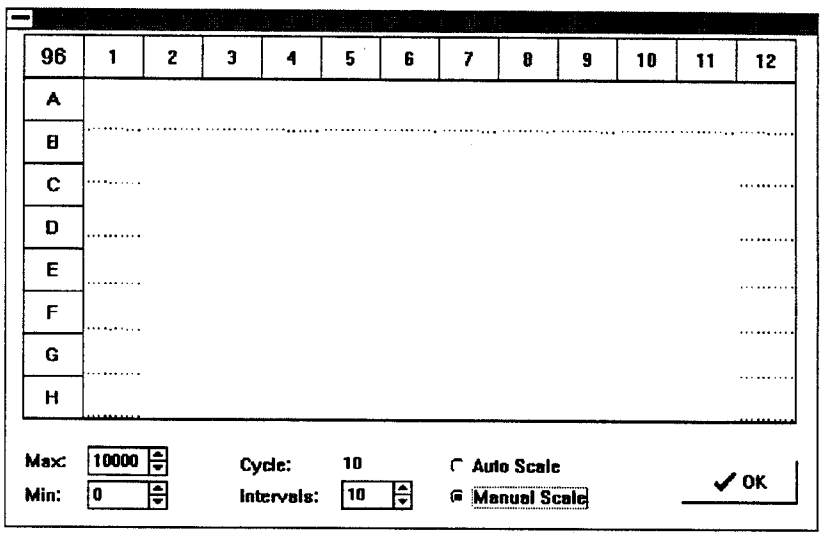


Fig. 5.7. Screen for real-time graphics.

The real-time graphics screen (Figure 5.7) displays real time graphic in defined microplate format (384, 96, 48, 24). During the test run all measured values will be automatically displayed as points. If you select Auto Scale minimum and maximum limits will be updated automatically. If you select Manual Scale minimum and maximum limits can be specified manually. You can specify the number of points to be shown in a well by manipulating the number of intervals.

Select buttons

Max	maximum limit for displayed values
Min	minimum limit for displayed values
Intervals	number of points to be shown. Default value is number of cycles defined in test definition.
OK	closes real-time graphics screen and saves measured values if test run is finished

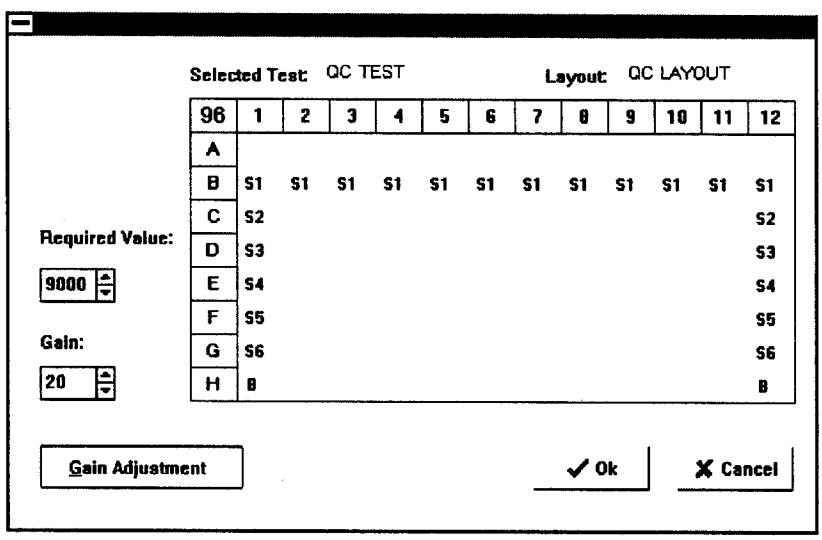


Fig. 5.8. Screen for Gain Adjustment.

The gain adjustment screen (Figure 5.8) performs gain adjustment for the plate. Specify the required value up to 9999. (Bio-Rad recommends using 9500). The gain adjustment button will determine the optimum gain for the selected well (for example the highest known standard).

Select buttons

Required value	required value of the result in relative fluorescent units
Gain optimum	gain for required result
Gain Adjustment	performs gain adjustment for the selected well in the layout above
OK	closes gain adjustment screen and saves gain value in corresponding test definition
Cancel	aborts gain adjustment

5.4 Incubating Plates (optional equipment)

Incubation (if installed) is implemented with either the [°C] icon, or by selecting the temperature option in the measurement menu.

After selecting this function the following screen will appear.

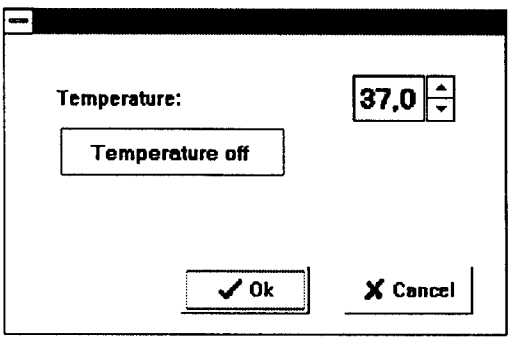


Fig. 5.9. Screen temperature selection.

Specify the target temperature of the incubator (25 °C to 45 °C), in steps of 0.1 °C using either the spinner or by typing directly into the selection box. The Temperature On button toggles to Temperature Off during incubation.

Select buttons

Temperature defines the target incubation temperature

Temperature off de-activates incubation

OK closes temperature screen

Cancel aborts last action, returns to main menu

If you have selected incubation, the measured temperature will be displayed in the tool bar. If the temperature is below the target temperature, the field will be red. If the target temperature has been reached, the field will be green.

5.5 Defining a Layout

Microplate layouts are defined using the  icon, or by selecting Layouts in the test setup menu. After selecting this function, all previously defined layouts will be listed.

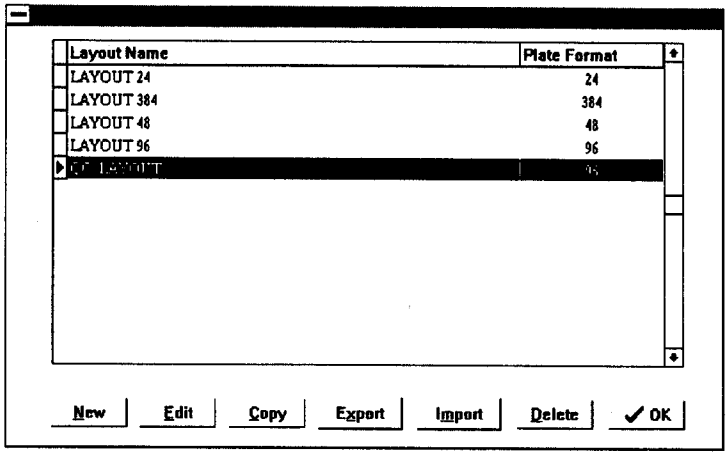


Fig. 5.10. List of all defined layouts.

The layouts screen (Figure 5.10) lists all previously defined and stored layouts.

Select buttons

New	creates a new microplate layout
Edit	modifies an existing microplate layout
Copy	copies an existing microplate layout
Export	exports an existing microplate layout
Import	imports an existing microplate layout
Delete	deletes an existing microplate layout
OK	closes the layout screen, and returns to main menu

When a new layout is defined, the plate format (384, 96, 48, or 24 wells) must be defined first.

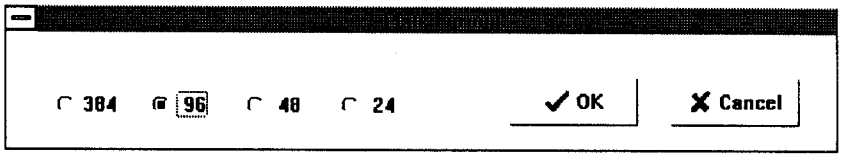


Fig. 5.11. Defining the plate format of a new layout.

The plate format screen (Figure 5.11) defines the plate format for the new microplate layout.

Select buttons

384	selects plate format to 384 wells
96	selects plate format to 96 wells
48	selects plate format to 48 wells
24	selects plate format to 24 wells
OK	begins definition of a new layout of the selected plate format
Cancel	aborts definition, returns to layouts screen

After selecting the plate format for the new microplate layout, the software proceeds to the corresponding layout definition screen.

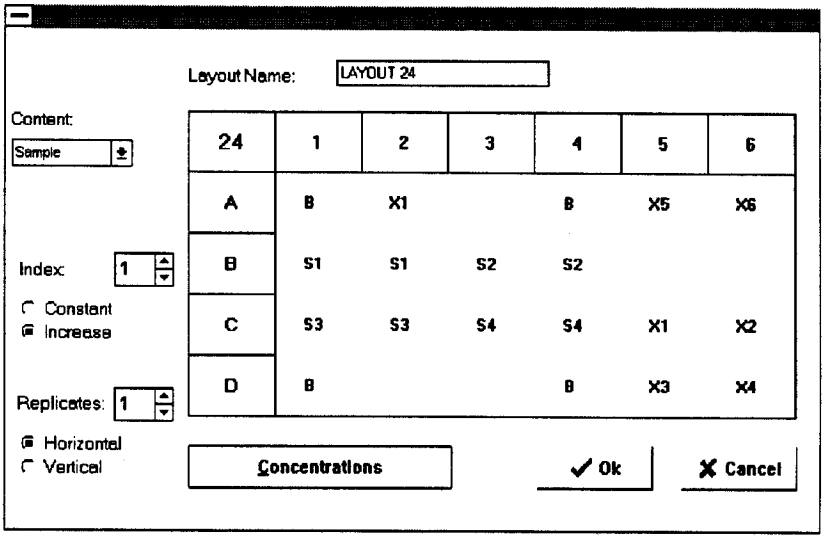


Fig. 5.12. Screen for modifying or defining a 24 well layout.

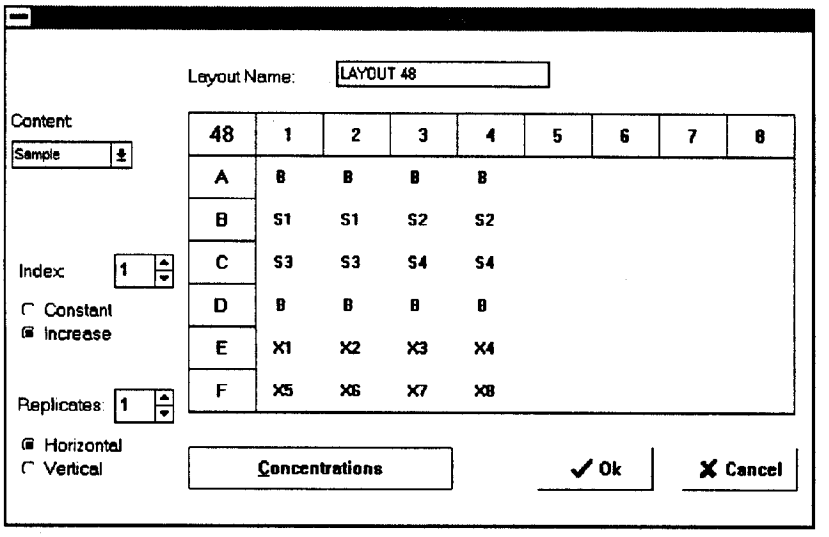


Fig. 5.13. Screen for modifying or defining a 48 well layout.

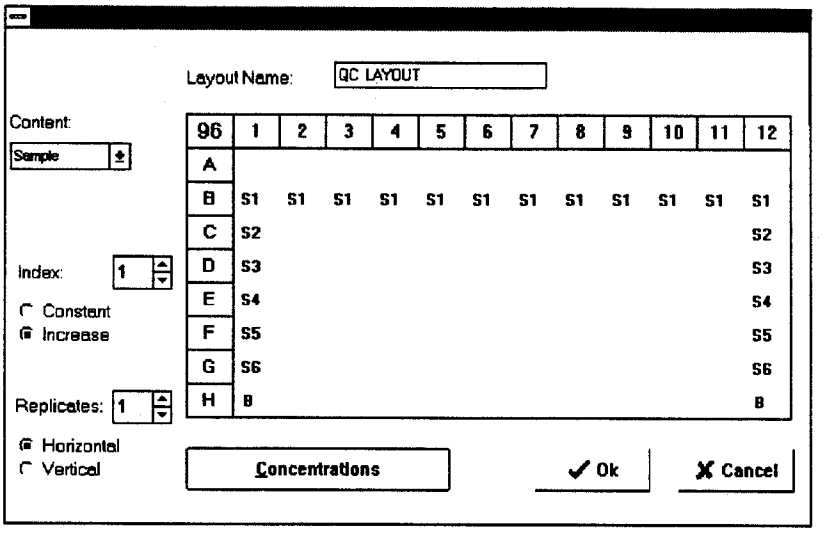


Fig. 5.14. Screen for modifying or defining a 96 layout.

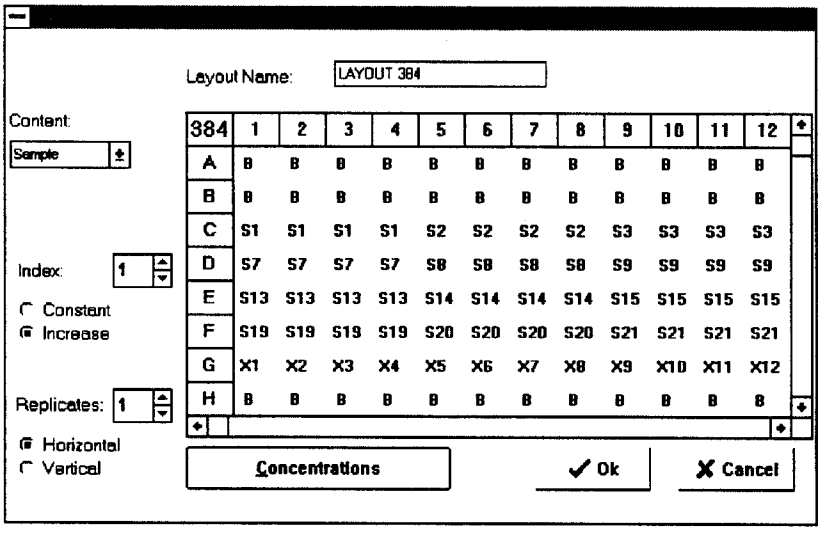


Fig. 5.15. Screen for modifying or defining a 384 layout.

The layout screens (Figure 5.13 through 5.15) are used to modify or to define a layout.

Select buttons

Content	Allows selection of well type (Empty, Samples, Blanks, Standards)
Index	Selects the number of the next well defined. (Define Auto Increment to automatically increase the index, or Constant Index for continuous replicates.)
Replicates	Selects the default number of replicates. Horizontal or Vertical determines the order of the replicates.
Concentrations	Switches to definition of standard concentrations (Figure 5.16)
OK	Saves all modifications and returns to Figure 5.10 (layouts)
Cancel	Returns to Figure 5.10 without saving

How to Modify A Layout

Define the appropriate type of sample for a given group of wells in the Content drop-down box. After defining the well type, select the well locations using the left mouse button, and dragging across all wells of that type. Wells can also be defined with the keyboard, by holding the shift key and highlighting the appropriate wells with the cursor keys. Wells can be defined by row or column by clicking the row or column label (A to H and 1 to 12). All wells of the microplate can be selected by clicking the field in the upper left border.

The concentrations screen (Figure 5.16) shows concentrations for the defined layout. Concentrations can be defined for individual wells by typing directly or by using the auto function. To use the auto function, define start value (concentration) and select factor, increment, or decrement and type in the offset or factor. Then select concentration by using the right mouse button and dragging across the fields you want to change. Fields can also be changed with the keyboard, by holding the shift key and highlighting the appropriate fields with the cursor keys.

Select buttons

Concentration defines the concentration of the available standards with the auto function

Layout Definition saves all modifications and returns back to Figure 5.10

Cancel returns to Figure 5.10 (layout definition) without saving

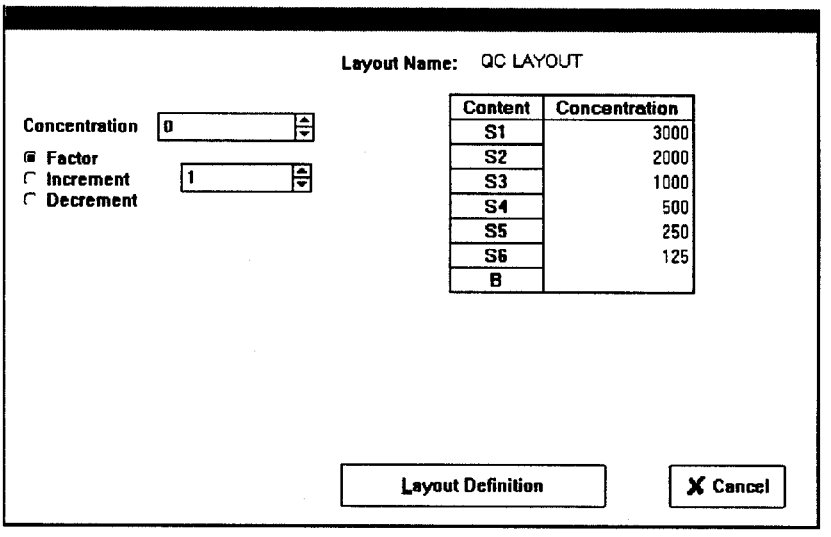


Fig. 5.16. Screen for defining concentrations.

The copy screen (Figure 5.17) is used to copy layouts, tests, or microplates

Select buttons

OK begin copying the selected layout, test or microplate

Cancel aborts copy function

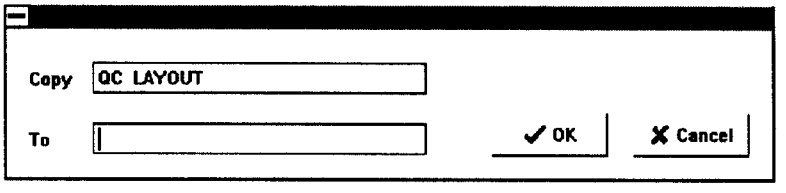


Fig. 5.17. Screen for copying layouts, tests or microplates.

The export screen (Figure 5.18) is to export layouts, tests or microplates.

Select buttons

OK	begin exporting the selected layout, test or microplate
Cancel	aborts export function

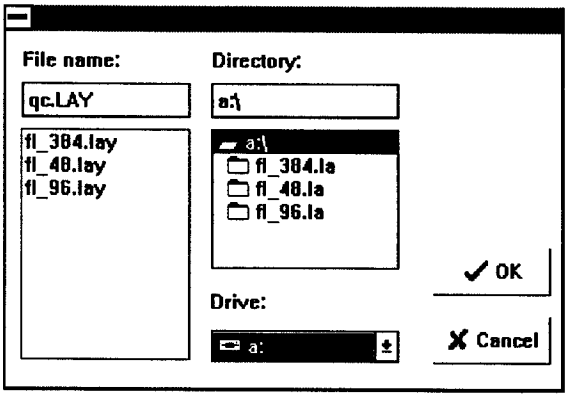


Fig. 5.18. Screen for exporting layouts, tests, or microplates.

The import screen (Figure 5.19) is to import layouts, tests, or microplates

Select buttons

OK	begin importing the selected layout, test or microplate
Cancel	aborts import function

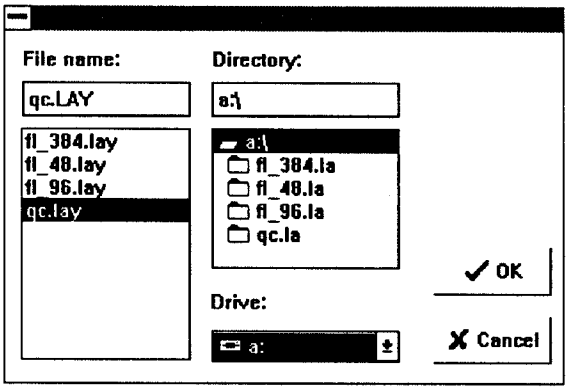


Fig. 5.19. Screen for importing layouts, tests, or microplates.

5.6 Defining a Test

Test procedures are defined using the  icon on the tool bar, or by selecting tests from the test setup menu. After selecting this function all defined tests will be listed.

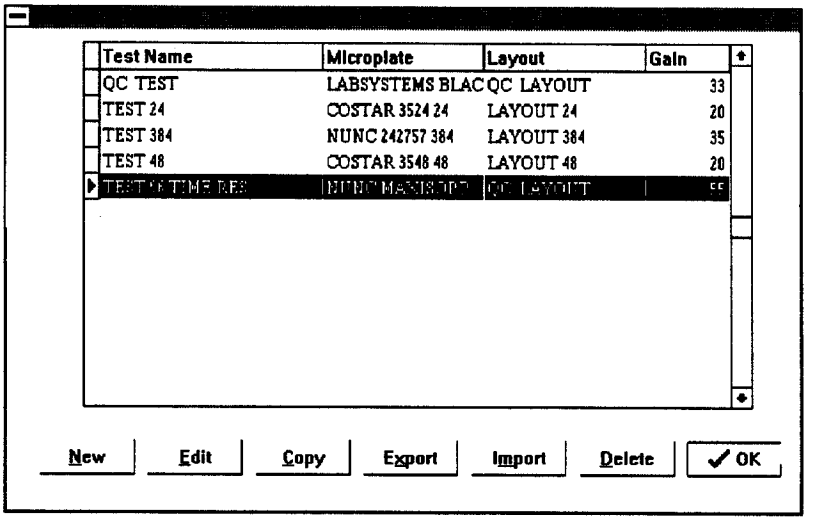


Fig. 5.20. List of all defined tests.

The tests screen (Figure 5.20) gives a list of all pre-defined tests.

Select buttons

New	defines a new test (Figure 5.21, test definition)
Edit	modifies a previously defined test (Figure 5.21, test definition)
Copy	copies an existing test (Figure 5.17, layout)
Export	exports an existing test (Figure 5.18, layout)
Import	imports an existing test (Figure 5.19, layout)
Delete	deletes an existing test
OK	returns to main menu, saving all modifications

When a new test procedure is defined, the software proceeds to the test definition screen to define the measurement procedure.

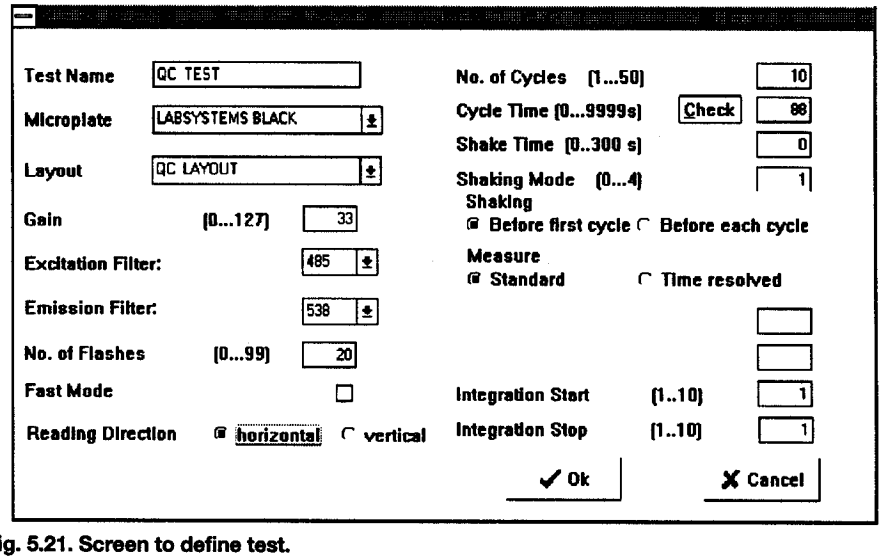


Fig. 5.21. Screen to define test.

Test screen (Figure 5.20)	to define a test
Test name	alpha numeric identifier for the test procedure
Microplate	selects the appropriate microplate type from a pre-defined list (default plates are included, and custom plates may also be defined in setup)
Layout	selects a pre-defined template, which designates the positions of Samples, Standards, etc. The Layout is used both for data reduction and to determine which wells should be measured.
Gain	adjusts voltage to the PMT, to provide optimum sensitivity for each assay
Excitation filter	selects the appropriate excitation filter type from a pre-defined list (defined in setup)
Emission filter	selects the appropriate emission filter type from a pre-defined list (defined in setup)
Fast mode	for small kinetic test runs you can select fast mode operation. Normally after inserting your microplate the transport system will be calibrated before measurement will begin. In fast mode no calibration will be done.
Reading direction	selects reading in horizontal or vertical direction
No. of cycles	defines the number of times the entire plate is measured, from 1 to 50 cycles; for every cycle, each well defined in the layout will be measured
Cycle time	defines the time interval between the start of one plate measurement to the start of the next cycle; this time includes the time to position the plate carrier, shake the plate (if used), the measurement time for each well, and a waiting time if necessary. If a Fluoromark fluorometer is connected, the check cycle time button is activated to validate the Cycle Time directly from the Fluoromark fluorometer.
Shake time	defines length of time to shake the plate before a cycle, from 0 to 300 seconds
Shaking Mode	defines mode for shaking. Mode 0 means high speed and low diameter (96 well plate) and mode 4 low speed and high diameter (24 well plate)
Shaking before first cycle / Before each cycle	selects if shaking is done only before the first or before each cycle
Measure standard/ Time resolved	selects mode for measure. Standard is done with fixed values for integration delay and integration time. For Time Resolved the values for integration delay and integration time can be defined in a range from 10 µs to 1,534 µs.
Integration start/ stop	in addition to using a measurement point from a single cycle for data analysis, the Excel template also has the ability to handle data from measurement points from several cycles; Integration Start/Stop selects the measurement points that will be used for data reduction (these integration limits can also be changed in Excel)
Select buttons	
OK	returns to test screen (Figure 5.20) saving all changes to the test definition
Cancel	returns to test screen (Figure 5.20) without saving any changes to the test definition

Section 6
Configuration of the Fluoromark and Definition of Microplates

Configuration of the communication port, Offset, definition of microplates, and configuration of the filters is initiated with the  symbol, or by selecting the Instrument Setup menu.

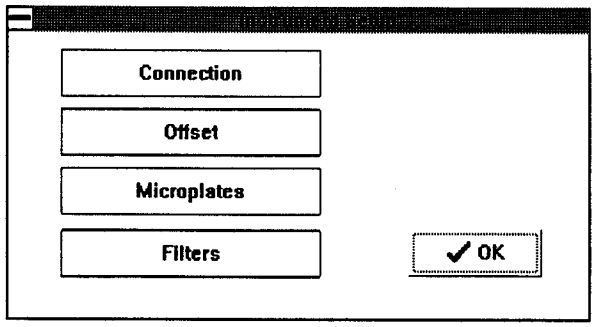


Fig. 6.1. Screen for instrument settings.

6.1 Serial Port For Communication PC to Fluoromark Microplate Fluorometer

To change the PC serial communication port, select Connection in the instrument setup menu.

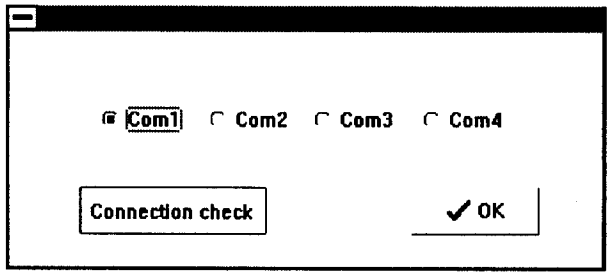


Fig. 6.2. Screen for selecting the com port.

Screen to select com port for the communication between PC and Fluoromark.

Select buttons

Com1	to select com port 1
Com2	to select com port 2
Com3	to select com port 3
Com4	to select com port 4
OK	return to main menu, saving changes
Connection Check	check the selected com port to verify the presence of a Fluoromark instrument

6.2 Definition of the Mechanical Offset

Every Fluoromark instrument is calibrated with a different offset position. This value is normally determined at the factory and is not changed. If service is performed to the plate transport or to the measuring head, the new offset position must be determined by a service technician. The calibrated offsets are posted on the back of each instrument.

Select Offset in the instrument setup menu to enter the offsets.

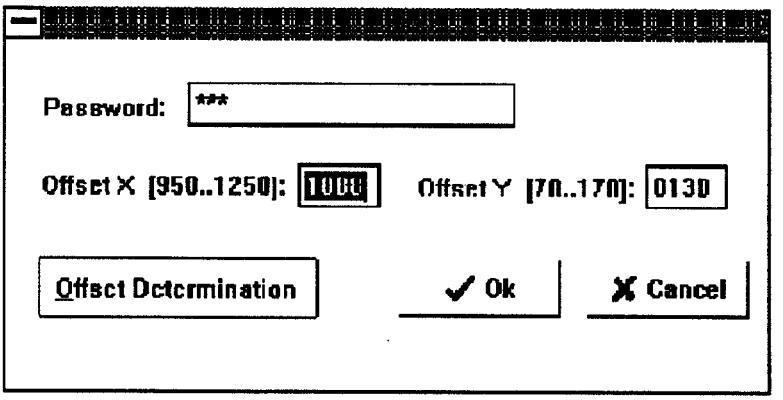


Fig. 6.3. Screen to define the offset.

Valid Offset calibration values are vital to optimum performance. Optical crosstalk and variability of results, can occur if incorrect offset values are used. Type Bio-Rad as the password, and enter the X and Y offset values from the back of the instrument.

If service has been performed, or if the offset values are not available, instruments can be field calibrated. To determine new offset values, fix the plate with the red locking pin. Then remove the pin without changing the position of the plate transport, and press Offset Determination button. The plate transport will move to the home position, and the new calibrated Offset values will be placed in the appropriate fields.

Password requires you to enter the password BMG. When the password is entered, the offset values can be changed, or automatically determined

Offset X field to enter the offset in x direction

Offset Y field to enter the offset in y direction

Select buttons

Offset Determination starts the automatic offset determination. Before you can start this, you have to type in the correct password. Then you can position the plate carrier under the measure head and check the right position with the stick. After this take out the stick and start the offset determination function.

OK saves the offset values, back to main menu

6.3 Definition of the Mechanical Dimensions of the Microplates

The Fluoromark software is supplied with the dimensions of standard microplates from all major microplate manufacturers. While most plates have the standard spacing and footprint, some plates have slightly different dimensions, and must positioned accordingly for optimum results. In addition, new plates can easily be defined by selecting Microplates in the menu instrument setup.

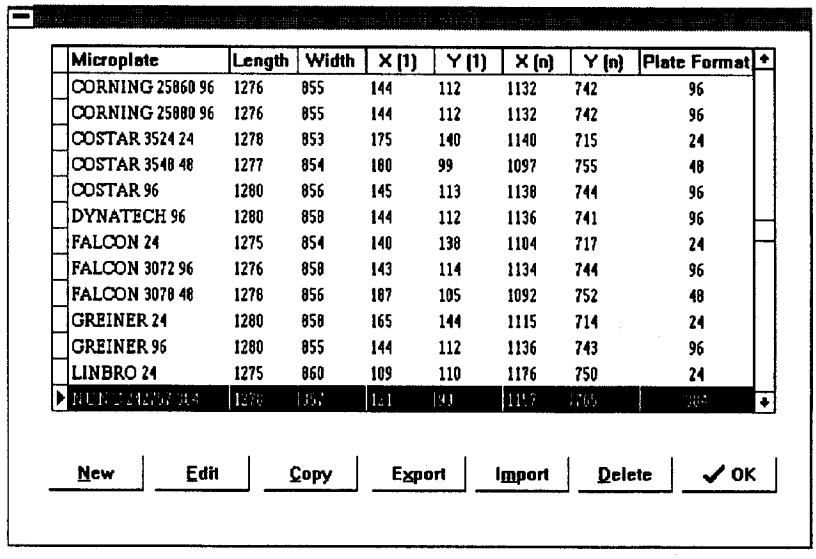


Fig. 6.4. List of defined microplates.

Microplate screen (Figure 6.4) lists all defined microplates with their specific mechanical dimensions.

Select buttons

New	proceeds to microplate screen (Figure 6.5) to create a new microplate name with no pre-defined dimensions
Edit	proceeds microplate screen (Figure 6.5) to change a defined microplate
Copy	copies an existing microplate layout screen (Figure 5.17)
Export	exports an existing microplate layout screen (Figure 5.18)
Import	imports an existing microplate layout screen (Figure 5.19)
Delete	deletes the currently selected microplate definition
OK	returns to the main menu, saving changes

Select New or Edit to proceed to microplate screen (Figure 6.3) for definition of the mechanical dimensions of a microplate, in steps of 0.1 mm.

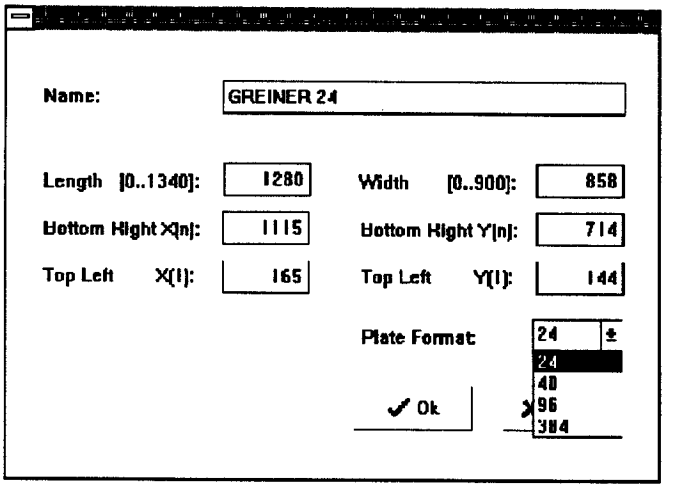


Fig. 6.5. Screen to define the mechanical dimensions of microplates.

Microplate screen (Figure 6.5) defines the plate format (384, 96, 48, or 24 wells) and the mechanical dimensions of microplates, in steps of 0.1 mm.

Name name for the definition of the microplate

Length outer length of the entire microplate, in the 1–12 dimension, in steps of 0.1 mm

Width outer width of the entire microplate, in the A–H dimension, in steps of 0.1 mm

X(n) distance from the center of the lower right well (normally H12) to the left outer edge of the microplate, in steps of 0.1 mm

Y(n) distance from the center of the lower right well (normally H12) to the top outer edge of the microplate, in steps of 0.1 mm

X(1) distance from the center of the upper left well (normally A1), to the left outer edge of the microplate, in steps of 0.1 mm

Y(1) distance from the center of the upper left well (normally A1) to the top outer edge of the microplate, in steps of 0.1 mm

Plate format selects the plate format of the Microplate (384, 96, 48 or 24 wells)

Select buttons

OK accepts current microplate name and dimensions, and returns to microplates screen.

Cancel returns to microplates screen without saving changes

6.4 Definition of the Filter Configuration

The Fluoromark can be configured with 8 excitation and 8 emission filters. To define the wavelength and position of the filters in your Fluoromark select Filters in the instrument setup menu.

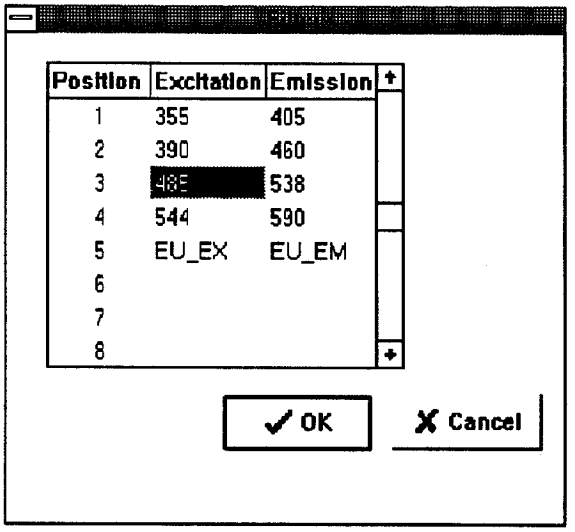


Fig. 6.6. Screen to define the filter configuration.

Select buttons

OK saves all positions and wavelength of the filters, and returns to main menu

Cancel returns to main menu without saving changes

Section 7
Microsoft Excel Data Reduction Routines

Note: Data Access must be installed with Excel, in order for the database functions to be enabled.

7.1 General Hints for Operation

Although no software can incorporate every conceivable feature, modern spreadsheet packages provide powerful data reduction with virtually unlimited flexibility. Pre-written data reduction templates in Microsoft's Excel display raw data, plot flash curves for single or multiple wells, calculate the average of replicates, perform blanking, calculate the concentrations of unknown samples, and graph standard curves.

Open Excel by pressing the  icon from the tool bar. Starting Excel this way, rather than by using Windows Program Manager, will automatically load the Fluoromark.XLT template. When the template is loaded, the name is changed to Fluoromark1.XLS to prevent accidentally overwriting the original template. (**Note:** the XLT extension denotes a template, and the XLS extension indicates a spreadsheet.)

Fluoromark1.XLS is actually several worksheets bound together in a workbook. The data from each experiment is divided into separate groups, and each sheet displays a different set of data. The name of each sheet appears on the tab at the bottom of the sheet, and users activates individual sheets by selecting the appropriate tab with the mouse.

The first sheet, called testruns, is a database of all previously measured plates, which lists the name, plate type, layout, and other parameters of each experiment. Data is recalled from the computer's hard drive by double-clicking anywhere on the line corresponding to the measurement of choice. Sheets for displaying raw data, graphs, and final data can be selected in any order.

An additional pull down menu Fluoromark in the Excel menu line allows tests to be imported into the database, or exported from the database to a separate file, add comment information to the test run, copy or delete a test run and a menu option switch back to Fluoromark software.

7.2 Performing Data Reduction in Excel

When the Testruns sheet is selected, a database of all previously measured plates is displayed.

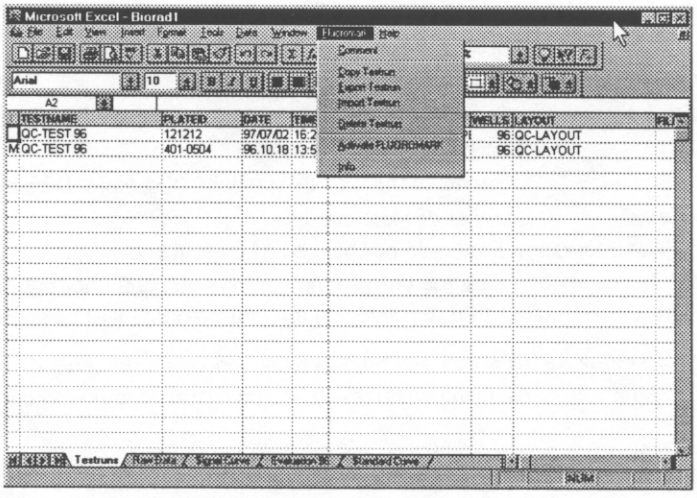


Fig. 7.1. Sheet 1 (testruns) in Excel.

Testruns screen (Figure 7.1) gives a list of all test runs with following information

Test Name	name of the corresponding test setup
Plate ID	measurement identification name of number
Date	date of the test run
Time	time of the test run
Microplate	name of the corresponding microplate definition
Wells	plate format of the corresponding microplate
Layout	name of the corresponding layout definition
Filename	file name of the test results

Select a test run by double clicking on the line corresponding to the measurement of interest.

Test runs can be copied, exported, imported and deleted with the menu item Fluoromark, and selecting the appropriate menu option. Additionally it is possible to add a comment to every test run. With the menu option Activate Fluoromark, you can switch back to the Fluoromark program. After selecting a test run, the program will automatically switch to sheet 2 (raw data).

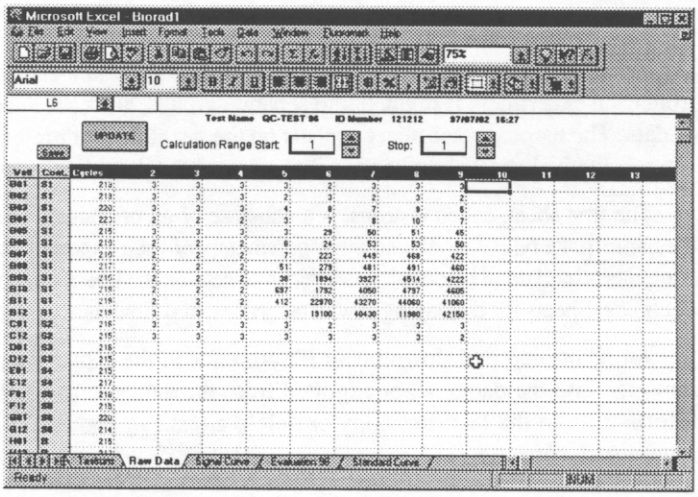


Fig. 7.2. List of all raw data for the selected test run AEQUORIN TEST.

Raw data screen (Figure 7.2) for the Fluoromark data analysis worksheet, displays the fluorescence signal data for each measured well. Relative fluorescence units for as many as 50 time intervals are shown after the well descriptions.
Each line gives the following information for each measured well.

Well	microplate well location
Cont.	contents of the well (S standard, X sample, B blank)
Interval	number of plate cycle

The integration limits can be changed by entering new values in the integration Start and Stop boxes, or by using the corresponding spinners, and clicking on the UPDATE button. After selecting UPDATE, the selected integration range will be shown in red.

To remove a measured well for data reduction, delete the content of the well and click on the UPDATE button. All manipulations will be stored permanently after clicking on the **Save** button.

Select button

UPDATE updates and saves your changes on integration start and stop and the selected wells for the following sheets

Save saves all manipulations in the test run file

To view a plot of the fluorescence curve of a single well or group of wells, highlight the well(s) of interest, select UPDATE, and switch to the Signal Curve sheet by clicking on the corresponding tab at the bottom of the sheet. A range of wells can be selected by clicking and dragging, and individual wells can be chosen by clicking on individual wells with the CTRL key depressed.

Signal curve screen (Figure 7.3) selects the signal curve of the selected wells from sheet 2. All formatting and mathematical functions of Excel are enabled, and can be used without restrictions.

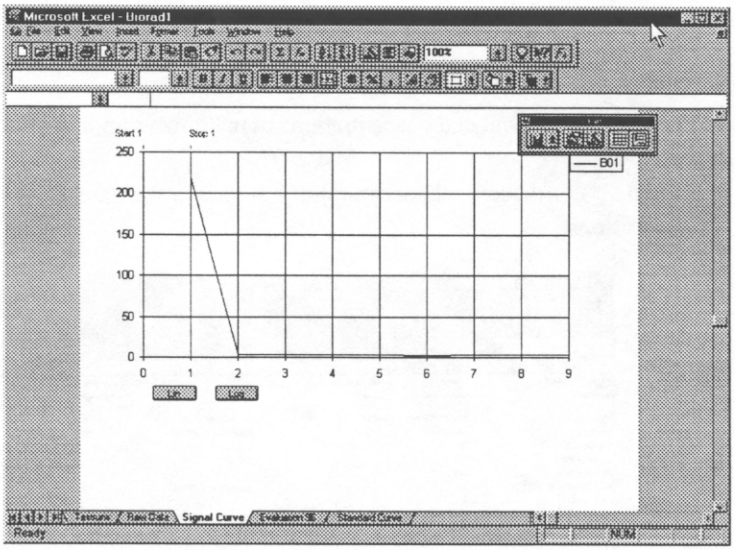


Fig. 7.3. Linear display of the signal curve.

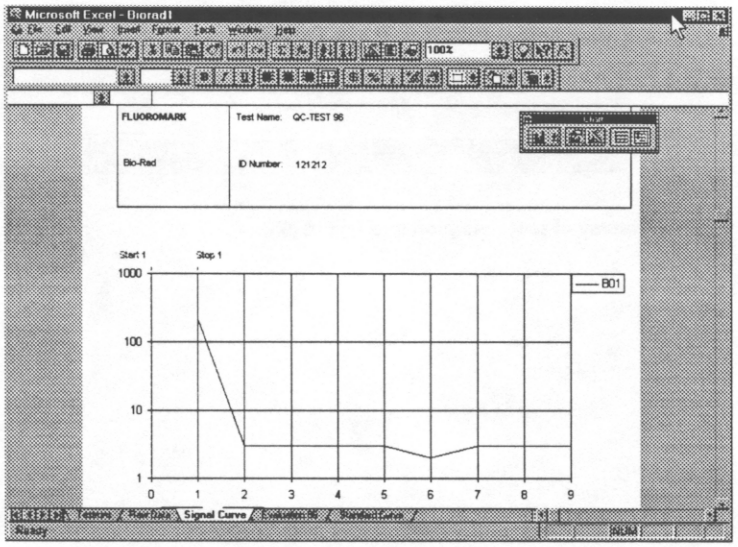


Fig. 7.4. Logarithmic display of the signal curve.

Switch buttons

- **Lin** changes Y axis (RFU) of signal curve to linear
- **Log** changes Y axis (RFU) of signal curve to logarithmic

Sheet 4, evaluation384, evaluation96, evaluation48, evaluation24, displays test definitions and calculated data. For displayed data, three tables in the microplate format are shown. The type of data (Raw, Blanked, Concentrations, etc.), can be selected using the drop-down menu on each table. The format of this sheet depends on the microplate used for the selected test run.

Select button

Layout	displays the microplate layout of the selected plate
Raw results	integral of the raw data from start to stop interval
Averages	displays the average integrals of replicate wells
Blank Correction (RLU)	displays averages the Blank values subtracted
Unknown concentrations	calculated concentrations of unknown samples
Standard concentrations	theoretical concentrations of known standards

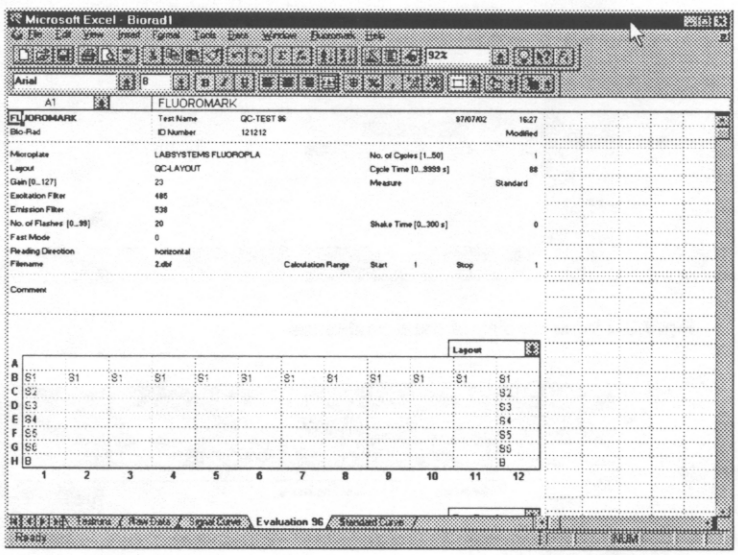


Fig. 7.5. Display of test definition and layout (96).

Sheet 5, standard curve, plots the measured RFU values for the standard concentrations, defined in the Test Setup, as a Standard Curve, or Calibration Curve. The Standard Curve can be displayed by selecting the Standard Curve tab at the bottom of the sheet. The Curve can be shown in linear or logarithmic scale.

Select buttons

Lin changes Y axis (RFU) and X axis (Conc.) to linear

Log correlation curve in logarithmic display

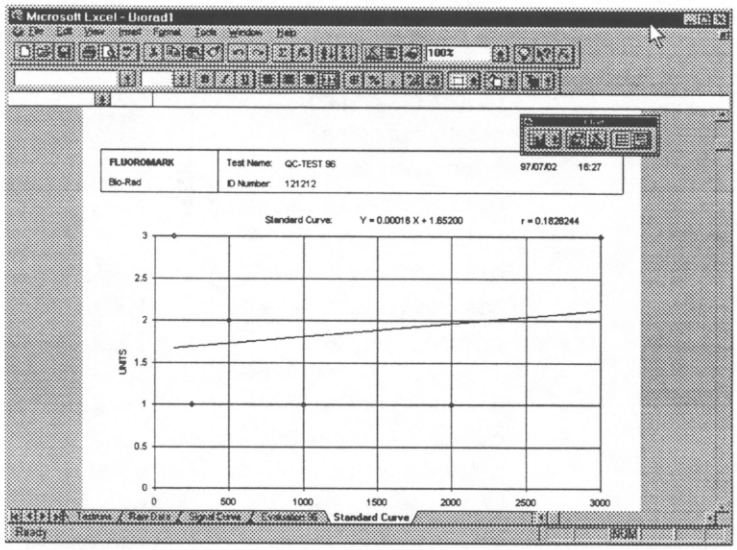


Fig. 7.6. Correlation curve in linear display.

Section 8
Error Codes

Error Code	Error Type	Error Message
0100	wrong command format	Interface error
0200	command character not OK	Invalid command
0300	command parameter not OK	Invalid parameter
0501	Error during writing in Fluo.init	
0502	no temperature defined	
0503	offset X not defined	
0504	offset Y not defined	
0505	no com port selected	
0506	no microplate def. for this test	
0507	no layout definition for this test	
0811	calibration error X-motor	initialization error transp. system
0812	calibration error Y-motor	initialization error transp. system
0813	calibration error X- and Y-motor; home sensor defect	initialization error transp. system
0814	calibration error ex. filter wheel	Initialization error ex. filter wheel
0815	calibration error em. filter wheel	Initialization error em filter wheel
0821	right XY area limit error	transport error
0822	left XY area limit error	transport error
0823	forward XY area limit error	transport error
0824	back XY area limit error	transport error
0851	error temperature sensor 1	signal temperature sensor 1 missing
0852	error temperature sensor 2	signal temperature sensor 2 missing
0853	temperature 1 higher than maximum	temperature 1 higher than maximum
0854	temperature 2 higher than maximum	temperature 2 higher than maximum

Section 9
Product Information

9.1 Product Information

Catalog Number	Product Description
170-6941	**Fluoromark Microplate Fluorometer, 110 V,** includes software, power cord, instruction manual, 5 excitation filters (355,390, 485, 544 nm, and time-resolved filter*), 5 emission filters (405, 460, 538, 590 nm, and time-resolved filter*)
170-6966	**Fluoromark Fluorometer, 100 V** (see description of 170-6941)
170-6970	**Fluoromark Fluorometer, 220 V EC version** (see description of 170-6941)
170-6942	**Fluoromark Microplate Fluorometer with built-in incubator,** includes the same accessory items as above.
170-6967	**Fluoromark Fluorometer with built-in incubator, 100 V** (see descriptions above)
170-6971	**Fluoromark Fluorometer with built-in incubator 220 V EC version** (see descriptions above)
170-6943	**Fluoromark Microplate Fluorometer with low UV read option down to 250 nm,** includes the same accessory items as above.
170-6968	**Fluoromark Fluorometer 220 V with low UV,** Japanese version (see descriptions above)
170-6972	**Fluoromark Fluorometer 220 V with low UV,** EC version (see descriptions above)
170-6944	**Fluoromark Microplate Fluorometer with built-in incubator and low UV read option down to 250 nm,** includes the same accessory items as above.
170-6969	**Fluoromark Fluorometer with built-in incubator and low UV,** 220 V Japanese version (see descriptions above)
170-6973	**Fluoromark Fluorometer with built-in incubator and low UV,** 220 V EC version (see descriptions above)
170-6946	**Microplate Manager Fluoromark Software with manual,** includes EXCEL version 5.0c
170-6947	**355 nm Excitation Filter**
170-6948	**390 nm Excitation Filter**
170-6949	**485 nm Excitation Filter**
170-6950	**544 nm Excitation Filter**
170-6951	**Time-Resolved Excitation Filter**
170-6958	**320 nm Excitation Filter**
170-6959	**515 nm Excitation Filter**
170-6960	**584 nm Excitation Filter**
170-6952	**405 nm Emission Filter**
170-6953	**460 nm Emission Filter**
170-6954	**538 nm Emission Filter**
170-6955	**590 nm Emission Filter**
170-6956	**Time-Resolved Emission Filter Eu**
170-6957	**Time-Resolved Emission Filter Sm**
170-6961	**555 nm Emission Filter**
170-6962	**612 nm Emission Filter**
170-6963	**96 Well Fluorescence Microplate,** solid black, 25
170-6964	**96 Well Fluorescence Microplate,** black with clear bottom, 25
170-6965	**384 Well Fluorescence Microplate,** solid black, 25

* Standard time-resolved excitation and emission filters support use of Europium and Samarium fluorophores.

9.2 Filter Table

Excitation	Emission
355 nm (standard)	405 nm (standard)
390 nm (standard)	460 nm (standard)
485 nm (standard)	538 nm (standard)
544 nm (standard)	590 nm (standard)
Time-resolved filter (standard)	555 nm (optional)
320 nm (optional)	612 nm (optional)
515 nm (optional)	Europium time-resolved filter (optional)
	Samarium time-resolved filter (optional)

Bio-Rad also offers filters for other custom applications. Contact your sales representative or Bio-Rad Laboratories directly at 1 (800) 4BIORAD.

9.3 Disinfection Certificate

This instrument and its inventory has 1) Never been in contact with any dangerous biological material, or 2) been disinfected according to the instructions of the operating manual of this instrument.

Name: _____
(Print) _____

Firm: _____
(Print) _____
_____
_____

Signature: _____ Date: _____

BIO-RAD

Declaration of Conformity

Bio-Rad Laboratories, Inc., 1000 Alfred Nobel Drive, Hercules, California, 94547, U.S.A., declares that the product

Fluoromark Microplate Reader (Catalog nos. 170-6941, 170-6942, 170-6943, 170-6944, 170-6970, 170-6971, 170-6972, 170-6973)

to which this declaration relates, is in conformity to the following standards or normative documents

EN61010 Part 1/03.94, IEC 1010-1/09.90 & Amendment 1/09.92, EU Low Voltage (73/23EWG), EN 60555, EN 55011, EN 50082-1, VDE Cert. No. 90337 G, checked for conformity with DIN EN 61010 Part 1/03.94, Classification VDE 0411 Part 1/03.94, UL Listing No. 171095-E168510, and with UL 3101-1, CAN/CSA C22.2 No. 1010-1

following the provisions of the Low Voltage,EMC Directive.

The product is imported into the EU by Bio-Rad Laboratories Ltd., Bio-Rad House, Maylands Avenue, Hemel Hempstead (London area), Hertfordshire HP2 7TD England.

July 30, 1997
date of issue

Norman Schwartz
Vice President

DC1706973 Rev A

BIO-RAD

Bio-Rad Laboratories

Life Science Group

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Canada Ph. (905) 712-2771, Fx. (905) 712-2990 **China** Ph. (86-10) 2046622, Fx. (86-10) 2051876 **Denmark** Ph. 39 17 9947, Fx. 39 27 1698
Finland Ph. 90 804 2200, Fx. 90 804 1100 **France** Ph. (1) 43 90 46 90, Fx. (1) 46 71 24 67 **Germany** Ph. 089 31884-0, Fx. 089 31884-100
Hong Kong Ph. 7893300, Fx. 7891257 **India** Ph. 91-11-461-0103, Fx. 91-11-461-0765 **Israel** Ph. 03 951 4127, Fx. 03 951 4129
Italy Ph. 02-21609.1, Fx. 02-21609.399 **Japan** Ph. 03-5811-6270, Fx. 03-5811-6272 **The Netherlands** Ph. 0313 18-540666, Fx. 0313 18-542216
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Sweden Ph. 46 (0) 8 627 50 00, Fx. 46 (0) 8 627 54 00 **Switzerland** Ph. 01-809 55 55, Fx. 01-809 55 00
United Kingdom Ph. 0800 181134, Fx. 01442 259118

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