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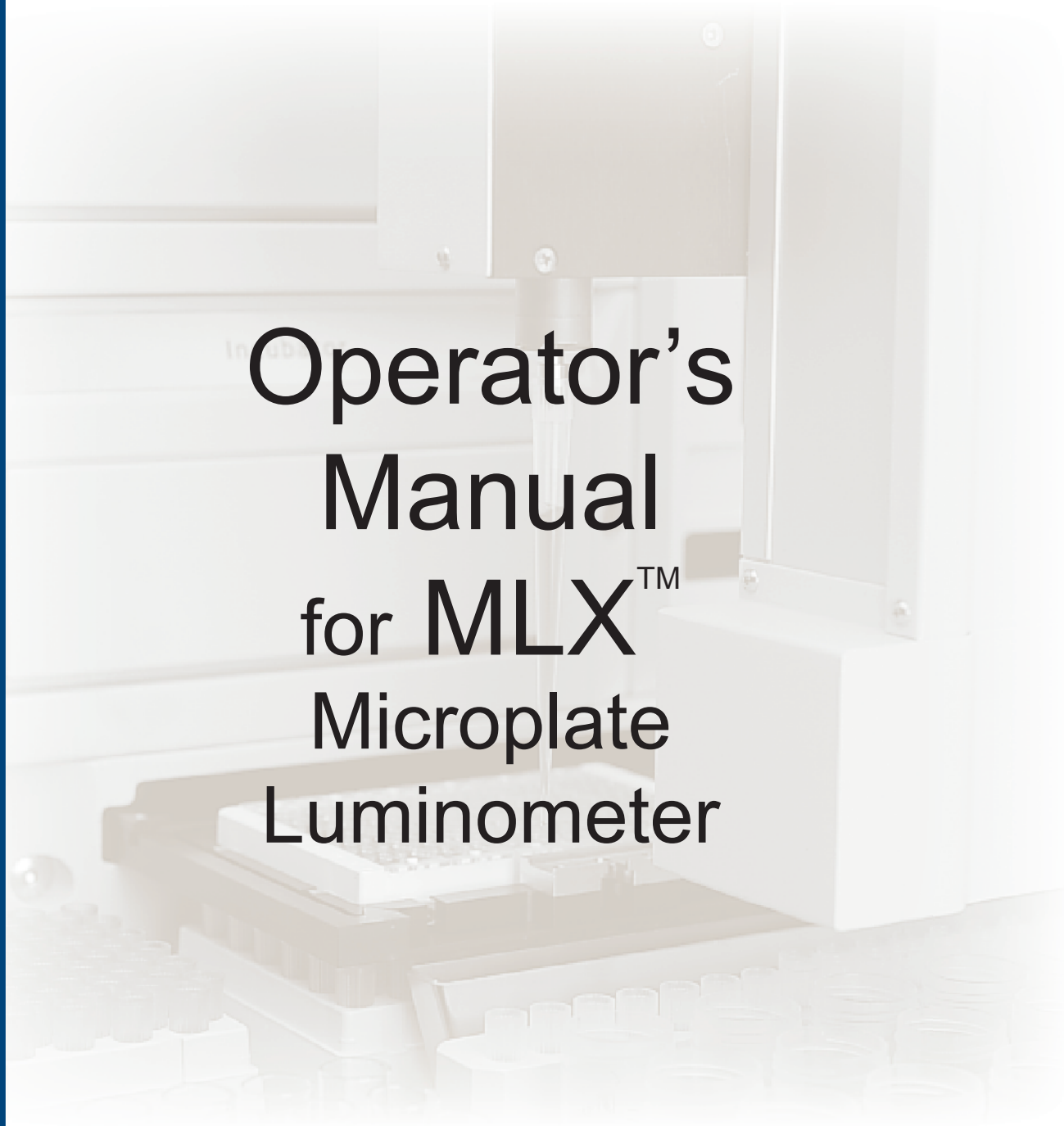
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Operator's Manual for MLX™ Microplate Luminometer

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MLX Microplate Luminometer

Operator's Manual

DYNEX Technologies, Inc.

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ATTENTION:

This MLX has been factory calibrated with the injector(s) in place. When running the Verification Plate, ensure the injector(s) are in place. This does not apply to No Injector units.

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How to Use this Manual

Before operating the MLX MicroplateLuminometer, take a few minutes to review this Operator's Manual. The manual contains the following chapters:

Chapter	Title	Contents
1	Overview	Provides an introduction to the MLX Microplate Luminometer system. The intended use of the system, instrument specifications, and components are outlined in this chapter.
2	Installation	Describes the procedures for installation of the system
3	Getting Started	Describes operation of the MLX Luminometer. Describes the Revelation window and the menus and commands that are contained on it. Outlines each of the procedures used during routine operation of the MLX system.
4	Defining an Assay	Describes the commands and procedures that are used during definition of an assay.
5	Analyzing a Plate	Describes the procedures that are followed when analyzing a plate.
6	Maintenance/ Troubleshooting	Describes the procedures for routine maintenance of the system. Describes procedures to follow if an error message appears.
7	Quality Control	Describes the procedures for quality control of the system.
	Appendix	
	Index	

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Table of Contents

1 Overview

Intended Use 1
Principles of Operation 1
Hardware Description 2
    Hardware Components 2
    Hardware Subsystems 3
Software Description 4
    Overview 4
    Menus 4
    MLX Configuration: the Utilities Menu 5
    Assay Definition: the Settings Menu 6
    Assay Execution 7
    Data Analysis 7
    Results Reporting 7
    User-Defined Data Verification 8
Specifications 9
    Instrument Dimensions and Weight 9
    Performance 9
    Power Requirements 9
    Operation 10
    Standards 10
Assay Overview 11
    Assay Types 11
    Fluidics Option 13
    Temperature Control 13
    Shaking 13
    Remote Control 13

2 Installation

Unpacking and Inspection 15
    Materials Provided 15
Placement of Components 16
Connecting the Communication Cables 17
Connecting the Power Cables 19
    Verifying the Voltage Setting 19
    Connecting the Power Cables 20
Installing the Optional Injectors 21
System Maintenance 24

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3 Getting Started

Starting the MLX Luminometer 25
The Revelation Window 27
Menus 28
Toolbars 39
Preparing the Dispensers 42
Positioning of Reagents 42
The Drain Plate 43
Initializing and Priming 44
Configuring Instrument Parameters 48
Select Language 48
Configure Reader 49
Heater Control 58
Results Font 59
Options 60
Changing the System Password 68
Naming of Wells 69
Labeling of Wells on the Plate 69
Well Types 69
Well Identification 70
Assigning Well IDs 71

4 Defining an Assay

Overview 73
Creating a New Assay 74
Modifying an Existing Assay 74
Assay Title 75
Reader Control: Glow-Endpoint Assays 76
Start Mode 77
Shake 78
Temperature 79
Reader Options 80
Reader Control: Kinetic Assays 81
Calculation Mode 82
Start/Stop Mode 83
Shake 85
Temperature 86
Reader Options 87
Kinetic Time Interval 88
Kinetic Graphs 89
Dispensing Options 90
Reader Control: Flash Assays 92
Calculation Mode 93
Start Mode 95
Shake 96
Temperature 97
Reader Options 98
Dispensing Options 99
Report Data 103
Data Graphs 105

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4 Defining an Assay (Continued)

Reader Control: Dual Flash Assays....106
Calculation Mode....107
Start Mode....109
Shake....110
Temperature....111
Reader Options....112
Dispensing Options....113
Report Data....115
Data Graphs....117
Template....118
Define....120
Blank Mode....122
Q.C.: Raw Data....125
Constructing an Expression....128
Multiple Expressions....130
Threshold....131
Threshold Equations....132
Pos/Neg Q.C.....133
Threshold Q.C.....134
Threshold Output Format....136
Curve Fit....138
Fits....139
Standards....140
Fit Type....142
Stored Curve....144
Graph....145
Sample Selection....147
Dilutions....148
Fit Q.C.....150
Calculated Q.C....152
Data Conversion....155
Results Flagging....157
Output Format....159
Ratio....161
Ratio Equations....162
Results Flagging....163
Output Format....165
Spreadsheet....167
Spreadsheet....168
Results Flagging....170
Output Format....172
Report Options....174
Report Layout....175
Data Matrix....176
Export to File....178
Header/Footer....180
Assay Options....181
Area Statistics....182
Processing Order....183
Sample ID Setup....184
Using the Wizard....185

5 Analyzing a Plate

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Starting an Assay 191
Using the Quick Operation Buttons 191
Using the Run Plate Command 195
Lot-Specific Data 196
Selecting Lot-Specific Data 197
Entering New Lot-Specific Data 198
Sample IDs 199
Entering Sample IDs at Run Time 199
Linking Sample IDs to a Separate File 199
Linking Sample IDs After the Plate is Run 201
Importing Data 202
User Definable Data File 202
Reading of the Plate 203
Status Messages 203
Display of Results 204
Results Presentation if QC Fails 206
Analysis of Results 206
Analyzing Results Using a New Assay 206
Removing Outliers and Re-Analyzing Results 207
Refresh 208
Fit Information 209
Kinetic Graphs 210
Titration Multiplier 211
Plate-Plate Statistics 211

6 Maintenance/Troubleshooting

General Cleaning 213
Cleaning the Mouse 213
Cleaning the Syringes 214
Cleaning the PMT Lens 215
General Precautions 217
Power ON/OFF 217
Software Modification 217
System Rebooting 217
Hazards 218
Spills 218
System Covers 218
Data Recovery 218
Operational Problems 219
Troubleshooting 221

7 Quality Control

Overview 225
Light Intensity Quality Control 226
Light Leak Testing 227
Light Intensity Accuracy and Precision Verification 228

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7 Quality Control (Continued)

Assay Quality Control 230
Monitoring of Results 230
Use of Control Materials 230
Reagent Dispensing 231

Appendix

Index

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This license agreement sets forth the terms and conditions of the license and the limited warranty for the Revelation™ software and the Microsoft® software that is provided as part of the MLX Luminometer system.

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1 Overview

Intended Use

The MLX Microplate Luminometer is a general-purpose instrument that is intended to automate the initiation of a reaction, measure the light that is emitted, and calculate the assay results that are obtained when using luminescence technology and microplates. The MLX is used in conjunction with an assay method and reagents that are provided by the user. Depending upon the assay that is being run, additional equipment may be required for incubation, separation, and washing of the reaction mixtures prior to reading and calculation of assay results on the MLX.

Principles of Operation

Light emitting chemical reactions and biological reactions have a wide range of analytical applications, ranging from detection of small molecules to the assay of large protein complexes. The utilization of a light-emitting reaction in the detection and/or quantitation of these substances is termed **luminescence**.

Advantages of luminescence over other technologies include superior sensitivity (with detection limits in the zeptomole range), speed, nonhazardous reagents, and simple procedures. Many luminescence procedures are single-step methods that involve simply addition of a single reagent or combination of reagents to the test sample. Procedures utilizing luminescence include immunoassay, protein blotting, and DNA probe methods.

Light produced from a luminescence reaction occurs either as a continuous emission that persists from several hours to several days (a **glow**) or as a short burst of light (a **flash**) that persists from several seconds to minutes after addition of a trigger reagent.

Depending upon the duration and stability of light emission, four types of assays are used. These are described below:

Assay Type	Description
Glow-endpoint	A single measurement of light intensity, to determine the intensity of the glow.
Kinetic	Two or more measurements of light intensity separated by time, to determine the increase or decrease in intensity of the glow. Usually, the measurements are taken within several minutes.
Flash	A continuous measurement of light intensity over a predetermined period of time, to determine the amount of light or other characteristics of light emitted in the flash.
Dual Flash	A compound flash assay that is triggered twice by using two different reagents.

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The intensity of emitted light, measured in Relative Light Units (RLU), is used to obtain qualitative or quantitative measurements. Usually, a series of standards (provided by the user) is run, and a calibration curve is prepared relating the resulting light intensity to the level in each standard. The intensity of light obtained for each unknown is compared to the intensity of emitted light for the standards, and the level of each unknown is then calculated.

The MLX Microplate Luminometer can be configured to automate any of the assays described. Optional reagent dispensers are available to support the automation of flash assays. The user can select from many calculation algorithms contained in the MLX Microplate Luminometer, and the results that are obtained can be reported in a wide variety of user-defined formats.

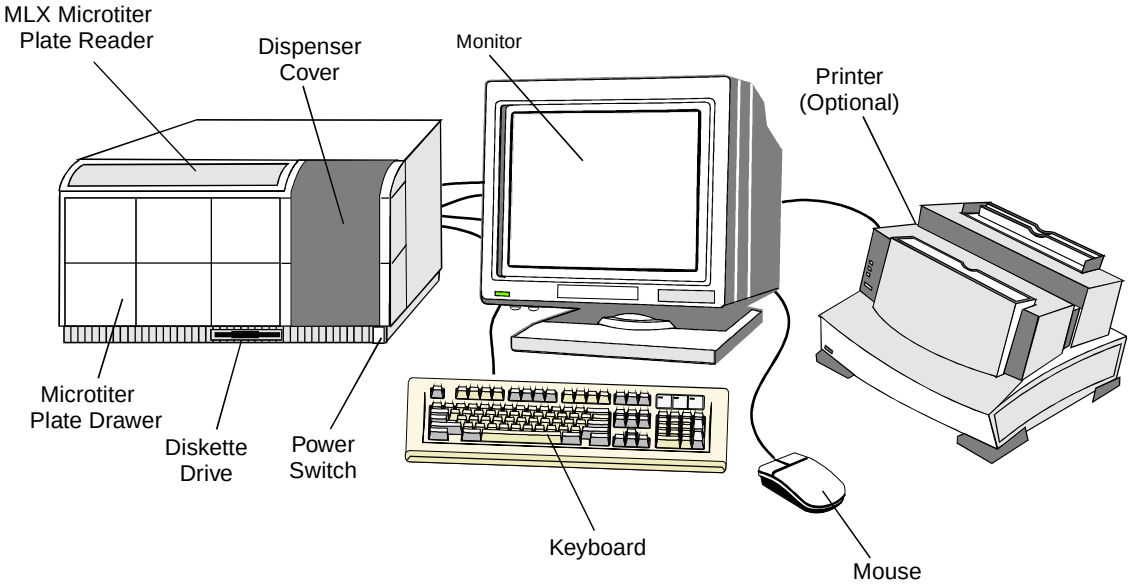
Hardware Description

Hardware Components

Your MLX Microplate Luminometer system (Figure 1-1) consists of the following hardware components:

- MLX Microplate Luminometer
- Monitor
- Keyboard
- Mouse
- Printer (optional)

Figure 1 -1 MLX Hardware Components



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Hardware Subsystems

The major subsystems of the MLX Microplate Luminometer are described below. The location of the subsystems is shown in Figure 1-2.

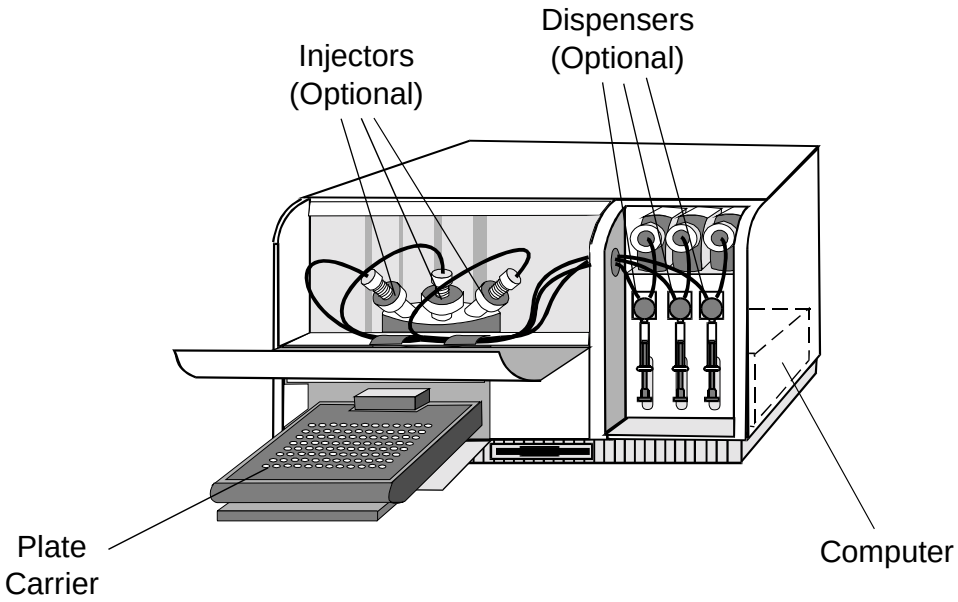
1. **Computer**. The computer provides a means for you to enter information and obtain results. The computer stores and retrieves assay profiles, executes the defined assay, and performs the various calculations needed for the assay. When executing the assay, the computer commands the micro-instrument to move to the target well and obtain light readings.

2. **Micro-instrument**. The micro-instrument consists of the **plate carrier** system, the **temperature control** system, and the **electro-optical** system. The plate carrier subsystem positions the Microplate in the instrument so that reagents can be dispensed into the wells and so that the light emitting from the wells can be read. The temperature control system maintains the temperature of the Microplate from 5° C above ambient temperature to 45° C. The electro-optical system measures the light that is emitted from each well in the Microplate.

3. **Barcode Reader** (optional). The barcode reader automatically reads the barcode that is printed on each Microplate. Use of the barcode reader allows you to automatically identify each Microplate.

4. **Fluidics** (optional). The fluidics subsystem consists of **dispensers** and **injectors** that are used during a flash assay. The dispensers consist of three 500 µL syringe pumps. Each dispenser is connected to a reagent bottle and an injector. The injectors allow precise dispensing of reagents into the Microplate well. Each injector is mounted inside an injector solenoid, which lowers the injector below the mask into the drain plate during a prime or purge operation.

Figure 1 -2 Hardware Subsystems



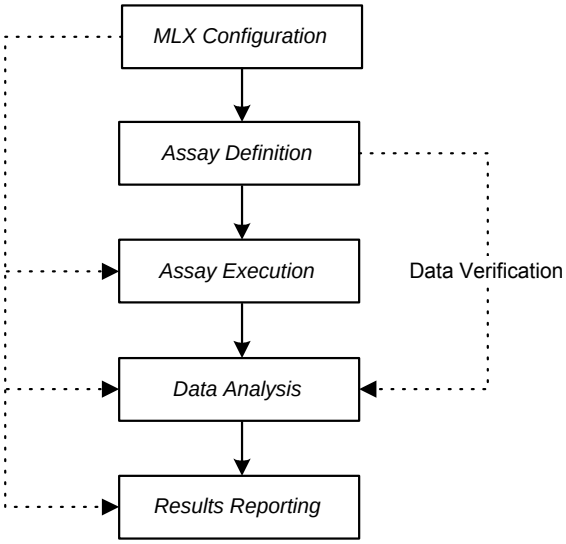
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Software Description

Overview

The Revelation™ software in the MLX Microplate Luminometer allows easy **configuration** of the MLX, **definition** of a luminescence assay, **execution** of the assay, **analysis** of data, and **reporting** of results (Figure 1 -3). The software includes an extensive menu of assay definition options that allow you to define any type of luminescence assay.

Figure 1 -3 Overview of Revelation Software



Menus

The menus on the MLX Microplate Luminometer are grouped according to software function. The menus and their use are shown below:

File	Settings	Data	View	Utility	Window	Help
Create a New Assay Open/Close Assay/Data File Import Data Save File Print File Run Plate	Define an Assay	Analyze Data	Define how Results are Displayed	Configure the MLX	Select from Open Files	Obtain On-Line Help

Note: Details of the menus are described in Chapter 3 (*Getting Started*).

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MLX Configuration: the Utility Menu

The **Utility** menu is used to configure the operating parameters for the MLX.

When the MLX is delivered to your laboratory, the operating parameters are configured to default values. However, you should verify that each operating parameter is configured according to your preference.

Note: Be sure to carefully read Chapter 3 (*Getting Started*) before you configure the MLX.

MLX Configuration Option	Action to Initiate
Prepare the Dispensers	Use the **Dispensers** command.
Select the Language	Use the **Select Language** command.
Select the Reader Type.	Use the **Configure Reader** command.
Specify Injector Speeds, Pickup Volumes, and Syringe Volumes	Use the **Configure Reader** command. Select the **Injector Speeds** tab.
Specify whether Dispensers are Installed	Use the **Configure Reader** command. Select the **General** tab.
Specify Barcode Reader Options	Use the **Configure Reader** command. Select the **Barcode** tab.
Specify Remote Control by an External Robotic Device	Use the **Remote Control** command.
Specify Remote/Data Port Communication Parameters	Use the **Configure Reader** command. Select the **Remote/Data Port** tab.
Select Frequency and Reporting Mode of Self Testing	Use the **Configure Reader** command. Select the **General** tab.
Specify whether Data from Glow Endpoint or Flash Assays are Saved Between Readings	Use the **Configure Reader** command. Select the **General** tab.
Maintain Plate Carrier Temperature Between Runs	Use the **Heater Control** command.
Select Font for Printed Reports	Use the **Results Font** command.
Create or Modify a Quick Operation Button	Use the **Options** command. Select the **Buttons** tab.
Specify that Results are Suppressed if QC Fails	Use the **Options** command. Select the **Preferences** tab.
Specify that a Plate ID is Automatically Assigned	Use the **Options** command. Select the **Preferences** tab.
Specify that Lot-Specific Data are Entered at Run Time	Use the **Options** command. Select the **Preferences** tab.
Specify Colors to Represent Well Types on the Template	Use the **Options** command. Select the **Colours** tab.
Specify Laboratory Name, etc. for Results Report Header	Use the **Options** command. Select the **Laboratory Information** tab.

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Assay Definition: the Settings Menu

The method that the MLX uses to run an assay is defined during Assay Definition. For example, the plate temperature during the assay, the time when the first reading of each well should be taken, and the plate agitation can be defined. In addition, the positions of various types of samples (test samples and controls, for example) on the plate can be defined, and the number of replicate readings that should be obtained on each well or sample type can be defined. Finally, the manner in which results are calculated can be defined.

Assay definition commands are accessed in the **Settings** menu, which is displayed whenever a new assay or an existing assay is open. Details of assay definition are provided in Chapter 4. After reviewing this chapter, you will have a good understanding of the wide variety of assay definition options that are available.

The assay definition options that are available include the following:

Definition Activity	Action to Initiate
Define MLX Parameters	Use the **Reader Control** command.
Define Sample Types and their Location	Use the **Template** command.
Define How Results are Calculated	Use the **Blank Mode** command to correct for the reagent blank.
	Use the **Threshold** command (for qualitative or semi-quantitative assays).
	Use the **Curve Fit** command (for quantitative assays).
	Use the **Ratio** command (for assays in which the result is to be expressed as a ratio or scaled).
	Use the **Spreadsheet** command to use the results of particular wells in calculating other results.
	Use the **Assay Options** command to specify the order in which results should be calculated and to specify summary statistical calculations.
Prompt for Control Values at Run Time	Use the **Options** command (on the **Utility** menu). Select the **Preferences** tab.
Prompt for Sample IDs at Run Time	Use the **Assay Options** command. Select the **Sample ID Setup** tab.
Specify the Content and Layout of the Results Report	Use the **Report Options** command.
Name the Assay	Use the **Assay Title** command.

Other assay definition options allow you to specify whether and how the data will be verified. These options are discussed in the section entitled *User-Defined Data Verification* on page 8.

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Assay Execution

After an assay has been defined, it can be run on the MLX. A run can be initiated by clicking on the **Quick Operation** button for the assay to be run or by activating the **Run Plate** command in the **File** menu.

Depending on the settings that you specified during configuration of the MLX and during definition of the assay, the MLX may prompt you for additional information such as control values or IDs of the samples that are being run. These and other details of assay execution are provided in Chapter 5.

Data Analysis

After an assay has been run, the data are analyzed and the results calculated according to the settings you selected during assay definition. If, after examining the results, you wish to reanalyze the data using a different method, you can do so from the **Data** menu.

Results Reporting

After an assay has been run, the data analyzed, and the results calculated, the results are reported. The data that are presented in the results reports and the format of the report are specified during configuration of the MLX.

During an assay, the MLX may display the result of testing each well and wait for your approval to proceed before the next well is assayed. This is useful in cases where expensive reagents are required for an assay. This too will depend on the settings that you specified during configuration of the MLX and during definition of the assay.

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User-Defined Data Verification

Revelation software allows you to specify multiple criteria for automatic verification of data during the assay execution, data analysis, and results reporting phases of the assay. These criteria include recovery of expected values on controls and criteria for flagging of individual results.

The data that are obtained and the manner in which the data are analyzed are determined by the user during assay definition. Extensive automated data verification can also be specified when defining an assay. Data verification can occur at various stages of an assay, as summarized below:

Stage of the Assay	Type of Verification
RLU Readings	**Q.C.: Raw Data.** RLU readings that are obtained when reading each well can be checked against criteria that you specify. Use the **Q.C.: Raw Data** command.
Results Calculation	**Threshold Q.C.** (For qualitative or semi-quantitative assays). RLU values corresponding to a cut-off value can be checked against criteria that you specify. Use the **Threshold** command. **Fit Q.C.** (For quantitative assays). The standard curve that is obtained for an assay can be checked against criteria that you specify. Use the **Curve Fit** command. **Calculated Q.C.** (For quantitative assays). The calculated value that is obtained for a sample can be checked against criteria that you specify. Use the **Curve Fit** command.
Results Report	(For quantitative assays). The calculated value that is obtained for a sample can be flagged when it meets criteria that you specify. For example, any value that exceeds the normal limit can be flagged as Abnormal High. Use the **Curve Fit**, **Ratio**, or **Spreadsheet** command, depending on how the results are being calculated.

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Specifications

Specifications for the MLX Microplate Luminometer are listed below.

Instrument Dimensions and Weight

Depth	450 mm	17.7 in
Width	500 mm	19.7 in
Height	250 mm	9.8 in
Weight	23 kg	50.7 lb

Performance

Dynamic Range	0.0008 to 10,000 RLU
Linearity	R^2 > 0.999 from 0.001 to 9,900 RLU
Precision	Better than 1.0% C.V. from 0.3 to 9,900 RLU Better than 2.5% C.V. from 0.03 to 0.2 RLU
Limit of Detection	Better than 10 femtograms of alkaline phosphatase or 1 attomol of recombinant aequorin
Resolution	± 0.0001 from 0.0001 to 10 RLU ± 0.002 from 10 to 1,000 RLU ± 0.02 from 1,000 to 10,000 RLU
Cross Talk	Less than 2.5 x 10^{-5} (ratio of dark well to light well)
Machine-to-Machine Variation	Less than ±10% for light intensity > 0.03 RLU
Spectral Response	400 - 650 nm

Power Requirements

	Voltage	Power	Frequency
Main Unit	100 - 120 V 220 - 240 V	175 VA 175 VA	60 Hz 50 Hz
Auxiliary Outlet	115 VAC 230 VAC	1.0 A 0.5 A	60 Hz 50 Hz
Line Voltage Variation	± 10%		
Line Frequency Variation	± 3 Hz		



The Auxiliary Outlet on the MLX Luminometer is used to provide power to the monitor. The voltage and current rating for that outlet are shown above. Do not exceed these ratings.

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Operation

Reading Time	User variable, depending upon chemistry
Read Modes	Glow-endpoint, kinetic, flash, dual flash
Processor	Pentium
Data Storage	800+ MB hard drive
Data Output	3.5 in diskette, hard drive, monitor and printer
Keyboard	101 key Qwerty

Standards

The instrument is designed in accordance with UL, IEC 1010, and FCC standards:

NRTL (UL 3101-1)	Nationally Recognized Testing Laboratory
CSA 22.2 #1010-1	Canadian Standards Association
EN 61010-1	Low Voltage Directive (European Normative)
EN 55022:1995 (B)	(Emissions)
EN 50082-1: 1992	(Generic Immunity, Light Industrial)
IEC 801-2: 1984	ESD
IEC 801-3: 1984	Radiated Immunity
IEC 801-4: 1988	Electrical Fast Transient/Burst

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Assay Overview

The MLX system utilizes a Windows® graphical user interface (GUI) so that the user can readily access, transfer, and analyze assay parameters and detailed test results. Test results can be copied or exported to Excel® or other Windows applications. A wide variety of reporting formats is also provided with the MLX system so that either summary test results or the detailed data obtained during each test can be reported and/or analyzed.

Assay Types

The light produced from a luminescence reaction occurs either as a continuous glow or as a short flash. Four types of assays (glow-endpoint, kinetic, flash, and dual flash) are used in luminescence measurements. Each of these assays can be configured and run on the MLX system.

Glow-Endpoint Assays

A single measurement of light intensity is made during a glow-endpoint assay. The measurement for a glow-endpoint assay is reported as:

- the average intensity observed during the measurement interval, **or**
- the sum of intensity readings obtained during the measurement interval.

The measurement for a glow-endpoint assay is taken:

- immediately, **or**
- at a certain time after starting the assay, **or**
- when the light intensity reaches a certain level, **or**
- when the rate of change in intensity reaches a certain level.

Kinetic Assays

A kinetic method measures the change in intensity of light emission over time. Two or more measurements of light intensity are made and analyzed. The measurement for a kinetic assay is reported as:

- the rate of change in intensity, **or**
- the maximum rate of change in intensity, **or**
- the time to achieve the defined rate or maximum rate of change in intensity.

Kinetic measurements are started:

- at a certain time after starting the assay, **or**
- when the light intensity reaches a certain level, **or**
- when the rate of change in intensity reaches a certain level.

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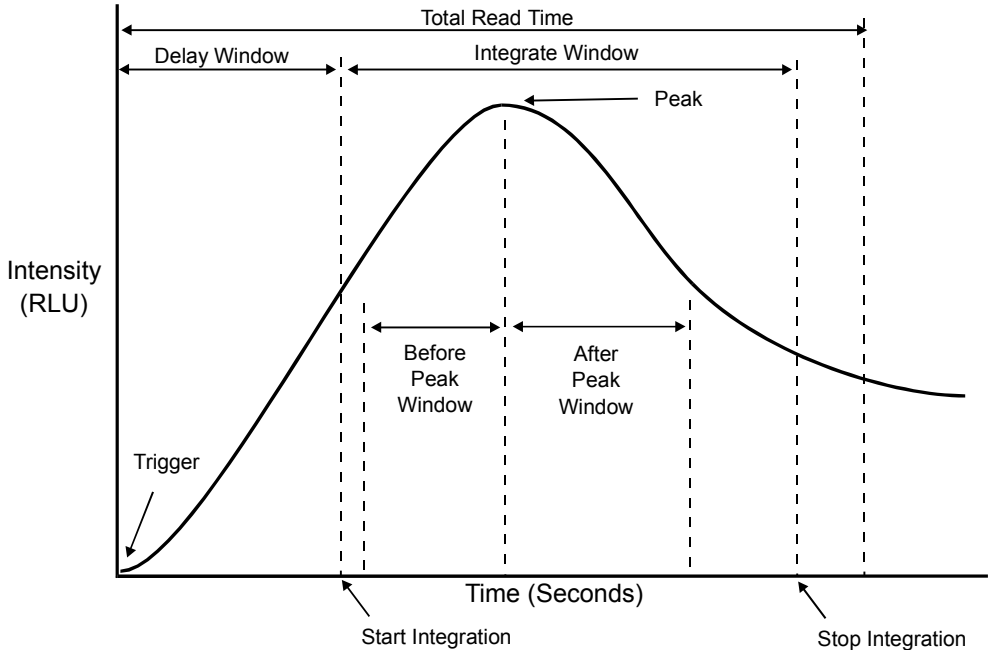
Kinetic measurements are ended:

- at a certain time after starting the assay, **or**
- when the light intensity reaches a certain level, **or**
- when the rate of change in intensity reaches a certain level, **or**
- after a specified number of measurements have been made.

Flash Assays

A flash assay records the instantaneous readings of light intensity (100 readings per second) during the flash of light. Readings are taken during the **Total Read Time** window (Figure 1-4). Additional windows for integrating light intensity are obtained as described.

Figure 1 -4 Flash Assay



The measurement for a flash assay is reported as:

- the total integral of light intensity during the *integrate window*, **or**
- the average of the total integral of light intensity during the *integrate window*, **or**
- the time of peak occurrence during the *integrate window*, **or**
- the light intensity at the peak, **or**
- the integral of light intensity in the *after peak window*, **or**
- the integral of light intensity in the *before peak window*, **or**
- the time of peak occurrence during the *delay window*, **or**
- the light intensity at the peak during the *delay window*.

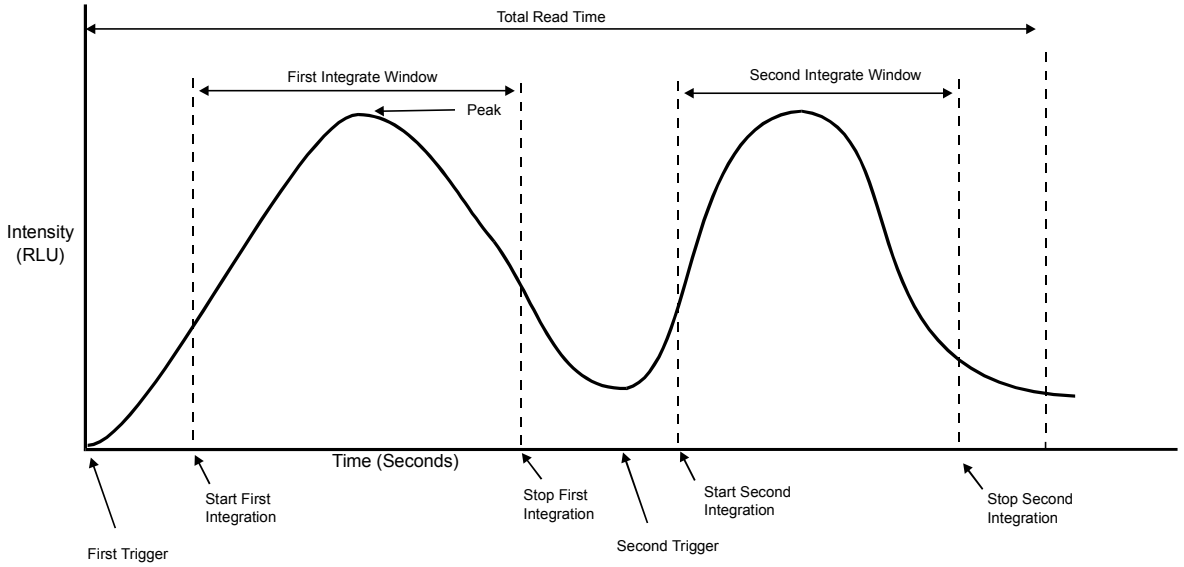
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Flash measurements are initiated by addition of the trigger reagent. This reagent is provided by the user.

Dual Flash Assays

A dual flash assay is read in the same manner as a flash assay, but the results are calculated differently. In the dual flash assay, two integrate windows are used in the calculation of results. The second integrate window can start anytime after the stop time of the first integrate window plus one second.

Figure 1 -5 Dual Flash Assay



The measurement for a dual flash assay is reported as:

- the total integral of light intensity during the *first integrate window*, **or**
- the average of the total integral of light intensity during the *first integrate window*, **or**
- the total integral of light intensity during the *second integrate window*, **or**
- the average of the total integral of light intensity during the *second integrate window*, **or**
- the ratio of total integral of light intensity during the *first integrate window* versus the *second integrate window*, **or**
- the ratio of total integral of light intensity during the *second integrate window* versus the *first integrate window*.

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Fluidics Option

If the fluidics option is installed, the MLX system can automatically dispense up to three reagents (provided by the user) to trigger a flash reaction. The amount and timing of each reagent dispensed is defined during configuration of the assay.

Note: A flash reaction must be defined in order to enable the fluidics.

Temperature Control

The MLX system can control the temperature of the Microplate from 5° C above ambient temperature to 45° C. Utilization of temperature control is selected during definition of the assay.

Shaking

The Microplate can be shaken during any of the assays described above. The enabling of shaking, the shaking rate, and the time when shaking is applied are specified during definition of the assay.

For glow-endpoint and kinetic assays, the plate can be shaken before each reading of the plate is started. For flash assays, the plate can be shaken during the reading of the well.

Remote Control

The MLX system can be set up so that it is controlled by an external robotic device that is used for automating Microplate preparation. This option must be installed on your MLX system before it can be activated.

If the remote control option is installed, the **Remote Control** command will be displayed on the **Utility** menu. Refer to Chapter 3 for a description of the **Utility** menu and the procedure for activating remote control of the MLX system.

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2 Installation

Unpacking and Inspection

Before unpacking the MLX Microplate Luminometer, determine the area where the system will be located. You will need an area that is approximately 91 cm (36 inches) wide, 61 cm (24 inches) deep, and 41 cm (16 inches) high.

To unpack the components:

1. Unpack the contents of each carton. Refer to the unpacking instruction sheet which is packed with this *Operator's Manual*.
2. Place the components in the position where they will be located.
3. Examine the packaging for each component to be sure that the power cord, connecting cables, or other materials have been removed.

Some components may contain additional protective packaging that must be removed. If such protective packaging is present, remove it.

Inspect each component for damage when unpacking it. If damage is observed, contact your shipper or service representative immediately. After all of the components have been unpacked and inspected, check to verify that all of the materials listed in the following section have been unpacked.

Do not discard the packaging material.

Materials Provided

The components of the MLX Luminometer are packaged in three separate cartons. The contents of each carton are listed below:

Instrument Carton

Article	Quantity
MLX Microplate Luminometer	1

Monitor Carton

Article	Quantity
Monitor	1

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Accessories Carton

Article	Quantity
Keyboard	1
Mouse	1
Mouse Pad	1
Instrument Power Cable	1
Monitor Power Cable	1
Microplate Starter Kit	1
Unpacking Instruction Sheet	1
Operator's Manual	1
Syringes (optional)	3
Injectors (optional)	3
Tubing Kit (optional)	1
Drain Plate (optional)	1
Reagent Bottles (optional)	3

Placement of Components

The MLX Luminometer must be placed in an area where there is at least six inches of space in front of the instrument to allow the plate carrier to automatically extend from the instrument.

CAUTION: Do not place the keyboard, the mouse, or any other material or equipment in front of the MLX Luminometer.

After positioning the MLX Luminometer, place the monitor to the left or right of the instrument. Place the mouse and keyboard in front of the monitor. If a printer is used, place the printer where it can be accessed.

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Connecting the Communication Cables

After the MLX Luminometer, the monitor, the keyboard, the mouse, the data interface or remote control robotic device, and the printer (if included) have been placed into position, they must be connected as outlined below.

Location of the receptacles for the cables depends upon whether dispensers are installed on your MLX system. For an MLX without dispensers, the receptacles are shown in Figure 2-1. For an MLX with dispensers, the receptacles are shown in Figure 2-2.

CAUTION: Do not connect any power cables at this time.

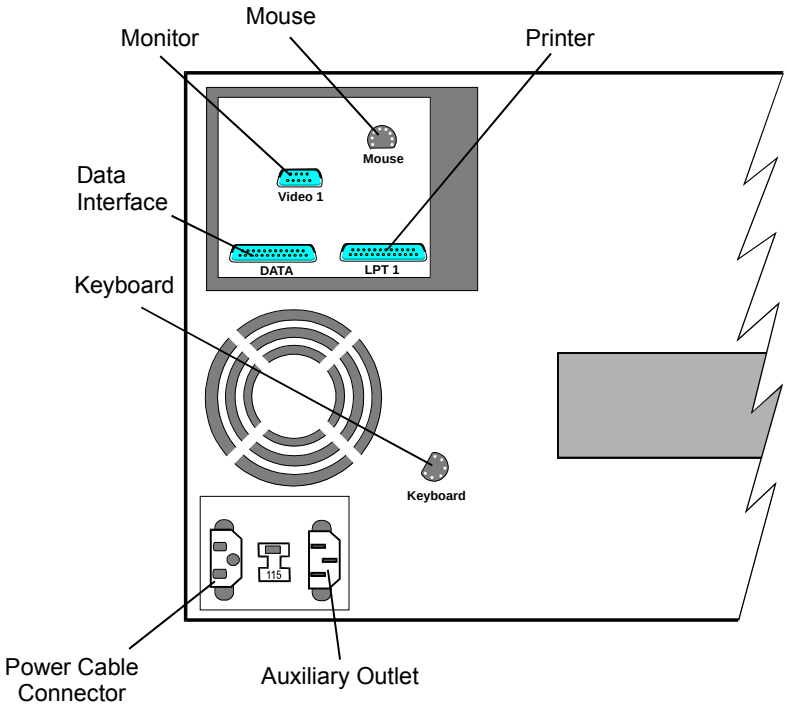
To connect the monitor:

1. Unwrap the monitor cable, if you have not already done so.
2. Plug the monitor cable into the **Video 1** receptacle at the rear of the instrument. See Figure 2-1 or Figure 2-2 for the location of the receptacle.

To connect the mouse and keyboard:

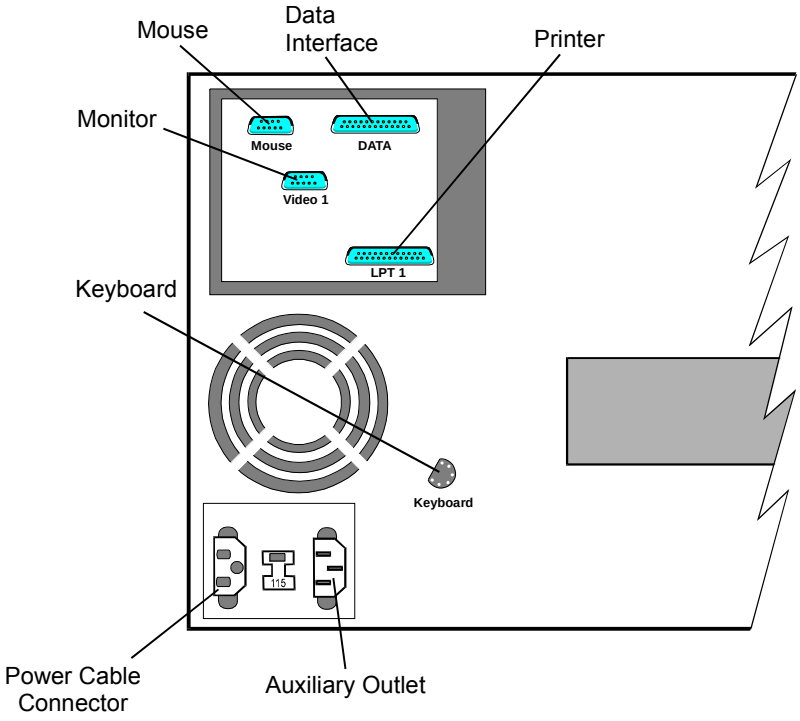
1. Unwrap the mouse cable and the keyboard cable, if you have not already done so.
2. Plug the mouse cable into the **Mouse** receptacle at the rear of the instrument. See Figure 2-1 or Figure 2-2 for the location of the receptacle.
3. Plug the keyboard cable into the **Keyboard** receptacle at the rear of the instrument. See Figure 2-1 or Figure 2-2 for the location of the receptacle.

Figure 2-1 Connecting the Cables (If Dispensers are Not Installed)



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Figure 2-2 Connecting the Cables (If Dispensers are Installed)



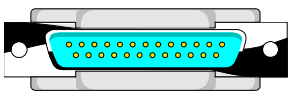
To connect an external device (if used) to the instrument:

1. Plug the communication cable from the external device into the **Data** receptacle at the rear of the instrument. See Figure 2-1 or Figure 2-2 for the location of the receptacle.
2. Refer to Chapter 3 for instructions on configuring the MLX system to accommodate the external device.

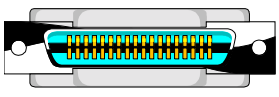
To connect the printer (if used) to the instrument:

1. Unwrap the printer cable, if you have not already done so. One end of the printer cable contains 25 pins (Figure 2-3).
2. Plug the 25-pin end of the printer cable into the **LPT 1** receptacle at the rear of the instrument.
3. Plug the other end of the printer cable into the printer cable receptacle marked **PARALLEL** at the rear of the printer.

Figure 2-3 Printer Cable Connectors



25-Pin End
Plug in to Instrument



Plug in to Printer
PARALLEL Port

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Connecting the Power Cables

Verifying the Voltage Setting

CAUTION: Ensure that the voltage setting on the instrument matches the voltage used in the laboratory before connecting the power cord to the power outlet.

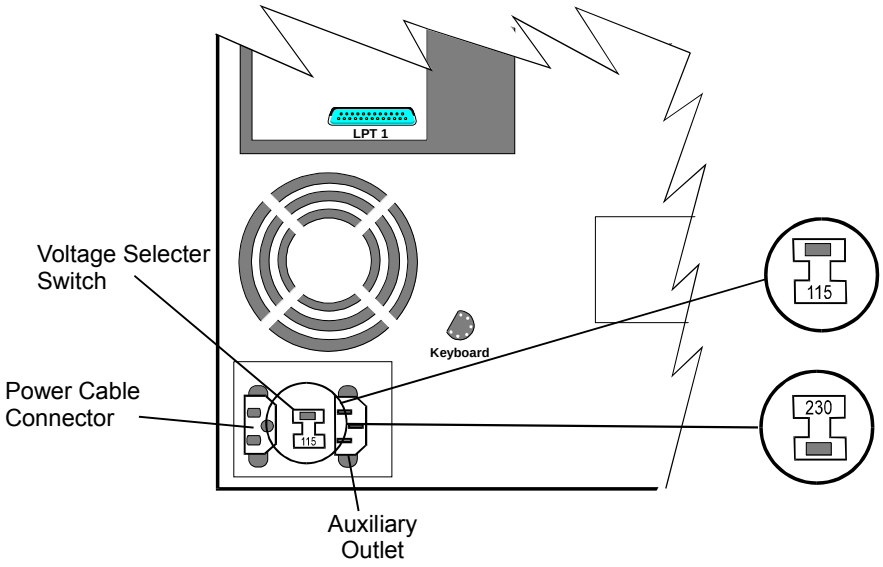
The voltage setting of the instrument must be checked before connecting the power cord to the power outlet in the laboratory. The voltage is checked at the point at the rear of the instrument where the power cord is connected to the instrument (Figure 2-4). The voltage setting is selected as outlined below.

Depending upon local electrical codes, an uninterruptible power system (UPS) may be required in your laboratory. A UPS is not provided with the MLX Luminometer system.

To verify (and change) the voltage setting:

1. Examine the voltage selector switch setting to determine the voltage setting. The voltage setting will be that which is displayed on the switch. Refer to Figure 2-4 for the location and appearance of the voltage selector switch.
2. If the voltage setting does not match the voltage supplied in your laboratory, use a pointed object such as a pencil to engage the recessed part of the switch and move the switch so the proper voltage setting is displayed.

Figure 2-4 Verifying Voltage Setting



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Connecting the Power Cables

You are now ready to connect the power cables. The components are either connected to electrical outlets (if an Uninterruptible Power System is not used) or they are connected to the UPS.

CAUTION: The MLX Luminometer components must be connected to properly grounded electrical outlets. Obtain assistance from a qualified electrician to verify that your electrical outlets are properly grounded.

Before connecting the power cables, be sure that the components have been connected to each other as outlined in the previous section.

To connect the power cables:

1. Unwrap the power cables for the MLX Luminometer, printer, and monitor, if you have not already done so.
2. Connect the monitor power cable to the MLX Luminometer, using the auxiliary outlet at the rear of the instrument (Figure 2-4).
3. Connect the MLX Luminometer to the laboratory electrical supply outlet.
4. If a printer is used, connect it to the laboratory electrical supply outlet.

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Installing the Optional Injectors

If your MLX Luminometer is configured for dispensing of reagents, then the following components are provided:

NOTE: If your assays require reagent dispensing, these components must be installed.

- Dispensers
- Injectors
- Reagent Bottles
- Tubing
- Drain Plate

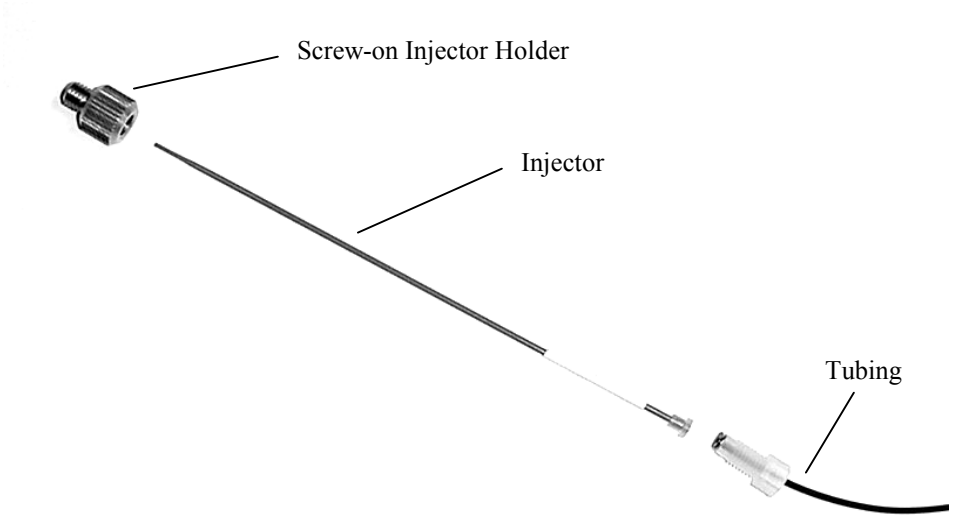
To install the injectors:

1. Turn the instrument ON (Refer to Chapter 3 for instructions). The Revelation window will appear automatically.
2. Eject the plate carrier by clicking on the Utility menu and then clicking on the Plate Out command.

   NOTE: To avoid damage to the injectors, be sure to eject the plate carrier before installing the injectors.

3. Unscrew the cap from the Injector "A" housing on the MLX Luminometer (see figure 2-6).
4. Assemble the Injector Assembly(s) by sliding the injector through the injector holder, and then screwing the threaded end of the tubing into the Injector Holder (see Figure 2-5).

Figure 2-5 Preparing the Injector Assembly



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5. Place the tip of one of the long injectors into the Injector “A” housing. Slide the injector into the housing and screw into place (see Figure 2-6).

> **NOTE:** Two of the injectors are longer and have a white collar. Be sure they are installed in the Injector “A” or Injector “C” housings.

6. Unscrew the cap from the Injector “B” housing on the MLX Luminometer.

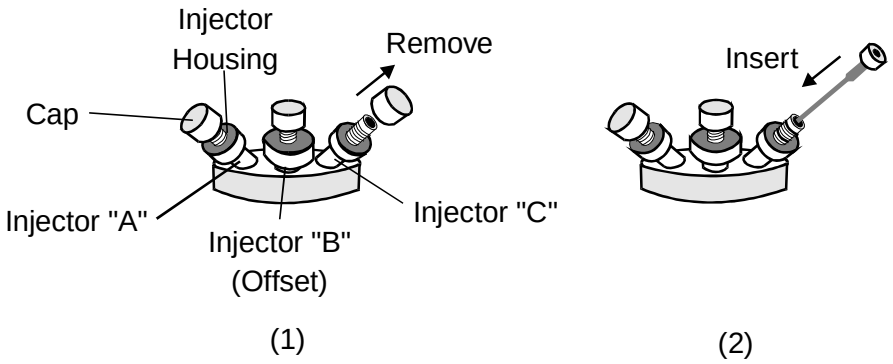
7. Place the tip of the short injector into the Injector “B” housing. Slide the injector into the housing and screw into place.

> **NOTE:** One of the injectors is shorter and has a yellow collar. Be sure that it is installed in the Injector “B” housing.

8. Unscrew the cap from the Injector “C” housing on the MLX Luminometer.

9. Place the tip of a long injector into the Injector “C” housing. Slide the injector into the housing and screw into place.

Figure 2-6 Installation of Fluid Injectors



To install the injector tubing:

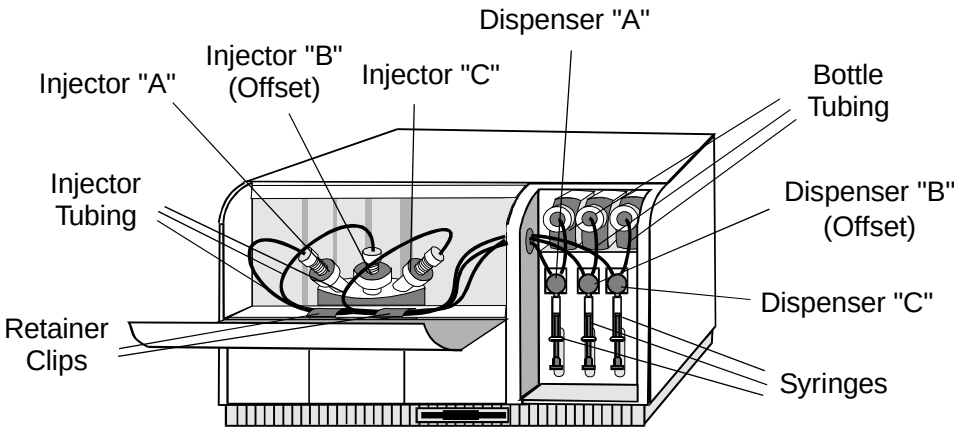
1. Locate the free end of the tubing that is attached to Injector “A”. Thread the injector tubing under the retaining clips and through the access hole in the front panel as shown in Figure 2-7.

2. Fasten the dispenser end of the tubing from Injector “A” to the left port of Dispenser “A” (see Figure 2-7).

3. Repeat Steps 1 and 2 for the tubing attached to Injector “B” and Injector “C”.

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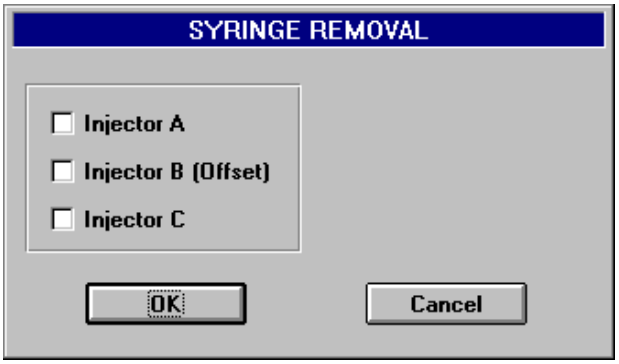
Figure 2-7 Installation of Tubing



To install the syringes:

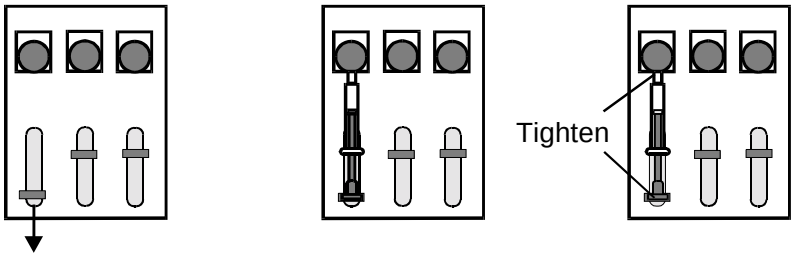
1. Activate the Dispensers command on the **Utility** menu (see Chapter 3 for instructions to start the MLX system). The Dispenser Utilities dialog box will appear.
2. Click on the Change Syringes... button. The Syringe Removal dialog box (Figure 2-8) will appear.
3. Select each syringe to be installed by clicking on the check box for that syringe. Then, click on the OK button. The syringe plungers will move to the end of their travel. Remove the first syringe from its protective package.
4. Place the first syringe into position. Fasten the syringe tip to the dispenser port (see Figure 2-9).
5. Fasten the syringe plunger to the dispenser arm (see Figure 2-9).
6. Repeat Steps 1 through 4 for the remaining dispensers.

Figure 2-8 The Syringe Removal Dialog Box



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Figure 2-9 Installation of Syringes



To install the bottles and bottle tubing:

1. Place the reagent bottle covers on the reagent bottles. Position the reagent bottles in the reagent tray.
2. Remove one of the bottle tubing sets from the protective packaging.
3. Fasten the bottle end of the tubing to the bottle marked “A”. Fasten the dispenser end of the tubing to the right port on the dispenser marked “A”.
4. Repeat Steps 2 and 3 for the “B” and “C” bottle tubing. When completed, the bottle tubing should be connected as shown in Figure 2-7.

System Maintenance

The MLX Microplate Luminometer is an electromechanical device that has few internal parts that are serviceable by the user. For a listing of those internal parts that may be replaced by the user, refer to the Maintenance section of this Operator’s Manual.

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3 Getting Started

Starting the MLX Luminometer

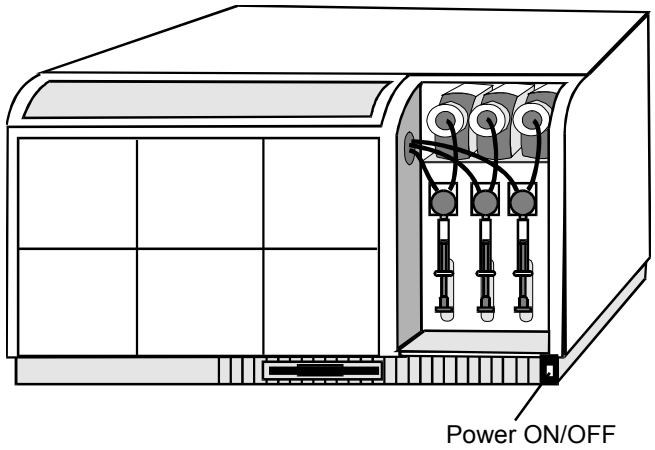
To start the MLX Luminometer:

1. Turn the power switch for the MLX instrument (Figure 3-1) and the monitor ON. The instrument will perform a series of self-tests. After the self-tests are done, the Revelation Window will be displayed. The results of the self-tests will be displayed in the Self-Test window (Figure 3-2).

   NOTE: The self-test results should indicate whether the system is operating properly. If a self-test failure is reported in the Self-Test window, it is recommended that you call Technical Service.

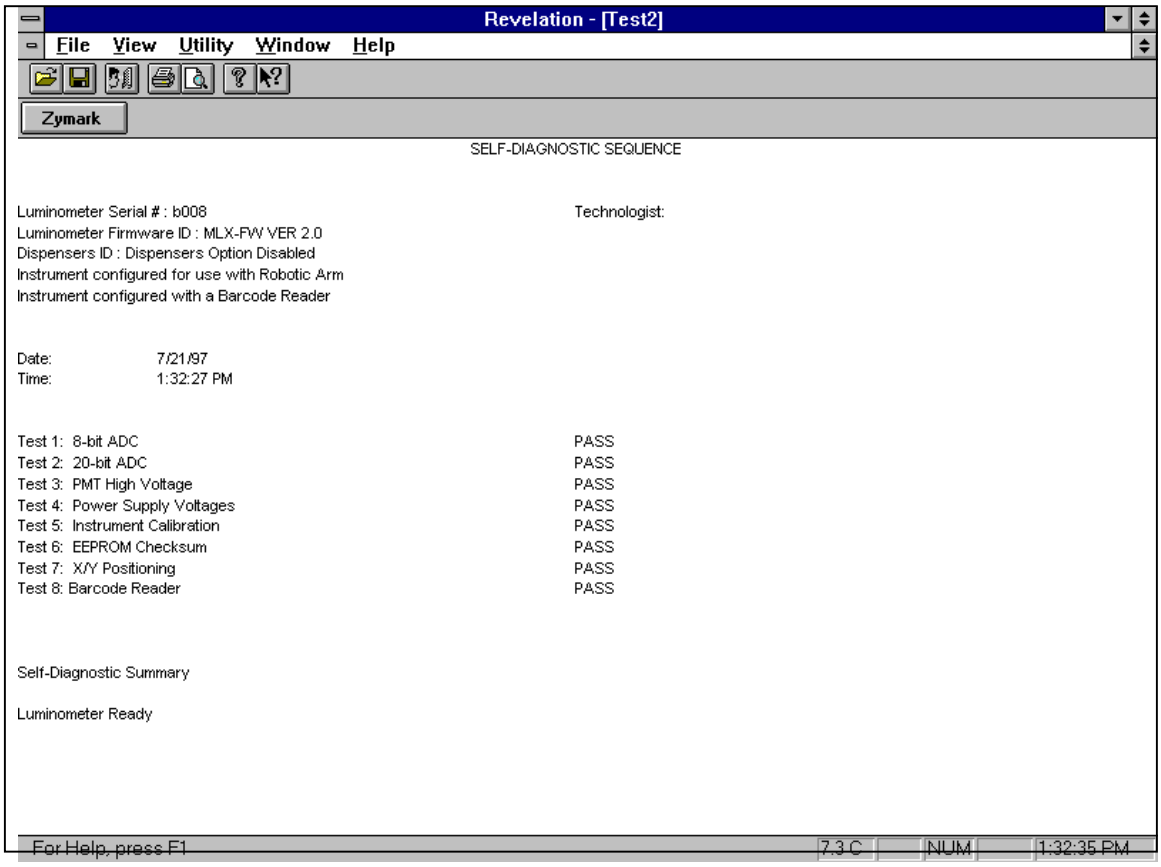
2. If a printer is used, turn the power switch to the printer ON.
3. If necessary, prepare the dispenser system as outlined later in this chapter.

Figure 3-1 Power ON/OFF



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Figure 3-2 The Self-Test Window



Note: The **Test 8: Barcode Reader** line will only be displayed in the Self-Test window if the barcode reader option is installed.

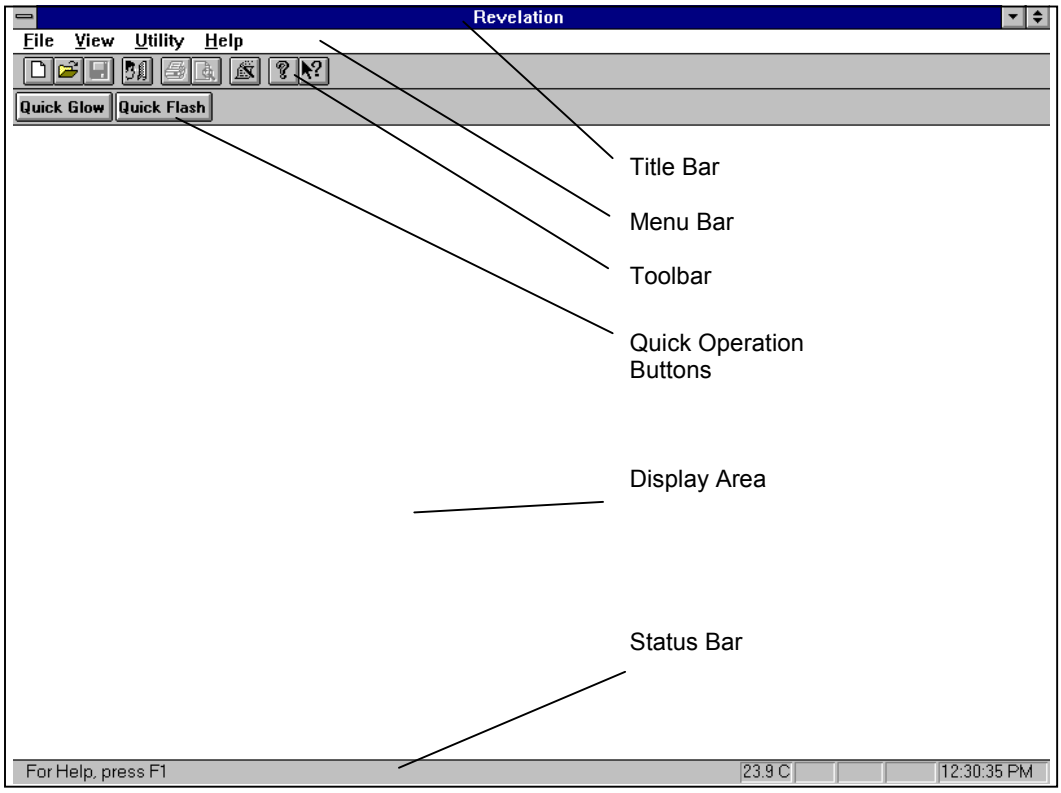
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The Revelation Window

The Revelation window (Figure 3-3) is displayed whenever you operate the MLX Luminometer. This window appears after you start up the MLX Luminometer.

Elements of the Revelation window are shown in Figure 3-3.

Figure 3-3 The Revelation Window



Elements of the Revelation window are described below:

Element	Description
Title Bar	Contains the title of the window.
Menu Bar	Provides access to the commands that are available.
Tool Bar	Provides easy access to commonly used commands.
Quick Operation Buttons	Provide ready access to assays that you specify. To create an Operation Button, see "Options " later in this chapter.
Display Area	This is the main area of the window, where dialog boxes and all other displays are presented.
Status Bar	Displays messages, temperature, keyboard status (Cap Lock, Num Lock and Scroll Lock), and the system time.

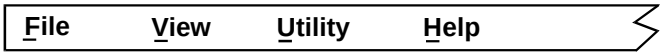
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Menus

The menus in the Revelation window are consistent with the active document.

On startup, the **File**, **View**, **Utility**, and **Help** menus are displayed (Figure 3-4).

Figure 3-4 Menus Displayed on Startup



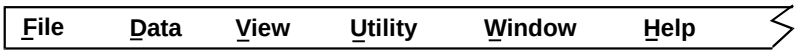
After you open or create an assay file and it is the active document, the **Settings** and **Windows** menus are added (Figure 3-5) and the **File** and **View** menus are expanded. These menus are displayed whenever at least one assay file is open and active.

Figure 3-5 Menus When an Assay File is Active



Whenever you complete a run, the results are displayed as the active document and the **Data** menu replaces the **Settings** menu (Figure 3-6).

Figure 3-6 Menus When a Plate is Run



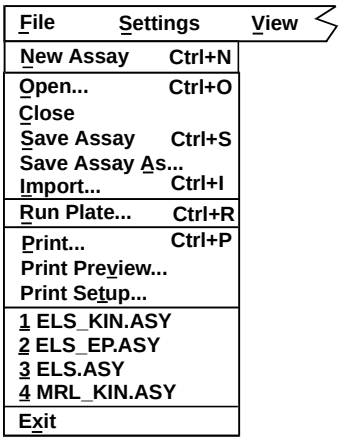
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The File Menu

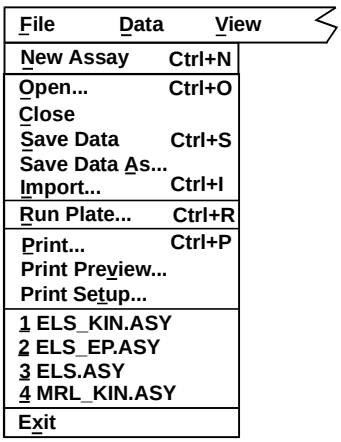
The **File** menu (Figure 3-7) provides commands to open and save files, print files on an installed printer, import files from other software packages, and read plates. The exact commands on the **File** menu depend on the active document.

Figure 3-7. The File Menu

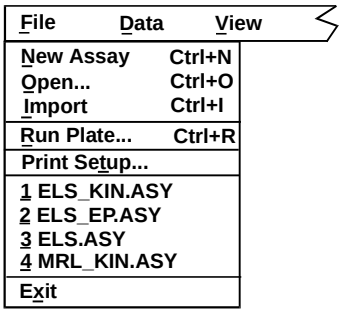
a) when Assay file is active



b) when Data file is active



c) when no file is open



Each command on the **File** menu is described below. Additional information is provided for some commands in the sections that follow.

File Menu Commands

Command	Description
New Assay	Create a new assay with all the default settings. See "Creating a New Assay" in Chapter 4.
Open	Open an existing assay. The File Open dialog box shows .ASY files by default, but other file types are available using the drop down list of file types. See "Modifying an Existing Assay" in Chapter 4.
Close	Close the current file. If you have made changes since you last saved, you will be prompted to save these changes. This command is only available if an assay file is open.
Save Assay (or Save Data)	Save the highlighted file. This command is only available if an assay (or data) file is open.
Save Assay As (or Save Data As)	Save the highlighted file under a new name. This option allows you to use an existing file as a basis for the new one without losing the original. This command is only available if an assay (or data) file is open.
Import	Import Sample ID files or import a user selectable data file from an external software package.

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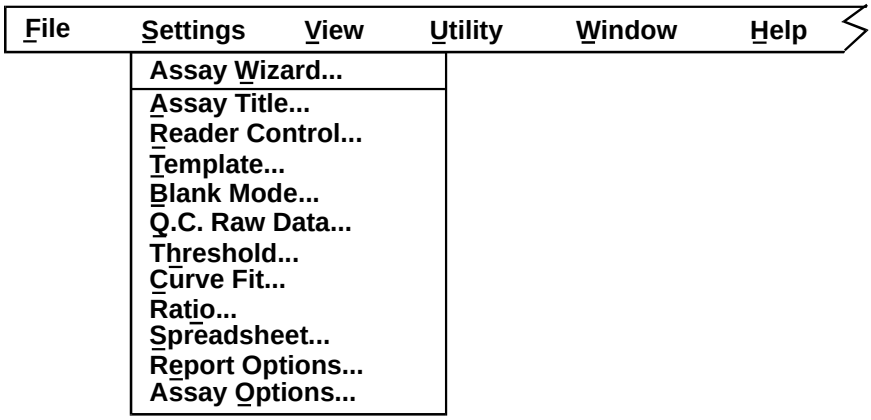
Command	Description
Run Plate	Displays the Run Plate dialog box. The plate can then be read after you select the assay to use and other information.
Print	Print a copy of the active assay (or data). This command is only available if an assay or data file is open.
Print Preview	View the active assay (or data) file as it will be printed. This command is only available if an assay or data file is open.
Print Setup	View and, if necessary, change the printer settings.
Exit	Exit Revelation. If there are unsaved data files or if you have made changes to any of the open files since you last saved them, Revelation will prompt you to save them.

The Settings Menu

The **Settings** menu (Figure 3-8) is only displayed when an assay file is open and active. It provides access to all of the commands necessary to display the dialog boxes needed to define an assay. Each of these commands is briefly described in the following table.

Note: Refer to Chapter 4 for complete details concerning all of the commands and dialog boxes associated with the Settings menu.

Figure 3-8 Settings Menu



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Settings Menu Commands

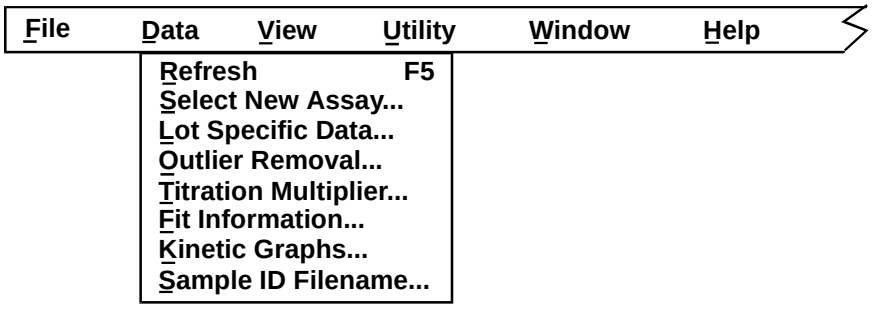
Command	Use this command to . . .
Assay Wizard	Create a new assay using the Wizard feature.
Assay Title	Enter the assay title, author, code and password.
Reader Control	Specify the start mode options, shake parameters, temperature control options, dispensing options, and reader options (gain level, scaling factor, and read time/well).
Template	Define the positions of the well type samples on the plate. This definition includes: location, well type, replicates, orientations, and fill direction.
Blank Mode	Select a pre-defined blank mode or define a custom blank mode.
Q.C. Raw Data	Define a quality control equation to be applied to the raw data collected when using this assay.
Threshold	Define threshold equations, positive/negative Q.C. ranges, threshold Q.C. equations, and the results output format.
Curve Fit	Specify all of the parameters required to formulate results using a user defined curve fit.
Ratio	Define the equation and output format required to report results as a ratio.
Spreadsheet	Perform arithmetic calculations on RLUs from different wells. The results of these calculations are output as a matrix or table of data.
Report Options	Select report formats, matrix type, and export files.
Assay Options	Specify area statistics, processing order, and sample ID setup.

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The Data Menu

The **Data** Menu (Figure 3-9) is only displayed after a "Run Plate" has been executed. Once a set of results has been displayed in the Data Reduction window, some of the **Data** Menu options will become available. The options available will depend on the contents of the test that was used to obtain the results.

Figure 3-9 The Data Menu



Data Menu Commands

Command	Use this command to . . .
Refresh	Reprocess data with new test parameters if changes have been made to a test file after a plate has been read and a data file produced. Save the test changes and select Refresh.
Select New Assay	Use the data from one test with the data processing options of another test.
Lot Specific Data	Displays the Lot-Specific Data dialog box. Then you enter the lot number and other lot-specific data for the reagents you are using.
Outlier Removal	Remove well results which fall outside the expected range from the calculations.
Titration Multiplier	Change the values of all the steps in a dilution block. This may be useful if you entered a nominal value dilution step (see "Define " in Chapter 4) and the change you specified in Printout Mode (see "Threshold Output Format " in Chapter 4) occurred at a different dilution step.
Fit Information	View the name, fit type and axes fit of a Curve Fit graph and store the curve parameters.
Kinetic Graphs	Plot individual well, replicate or whole plate Kinetic Graphs. This option works in the same way as the Settings menu, Kinetic Graphs option (refer to "Kinetic Graphs Dialog Box" in Chapter 5). This option only applies to data from a glow-kinetic assay.
Sample ID File Name	Enter a new file name for the Sample ID file. Select the Use Sample ID File check box, click on **Change**, then enter an appropriate file name.

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The View Menu

The **View** menu provides commands to display (or not display) the Toolbar and Status Bar. Other commands are displayed on the **View** menu, depending upon whether a data file is open:

- If there are no data files open, the **View** menu shows only the Tool Bar and Status Bar options (Figure 3-10).
- If there is a data file open, the menu shows all the options you can include in an assay, but dims the ones which do not apply to the current file (Figure 3-11).
- If plate statistics are displayed, the **View** menu shows the mean, standard deviation and coefficient of variation (CV) options (Figure 3-12).

The table that follows describes each command available from the **View** menu.

Figure 3-10 The View Menu When No Data Files Are Open

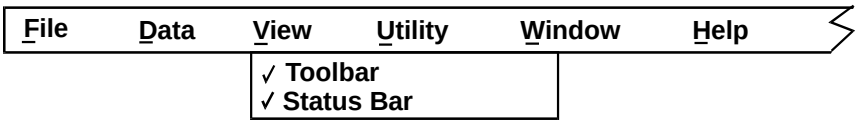


Figure 3-11 The View Menu When a Data File is Open

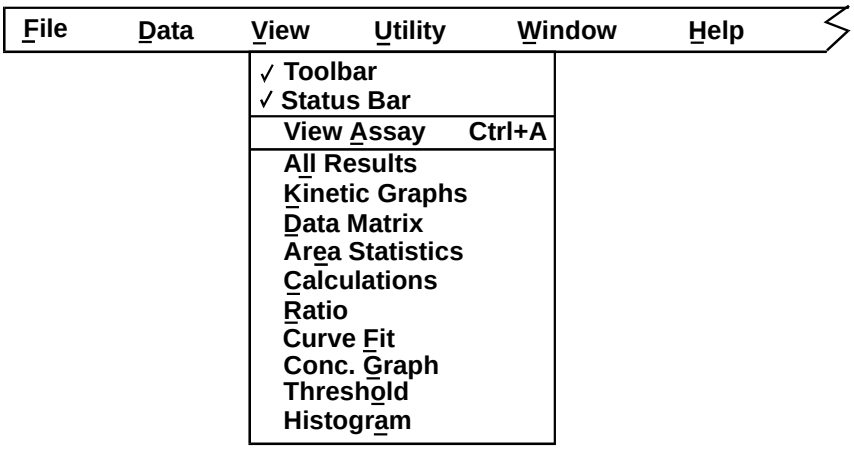
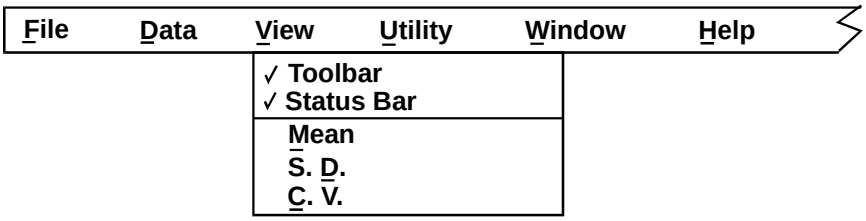


Figure 3-12 The View Menu With Plate Reproducibility



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View Menu Commands

Command	Use this command to . . .
Toolbar	Show or hide the Toolbar at the top of the Revelation window.
Status Bar	Show or hide the Status Bar at the bottom of the Revelation window.
All Results	Show all results in the plate data window.
Kinetic Graphs	Show only Kinetics Graphs.
Data Matrix	Show only the Data Matrix.
Area Statistics	Show only the Area Statistics results.
Calculations	Show only Calculations results. These will be displayed as you specified in the Output Format box.
Ratio	Show only Ratio processing results. These will be displayed as you specified in the Output Format box.
Curve Fit	Show only Curve Fit results. These will be displayed as you specified in the Output Format box.
Conc. Graph	Show only the Curve Fit concentration graph in the plate data window.
Threshold	Show only Threshold processing. These will be displayed as you specified in the Output Format box.
Histogram	Show only the Threshold processing histogram.
Mean	Display the mean of the plate statistics when the plate reproducibility test is run.
S.D.	Display the standard deviation of the plate statistics when the plate reproducibility test is run.
C.V.	Display the coefficient of variation of the plate statistics when the plate reproducibility test is run.

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The Utility Menu

The **Utility** menu (Figure 3-13) provides commands to select the Reader type, configure the Reader to meet the particular needs of the assays to be run, verify accuracy and precision of the luminometer readings, transfer control to a robotic arm system, and perform simple manual plate operations.

The specific commands that are displayed on the **Utility** menu depend on the following:

- whether Dispensers are installed
- whether the Remote Control option is installed
- whether control has been transferred to a robotic arm system (this applies if the Remote Control option is installed)

All possible **Utility** menu commands are shown in Figure 3-13. The Utility menu commands that are displayed when control has been transferred to a robotic arm system are shown in Figure 3-14. Finally, all **Utility** menu commands are described in detail in the following section.

Figure 3-13 Utility Menu (All Commands)

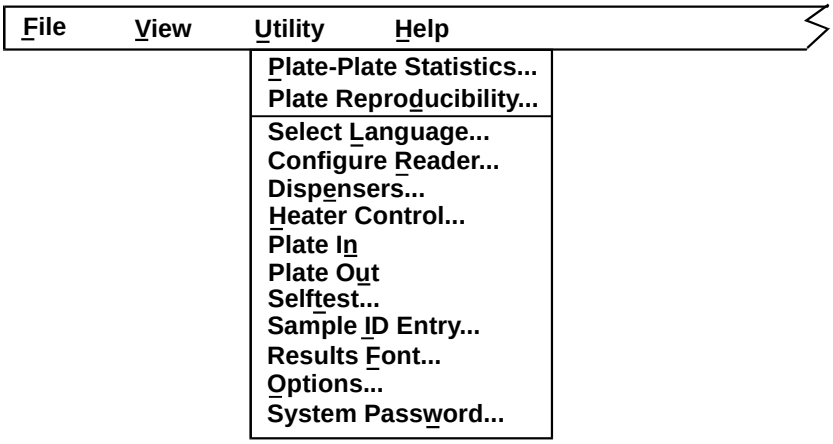
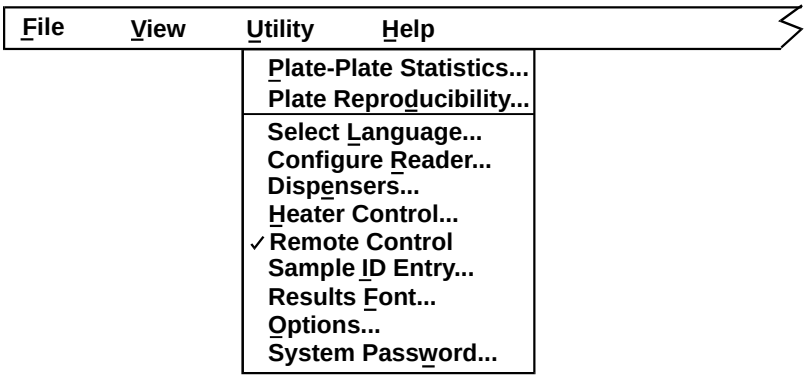


Figure 3-14 Utility Menu Commands (When MLX is Under Remote Control)



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Utility Menu Commands

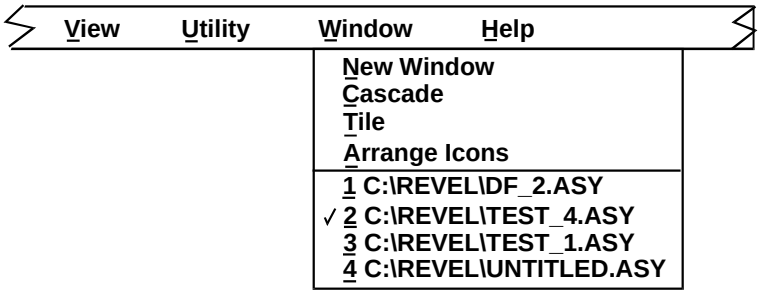
Command	Description
Plate-Plate Statistics	Compare plate data statistically, and use data from one test with the data processing options of another.
Plate Reproducibility	Calculate statistical data for a single plate over several readings.
Select Language	Choose the display menu language. (Only available when no files are open.)
Configure Reader	Specify the Reader type and set it up to suit your applications.
Dispensers	Initialize, prime, purge, wash, or remove reagents from the dispenser assemblies. This command displays the Dispenser Utility dialog box, which allows you to select the appropriate dispenser setup function.
Heater Control	Specify the manner in which temperature of the plate wells is controlled.
Plate In	Allows the user to retract the plate carrier inside the instrument.
Plate Out	Brings the plate carrier out of the instrument.
Selftest	Runs the series of self-tests (see page 25).
Verification	Verify accuracy and precision of the MLX Luminometer by running the Reference Plate (see Chapter 7). Also, add or modify Reference Plate information. **Note:** The **Verification** command will not be displayed if a check mark is next to the Remote Control command (if the MLX system is being controlled by a remote robotic rdevice).
Remote Control	Specify that the MLX Luminometer is controlled by a remote controlled robotic arm device. A check mark is displayed next to the **Remote Control** command when the MLX system is being controlled by a remote robotic device. **Note:** The **Remote Control** command will not be displayed if the remote device option is not installed on your MLX Luminometer.
Sample ID Entry	Specify and apply Sample IDs to well types.
Results Font	Select fonts for all printouts.
Options	Allow user specification of special command buttons, default operational preferences, and well type colors, and also displays the revelation sub-directory structure.
System Password	Assign a password to the system which limits access to certain dialog boxes and programs.

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The Window Menu

The **Window** Menu (Figure 3-15) is only available when assay files and/or data files are open. This menu provides commands to create new windows, tile and cascade existing windows, and arrange icons. The following table describes each command available from the **Window** Menu.

Figure 3-15 Window Menu



Window Menu Commands

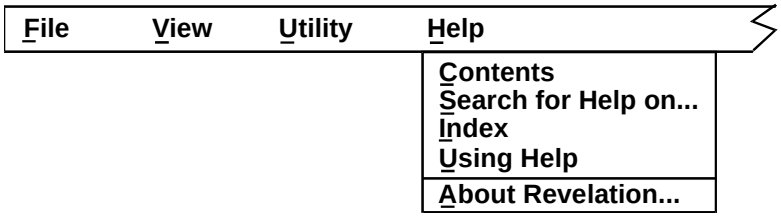
Command	Use this command to . . .
New Window	Create a new window exactly the same as the current window. The old window will be titled with the original name with :1 at the end and the new window will be titled with the original name with :2 at the end. Any changes you made to copied windows will be copied to the original as well. If you close any copied windows having made changes, Revelation will only ask if you want to save the changes when you attempt to close the final window.
Cascade	Arrange and resize all the open windows. The windows will all overlap but the title bar of each one will remain visible.
Tile	Resize and arrange all the open windows. They are placed side by side so that they fill the whole screen and the contents of each can be viewed.
Arrange Icons	Arrange all the icons in evenly space rows.
Window List	The end of the Windows menu shows a list of all the Windows open in the Revelation Window. If there are more than 10 windows open, the list refers to **More Windows**. To bring any one of these windows to the front of the screen, click on the required window.

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The Help Menu

The **Help** Menu (Figure 3-16) provides commands to obtain on-line context sensitive help. The commands available from this menu are described in the following table.

Figure 3-16 The Help Menu



Help Menu Commands

Command	Use this command to . . .
Contents	The Table of Contents of the Help.
Search for Help on	Locate information about a topic from an alphabetical list.
Index	Access the program help file so that you can search for help on any topic.
Using Help	Brief tutorial titled "Help Basics".
About Revelation	View information about the copyright and version of the Revelation program.

Note: Within the Help menus, words that are underlined or dotted underlined are keywords. Click on a keyword to find out more about that topic.

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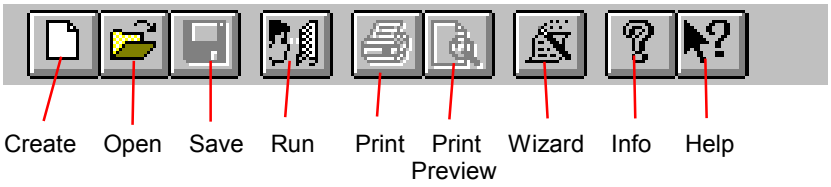
Toolbars

The Starting Toolbar

The starting toolbar provides quick access to the most commonly used commands available from the menus. To use the toolbar, click on the tool button once with the left mouse button. The command represented by that tool will be activated.

The buttons that are contained on the toolbar depend upon the menus that are displayed. For example, before an assay file is opened or created, the starting toolbar is displayed (Figure 3-17). The tools contained on the starting toolbar correspond to the commands that are contained on the starting menu shown in Figure 3-1.

Figure 3-17 Starting Toolbar



Each button on the starting toolbar is described below. For additional information, refer to "The File Menu " on page 29.

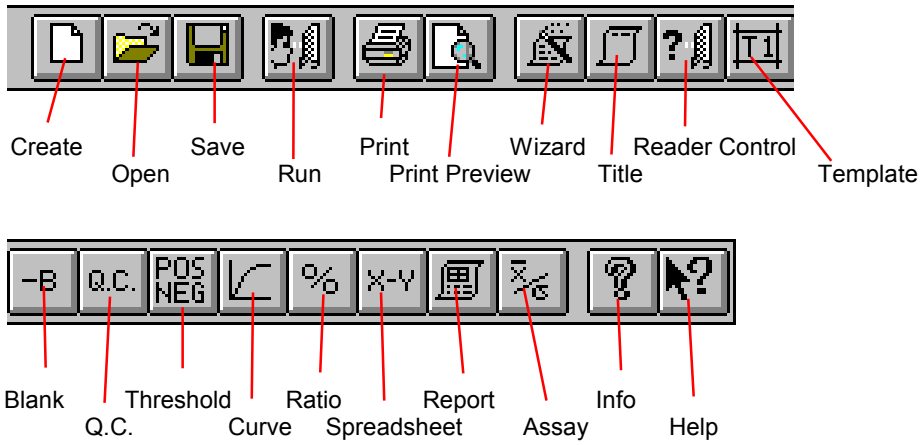
Button	Description
Create	Open a new assay with all the default settings.
Open	Open an existing assay.
Save	Save the highlighted file.
Run	Read the plate using the currently selected assay.
Print	Print a copy of the currently displayed report.
Print Preview	Display a full page as it will be printed.
Wizard	Access the Wizard feature to assist you with system operation.
Info	Display software version information concerning Revelation.
Help	Access context-sensitive on-line help.

The Settings Toolbar

The Settings toolbar is substituted for the starting toolbar whenever an assay is being created or edited. The buttons contained on the Settings toolbar (Figure 3-18) correspond to the commands that are available when the **Settings** menu is displayed.

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Figure 3-18 The Settings Toolbar



Each button on the Settings toolbar is described below. For additional information, refer to Chapter 4.

Button	Description
Create	Open a new assay with all the default settings.
Open	Open an existing assay.
Save	Save the highlighted file.
Run	Read the plate using the currently selected assay.
Print	Print a copy of the current assay.
Print Preview	Display a full page as it will be printed.
Wizard	Access the Wizard feature to assist you with system operation.
Title	Enter the assay title, author, code and password.
Reader	Specify the start mode options, shake parameters, temperature control options, and reader options (gain level, scaling factor, and read time/well).
Template	Define the positions of the well type samples on the plate. This definition includes: location, well type, replicates, orientations, and fill direction.
Blank	Select a pre-defined blank mode or define a custom blank mode.
Q.C.	Define a quality control equation to be applied to the raw data collected when using this assay.
Threshold	Define threshold equations, positive/negative Q.C. ranges, threshold Q.C. equations, and the results output format.

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Button	Description
Curve	Specify all of the parameters required to formulate results using a user defined curve fit.
Ratio	Define the equation and output format required to report results as a ratio.
Spreadsheet	Prepare a spreadsheet containing the results formatted so that well RLUs can be added to or subtracted from one another.
Report	Select report formats, matrix type, and export files.
Assay	Specify area statistics, processing order, and sample ID setup.
Info	Display software version information concerning Revelation.
Help	Access context-sensitive on-line help.

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Preparing the Dispensers

Before you run a flash assay, the reagent dispensers must be prepared. Preparation of the dispensers consists of:

- Placing each required reagent in the appropriate dispenser bottle.
- Initializing and priming the dispensers.

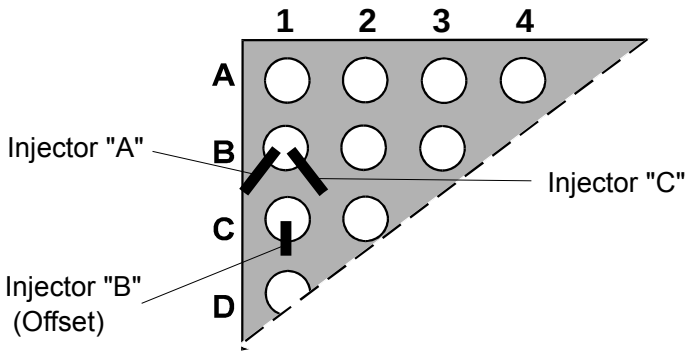
Positioning of Reagents

The reagent bottle [Dispenser A, B (Offset), or C] that you use for each reagent depends upon the definition of the flash assay. Each dispenser is used for a particular purpose, as outlined below:

Dispenser	Purpose
A	Triggers the flash reaction. Reading of the well commences at the same time that reagent is dispensed into the well from Injector A.
B (Offset)	Dispenses a reagent into the well before the reaction is triggered or reading has commenced. This may be a reagent to prepare the sample for reading. For example, a lysing reagent may be dispensed from the B (Offset) injector.
C	Dispenses a reagent into the well when reading or after reading has commenced.

The Offset (B) injector is mounted so that it is positioned one well ahead of Injector A and Injector C (Figure 3-19). When dispensing reagents, the Offset (B) reagent is always dispensed into a well first. Then the plate carrier advances one well position causing Injector A and Injector C to be positioned over the well and Injector B (Offset) to be positioned over the next well. The dispenser/reader head remains in this position until reagent has been dispensed from Injector A and from Injector C and the readings are completed at the times specified in the assay Settings. Detailed procedures for configuring the dispensers for a flash assay are outlined in Chapter 4.

Figure 3-19 Positions of Injectors



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The Drain Plate

The Drain Plate (Figure 3-20) must be used whenever the dispensers are primed or washed. The Drain Plate is inserted in the plate carrier in the same manner as a Microplate. The reagents that are expelled from the injectors during the prime or wash cycles are collected on the Drain Plate. After the prime or wash cycles are completed, the Drain Plate can be removed and the waste reagents disposed. Each reservoir in the Drain Plate can contain up to 20 mL of fluid.

Note: You will be prompted to insert the Drain Plate whenever you carry out an operation that requires it. The procedure below is only for an occasion when you want to insert the Drain Plate before carrying out a procedure that requires it.

Note: When not using the Drain Plate, store it exposed to ambient light so that its glow plug will glow when placed in the MLX

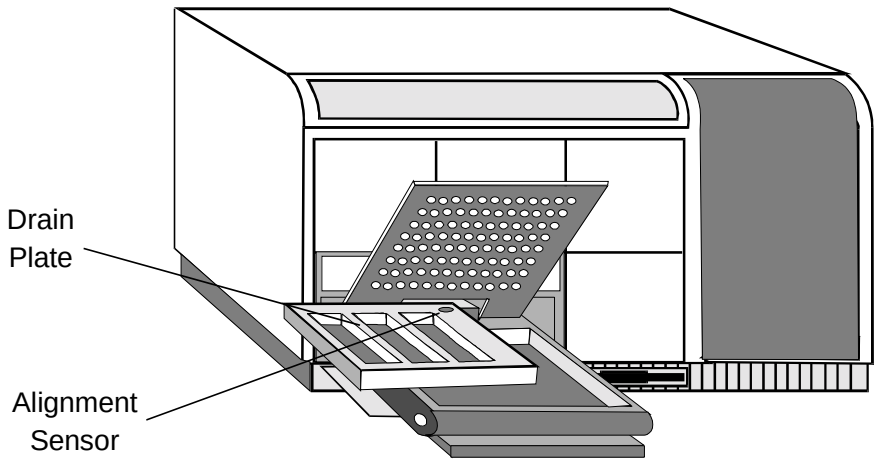
Note: If a robotic arm with remote control is used with your system, the appearance of the Drain Plate will be slightly different than the one shown.

To insert the Drain Plate:

1. Click on **Utility** and then click on **Plate Out** to eject the plate carrier.
2. Place the Drain Plate on the plate carrier (Figure 3-20).

   NOTE: Be sure that the alignment well (located at well A13) of the Drain Plate is positioned in the upper right corner (see Figure 3-10) when inserting the Drain Plate.
3. Click on **Utility** and then click on **Plate In** to close the plate carrier.

Figure 3-20 Inserting the Drain Plate



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Initializing and Priming

Before a flash assay is run, the injectors must be initialized and primed. You may also want to wash (or purge) the injectors if they have not been used for an extended period. Finally, you can remove (or recover) reagents from the injectors and tubing by drawing them back into the syringe.

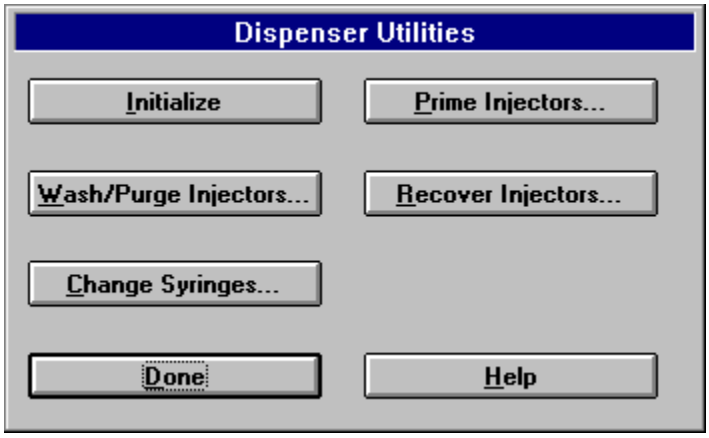
Each of these actions is summarized below.

Action	Description
Initialize	Electronically initialize the syringe pumps.
Prime	Dispense a small amount of reagent from the reagent supply bottle to fill the tubing and injector in preparation for a dispense operation. This will eject any air bubbles that may have formed at the end of the injectors.
Wash/Purge	Dispense a larger amount of reagent from each injector, to replace reagent that remained in the injector during the shut-down period.
Recover	Recover reagent from the injector and injector tubing and return the reagent to the supply bottle.

To initialize the injectors:

1. Click on **Utility** and then click on **Dispensers**. The Dispenser Utilities dialog box (Figure 3-21) will be displayed.
2. Click on **Initialize**. The injectors will be initialized.

Figure 3-21 The Dispenser Utilities Dialog Box



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To prime the injectors:

1. Click on **Utility** and then click on **Dispensers**. The Dispenser Utilities dialog box (Figure 3-21) will be displayed.
2. Click on **Prime Injectors...**. The Prime dialog box (Figure 3-22) will appear.

   NOTE: If you have not initialized the injectors, the **Prime Injectors** button will be inactive.

3. Select an injector to be primed by placing an **X** in the check box for that injector. Then, select the number of priming cycles by dragging on the scroll bar.
4. Repeat Step **3** for the other injectors, if needed.
5. Click on **OK**. If you have specified priming of at least one injector, the plate carrier will eject and a message box will prompt you to insert the Drain Plate (Figure 3-23).
6. Place the Drain Plate on the plate carrier (see previous section) and then click on the **OK** button in the message box. The prime cycles will be carried out.
7. At the end of the priming, the plate carrier will eject and a message box will prompt you to remove the Drain Plate
8. Remove the Drain Plate from the plate carrier. Properly dispose of the reagents on the Drain Plate.

Figure 3-22 The Prime Dialog Box

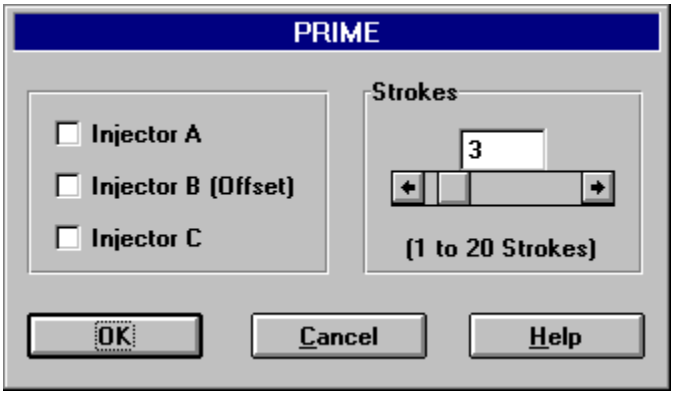


Figure 3-23 The "Insert Drain Plate" Message Box



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To wash or purge the injectors:

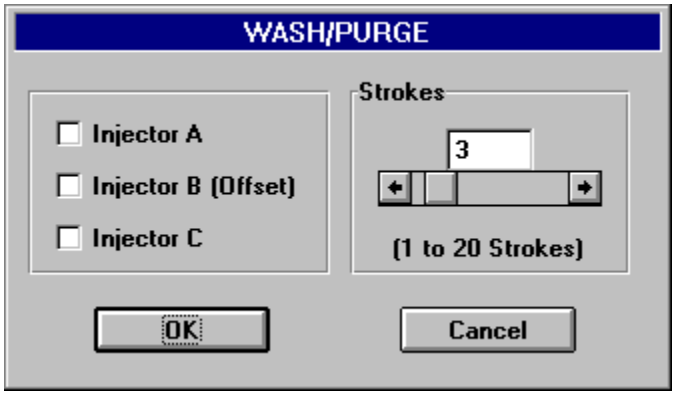
> **CAUTION:** Wash/Purge is similar to priming, except that a larger volume of reagent is expelled. Be sure to remove and empty the Drain Plate after this procedure has been completed, to avoid overflow of the Drain Plate and damage to the MLX.

1. Click on **Utility** and then click on **Dispensers**. The Dispenser Utilities dialog box (Figure 3-21) will appear.
2. Click on **Wash/Purge Injectors...**. The Wash/Purge dialog box (Figure 3-24) will be displayed.

> **NOTE:** If you have not initialized the injectors, the **Wash/Purge Injectors** button will be inactive.

3. Select an injector to be purged by placing an **X** in the check box for that injector. Then, select the number of purging cycles by dragging on the scroll bar.
4. Repeat Step **3** for the other injectors, if needed.
5. Click on **OK**. If you have specified purging of at least one injector, the plate carrier will eject and a message box will prompt you to insert the Drain Plate (Figure 3-23).
6. Place the Drain Plate on the plate carrier (see previous section) and then click on the **OK** button in the message box. The purging cycles will be carried out.
7. At the end of the purging, the plate carrier will eject and a message box will prompt you to remove the Drain Plate
8. Remove the Drain Plate from the plate carrier. Properly dispose of the reagents on the Drain Plate.

Figure 3-24 The Wash/Purge Dialog Box

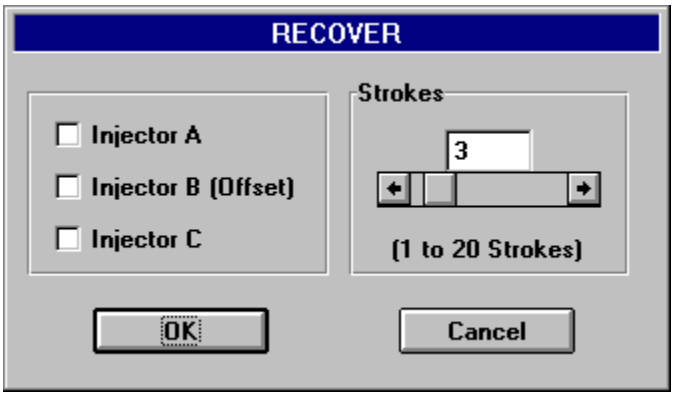


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To recover the reagents:

1. Click on **Utility** and then click on **Dispensers**. The Dispenser Utilities dialog box (Figure 3-21) will appear.
2. Click on **Recover Injectors...**. The Recover dialog box (Figure 3-25) will be displayed.
3. Select a reagent to be recovered by placing an **X** in the check box for that reagent. Then, select the number of recovery cycles by dragging on the scroll bar.
4. Repeat Step **3** for the other reagents, if needed.
5. Click on **OK**. The recovery cycles will be carried out.

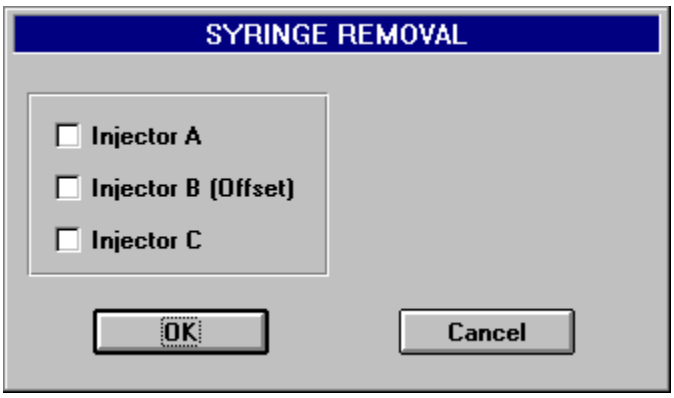
Figure 3-25 The Recover Dialog Box



To change syringes:

1. Click on **Utility** and then click on **Dispensers**. The Dispenser Utilities dialog box (Figure 3-21) will appear.
2. Click on **Change Syringes...**. The Syringe Removal dialog box (Figure 3-26) will be displayed.
3. Install or change the syringes as outlined in Chapter 2.

Figure 3-26. Syringe Removal Dialog Box



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Configuring Instrument Parameters

Before you define your first assay on the MLX, certain instrument parameters should be configured. The commands for doing this are contained on the **Utilities** menu:

Command	Purpose
Select Language	Change the language in which menus are displayed. See page 48 for more information.
Configure Reader	Specify the Reader type and the setup parameters for the Reader. See page 49 for more information.
Heater Control	Specify the manner in which temperature of the plate wells is controlled. See page 56 for more information.
Results Font	Specifies the font that is used when printing reports. See page 59 for more information.
Options	Select user preferences, specify laboratory information for the report header, specify colors to represent the well types, and create Quick Operation Buttons. See page 60 for more information.

Select Language

Use this command to display the Language dialog box (Figure 3-27). You can then change the language in which menu commands are displayed. This command is only available when all assay files and data files are closed.

Note: The languages that are available depend upon the version of software purchased.

Figure 3-27 Language Dialog Box



To change the language:

1. Close all assay files and data files.
2. Click on **Utility** and then click on **Select Language**. The Language dialog box (Figure 3-27) will be displayed.
3. Select the language you want
4. Click on **OK**.

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Configure Reader

Use this command to select a different reader type or to modify the setup parameters of your MLX instrument.

To select a different reader type:

1. Close all assay files and data files.
2. Click on **Utility** and then click on **Configure Reader**. The Reader Type dialog box (Figure 3-28) will be displayed.

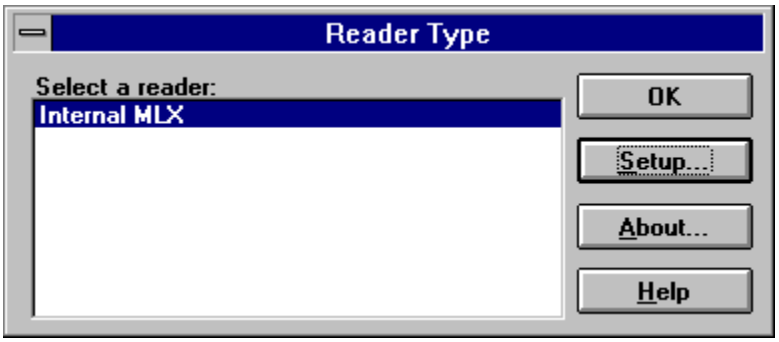
   Note: If one or more assay or data files are open when you click on **Configure Reader**, the Reader Type dialog box will not be displayed. Instead, you will be prompted to enter the password to modify the setup parameters of the current configured reader.

3. Use the Reader Type dialog box to select a reader other than the Internal MLX.

   Note: If no other readers are used, only the Internal MLX reader will be listed. If this dialog box does not display any reader types, you will need to re-install Revelation.

4. Click on **OK**.

Figure 3-28 Reader Type Dialog Box



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To modify the MLX setup parameters:

1. Click on **Utility** and then click on **Configure Reader**. The Reader Type dialog box (Figure 3-28) will be displayed.

   Note: If one or more assay or data files are open, the System Password dialog box will be displayed instead of the Reader Type dialog box. In this case, skip Step 2 below and proceed to Step 3.

2. Click on **Setup**. The System Password dialog box will be displayed.

3. Type the system password in the Enter Password field. Then click on **OK**.

   Note: If you enter an incorrect password, the system will not respond. If this occurs, delete the password entry (shown as asterisks) by backspacing or highlighting it. Then, retype the password.

4. Six tabbed MLX Setup dialog boxes will be displayed. Select the appropriate setup tab as outlined below:

Tab	Purpose
General	Specify whether dispensers are installed, select the frequency and reporting mode of self testing, specify whether data from glow-endpoint or flash assays are saved between well readings, specify whether data are to be exported through a serial port, and specify a range of acceptable RLU values.
Instrument	Specify the parameters for communication to the MLX micro-instrument.
Injectors	Specify which injectors are installed on the MLX instrument and the communication parameters for the injectors.
Injector Speeds	Specify the dispensing and aspiration speeds, the syringe size, and the pickup volume for each injector.
Remote/Data Port	Specify communication parameters for exporting data through the RS232 port. **Note:** The Remote/Data Port tab is displayed even if the Remote/Data Port option is not installed on your MLX system.
Barcode	Specify the type of barcode that is read and check the reading of a barcode. **Note:** The Barcode tab is not displayed if the Barcode Reader option is not installed on your MLX system.

5. Instructions for using each dialog box are contained in the following sections.

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General

The General dialog box is shown in Figure 3-29.

To specify general parameters:

1. ***Installed Options*:** If dispensers are installed, place an **x** in the **Dispensers** check box.
2. ***Self-Test*:** If you want the results of the self-test (page 26) to be printed each time the self-test is run, place an **x** in the **Print Selftest Report** check box.

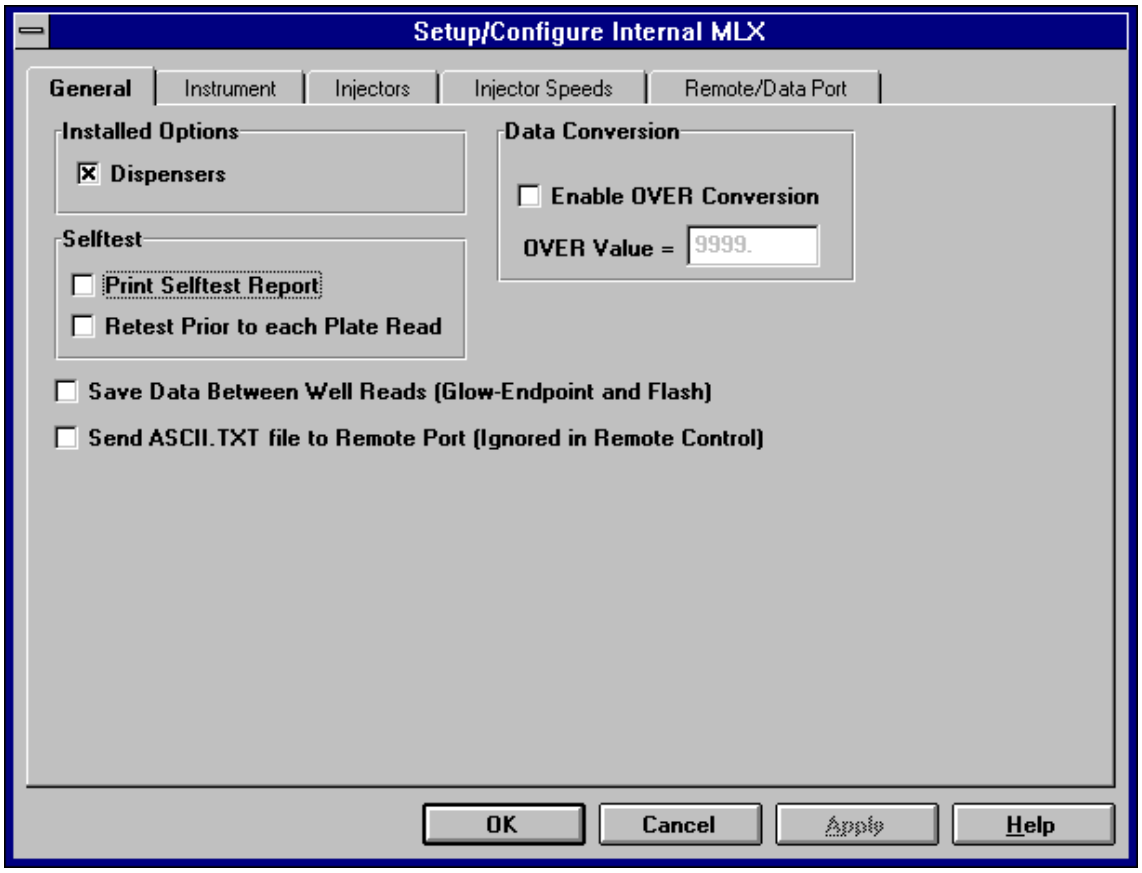
   If you want the self-test to be run before each plate is read, place an **x** in the **Retest Prior to Each Plate Read** check box.
3. ***Save Data between Well Reads*:** If you want the data obtained from glow-endpoint or flash assays to be temporarily saved between well readings, place an **x** in the **Save Data Between Well Reads** check box. See Chapter 4 for additional information.
4. ***Send ASCII Text File to Remote Port*:** If you want data to be exported through the RS232 serial port to another system, place an **x** in the **Send ASCII.TXT File to Remote Port** check box. Be sure to configure the Export to File options in the assay definition (see page 178).

   Note: If your MLX system is being operated under remote control, this selection is ignored.
5. ***Data Conversions*:** If you want to extend the range of acceptable RLU values, place an **x** in the **Enable OVER Conversion** check box. Then, enter the maximum allowable RLU value in the **OVER Value** text box.
6. Click on **OK**.

   Note: The General dialog box and many other MLX dialog boxes contain an Operation Button labeled **Apply**. This Operation button is for a different application and is inactive on the MLX.

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Figure 3-29 The General Dialog Box



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Instrument

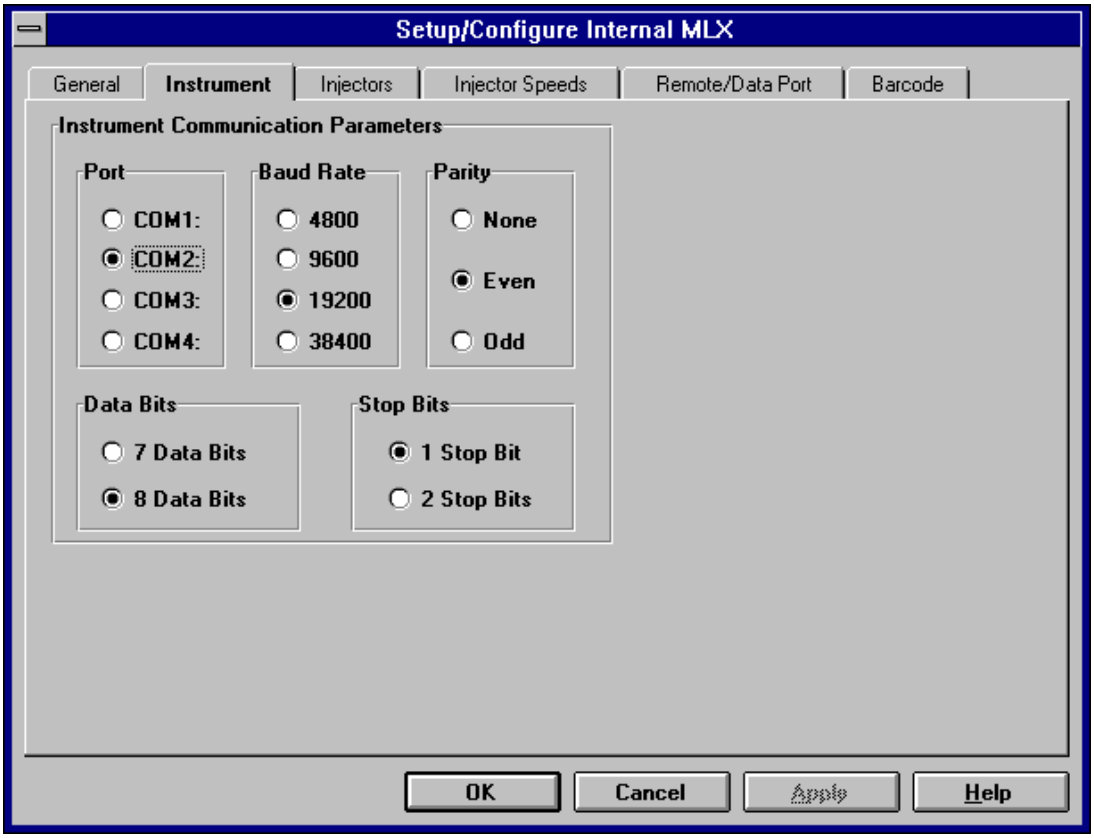
The Instrument dialog box is shown in Figure 3-30.

Warning: The settings shown below are default settings and should not be changed, except by qualified technical service personnel.

To change communication parameters for the instrument:

1. ***Port*:** Select the port for which the communication parameters are being established by clicking on the appropriate radio button.
2. ***Baud Rate*:** Select the speed (baud rate) of the port.
3. ***Parity*:** Select the parity error-checking method that is used.
4. ***Data Bits*:** Select the number of data bits.
5. ***Stop Bits*:** Select the number of stop bits.
6. Click on **OK**.

Figure 3-30 Instrument Communication Parameters Dialog Box



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Injectors

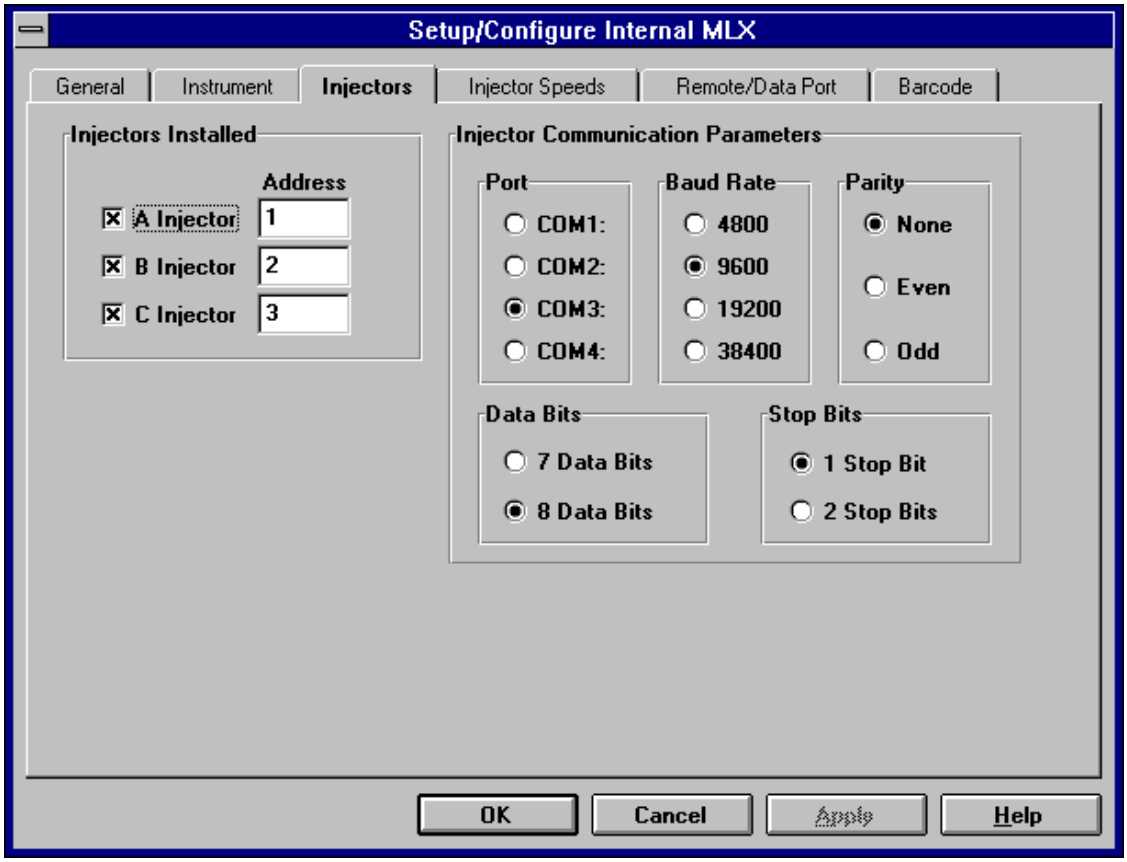
The Injectors dialog box is shown in Figure 3-31.

Warning: The settings shown below are default settings and should not be changed, except by qualified technical service personnel.

To change communication parameters for the injectors:

1. ***Injectors Installed*:** Specify the injectors that are installed and the communication address for each by placing an **X** in the appropriate check box opposite each installed injector. Then, type the communication address for that injector.
2. ***Port*:** Select the port for which the injector communication parameters are being established.
3. ***Baud Rate*:** Select the speed (baud rate) of the port.
4. ***Parity*:** Select the parity error-checking method that is used.
5. ***Data Bits*:** Select the number of data bits.
6. ***Stop Bits*:** Select the number of stop bits.
7. Click on **OK**.

Figure 3-31 Injectors Dialog Box



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Injector Speeds

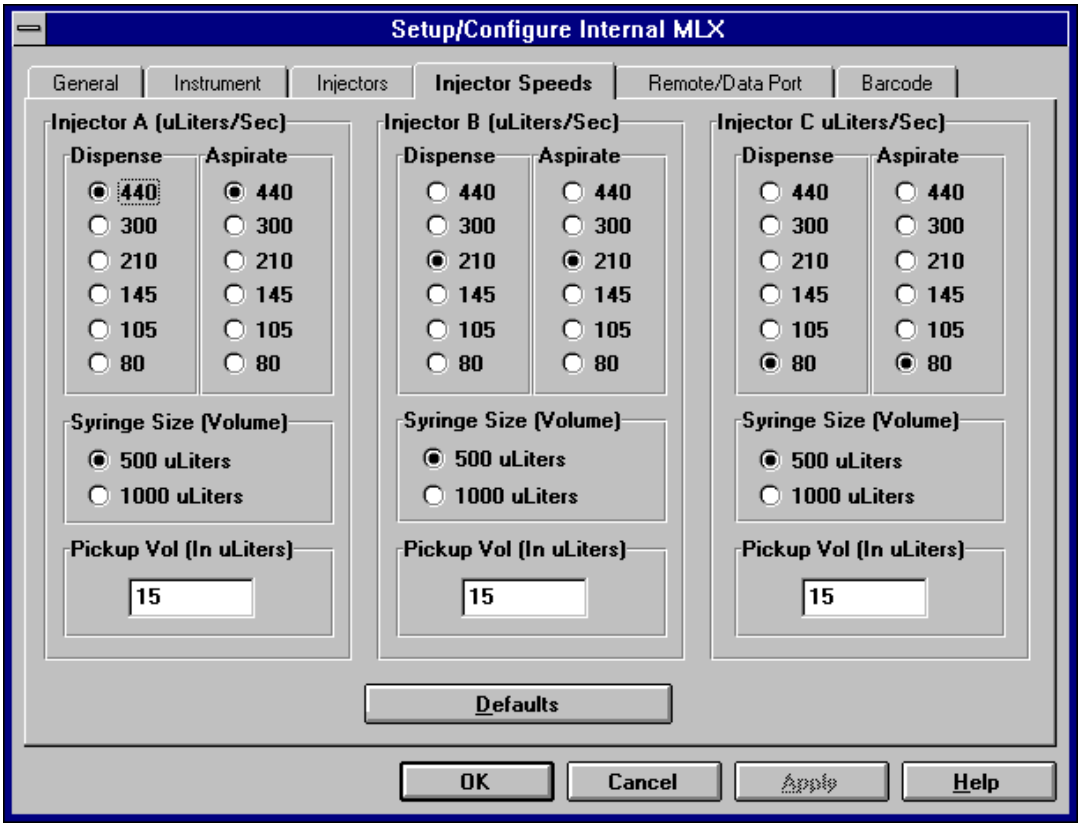
The Injector Speeds dialog box is shown in Figure 3-32.

To change injector speeds:

1. ***Injector A: Dispense*:** Select the rate (in µL/sec) at which liquid is to be dispensed from Injector A by clicking on the appropriate radio button.
2. ***Injector A: Aspirate*:** Select the rate (in µL/sec) at which liquid is to be aspirated from Injector A.
3. ***Injector A: Syringe Size*:** Select the size of the syringe that is connected to Injector A.
4. ***Injector A: Pickup Volume*:** Type the volume (in µL; range: 0 to 50 µL) of liquid that is withdrawn back into Injector A after dispensing, to prevent droplet formation on the injector tip.
5. Repeat Steps **1** through **4** for Injector B (if installed) and Injector C (if installed).
6. Click on **OK**.

Note: Figure 3-32 shows the default values for these settings. If you want to return to the default values for all of the injectors, click on **Defaults** before you click on **OK**.

Figure 3-32 Injector Speeds Dialog Box



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Remote/Data Port

The Remote/Data Port dialog box is shown in Figure 3-33.

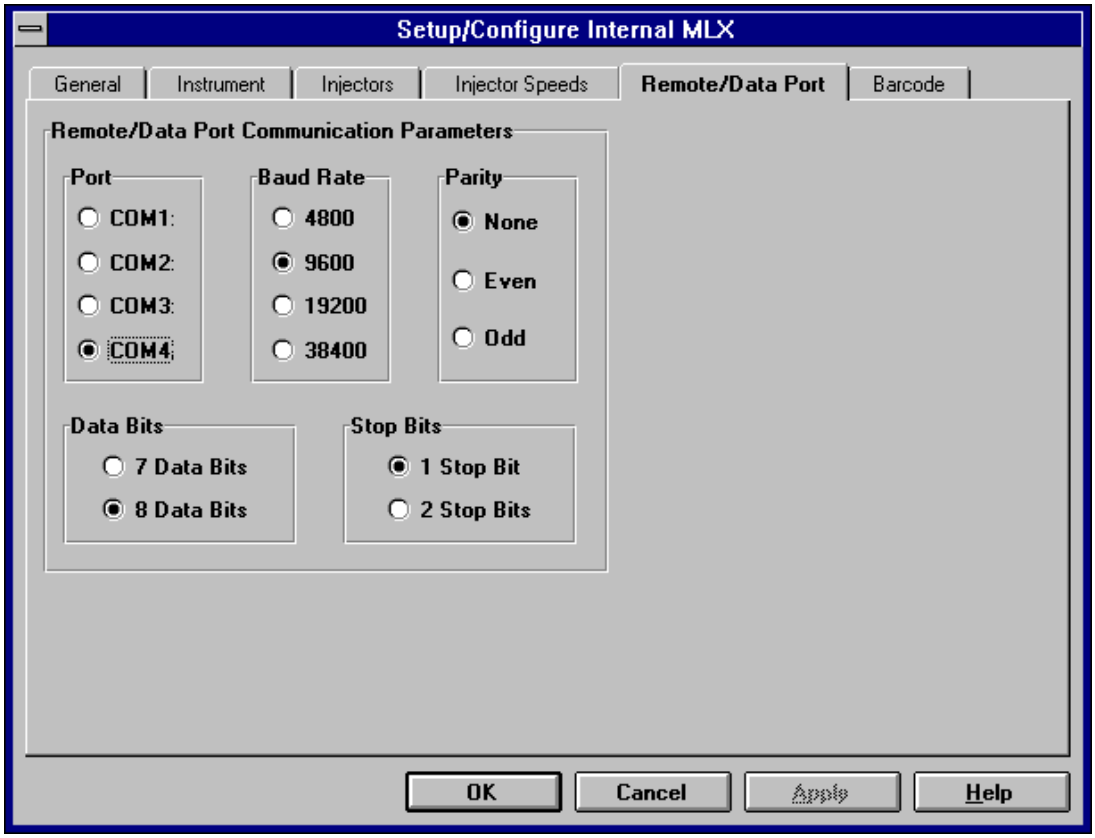
Note: The Remote/Data Port dialog box tab is displayed even if this option is not installed on the MLX system.

Warning: The settings shown below are default settings and should not be changed, except by qualified technical service personnel.

To change communication parameters for the remote/data port :

1. ***Port*:** Select the port for which the communication parameters are being established by clicking on the appropriate radio button.
2. ***Baud Rate*:** Select the speed (baud rate) of the port.
3. ***Parity*:** Select the parity error-checking method that is used.
4. ***Data Bits*:** Select the number of data bits.
5. ***Stop Bits*:** Select the number of stop bits.
6. Click on **OK**.

Figure 3-33 Remote/Data Port Dialog Box



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Barcode Reader

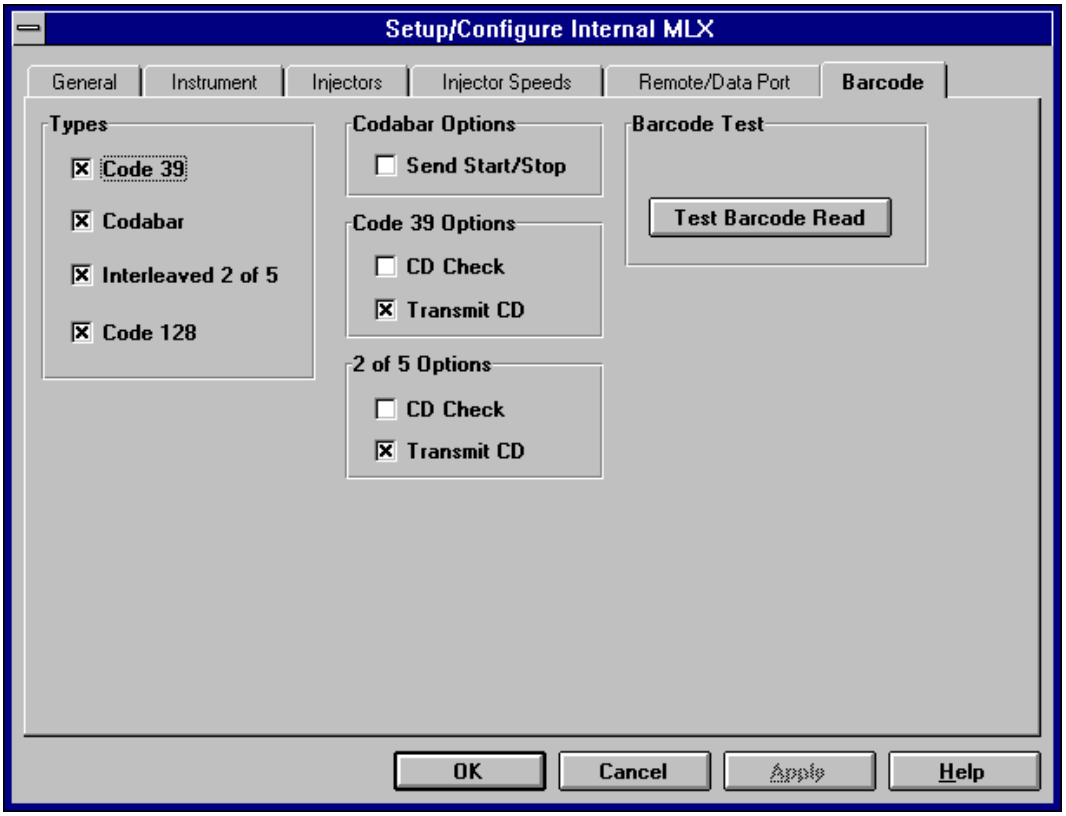
The Barcode Reader dialog box is shown in Figure 3-34.

Note: The Barcode Reader dialog box is available only if the barcode reader option is installed on the MLX system.

To specify barcode reader options:

1. ***Types*:** Specify the types of barcode labels that are to be read by placing an **X** in the appropriate check box opposite each barcode type. More than one box can be checked.
2. ***Codabar Options*:** Place an **X** in the **Send Start/Stop** check box if you want the Start and Stop characters to be read.
3. ***Code 39 Options*:** Place an **X** in the **CD Check** or the **Transmit CD** check box, depending on the option you want.
4. ***2 of 5 Options*:** Place an **X** in the **CD Check** or the **Transmit CD** check box, depending on the option you want.
5. ***Barcode Test*:** To verify the proper reading of a test barcode label, place a test plate in the MLX system and then click on **Test Barcode Read**.
6. Click on **OK**.

Figure 3-34 Barcode Reader Dialog Box



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Heater Control

Use this command to display the Master Temperature Control dialog box (Figure 3-35).

Generally, temperature control is specified within the assay by using the Reader Control command on the **Settings** menu. However, if you wish to enable heating to maintain the plate carrier at a specified temperature between runs or if you wish to override the assay settings, the Master Temperature Control dialog box allows you to do this.

To use the master temperature control:

1. Click on **Utility** and then click on **Heater Control**. The Master Temperature Control dialog box (Figure 3-35) will be displayed.
2. ***Enable Heating*:** If you want the plate carrier to be maintained between runs at the temperature entered in the **Set Temperature** box, place an **X** in the **Enable Heating** check box.

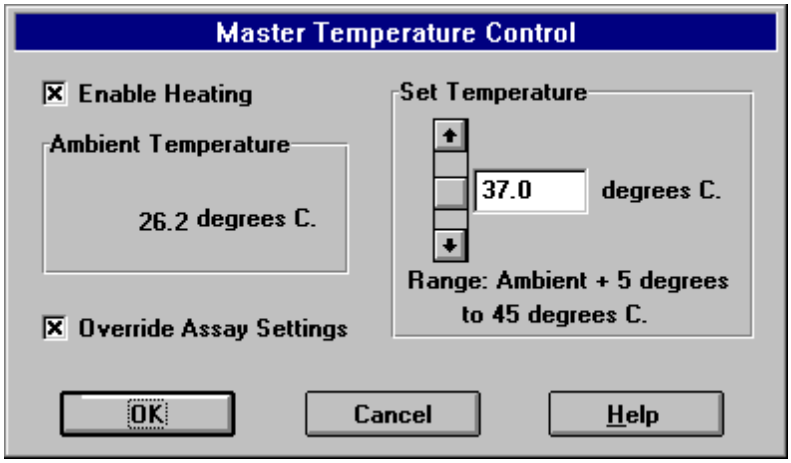
   Note: The **Ambient Temperature** field displays the current ambient temperature.

3. ***Set Temperature*:** Use the scroll bar to specify the temperature at which the plate carrier is to be maintained between runs (see above).
4. ***Override Assay Settings*:** Place an **X** in this check box to specify that the well temperature during an assay will be controlled by the master temperature control rather than the assay. The plate wells will be maintained at the Set Temperature rather than the temperature specified by the Assay Settings (see Chapter 5) whenever the Override Assay Settings box is checked

   Important: If **Override Assay Settings** is checked, temperature settings specified for an assay in the Settings menu are disabled. Therefore, if **Enable Heating** is not checked and **Override Assay Settings** is checked, temperature control specified for an assay will be **disabled**.

5. Click on **OK**.

Figure 3-35 Master Temperature Control Dialog Box



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Results Font

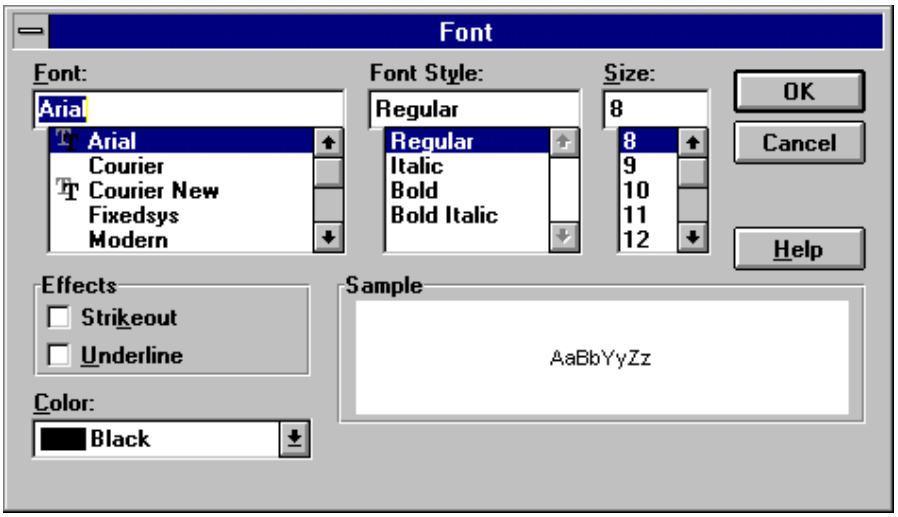
Use this command to display the Results Font dialog box (Figure 3-36). Then, you can select the font properties that will be used in all printed reports:

Property	Description
Font	The fonts installed on your system. For example: Arial or `Courier`.
Font Style	The style to be applied. For example: *Italic* or **Bold**.
Size	The font size. For example: 10pt, 12pt or 14pt.
Effects	Special effects: For example: ~~Strikeout~~ or Underline.
Color	Color of the font.

To select font properties:

1. Click on **Utility** and then click on **Results Font**. The Font dialog box (Figure 3-35) will be displayed.
2. ***Font*:** Select the font you want. Use the scroll bar to display additional fonts.
3. ***Font Style*:** Select the style you want. Use the scroll bar if needed.
4. ***Size*:** Select the font size you want. Use the scroll bar if needed.
5. ***Effects*:** If you want either the strikeout or underline effect, place an **x** in the appropriate check box.
6. ***Color*:** Select the font color you want. Use the drop-down arrow to display the available color choices.
7. Click on **OK**.

Figure 3-36 Font Dialog Box



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Options

Use this command to display the five Options tabs:

Tab	Purpose
Buttons	Create, label, and position Quick Operation buttons. See page 61 for more information.
Preferences	Specify whether results are to be suppressed if QC fails, whether a Plate ID is to be automatically assigned, whether lot-specific data are entered at run time, and other preferences. See page 63 for more information.
Colours	Select the colors used to represent well types on the template display and in the results display. See page 65 for more information.
Directories	Display the current sub-directory structure of the Revelation directory showing you where all the different Revelation file types will be placed. See page 66 for more information.
Lab Information	Enter your laboratory name, address, and phone numbers so that this information will appear on report headers. See page 67 for more information.

Instructions for using each dialog box are contained in the following sections.

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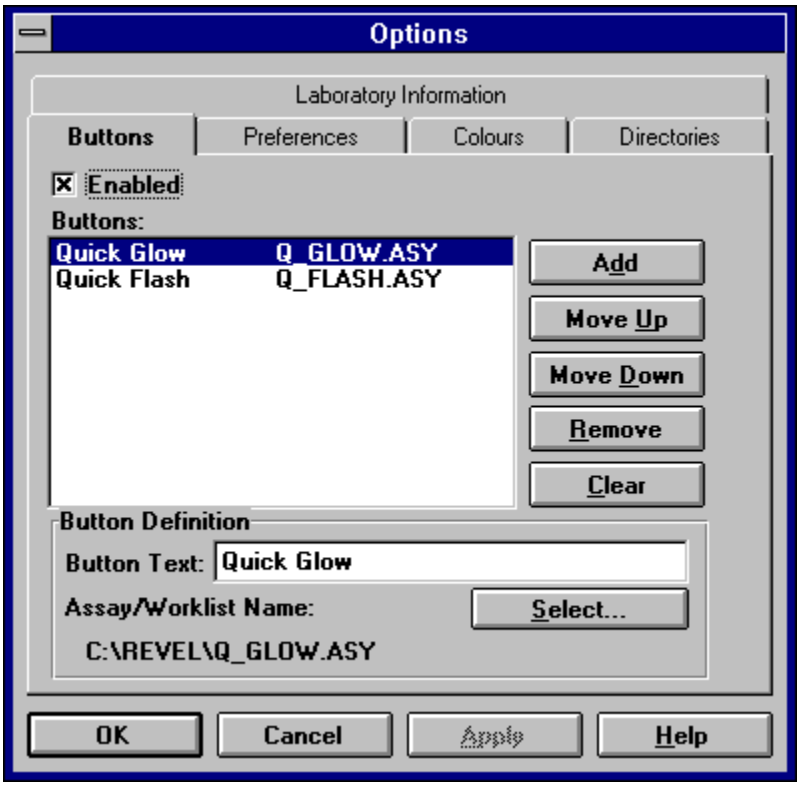
Buttons

Use the Buttons tab (Figure 3-37) to create, label, and position Quick Operation buttons.

To create a Quick Operation button:

1. Click on **Utility** and then click on **Options**.
2. Click on the **Buttons** tab. The Buttons dialog box (Figure 3-37) will be displayed.
3. Place an **X** in the **Enabled** check box, if an **X** is not already present.
4. Click on **Add**.
5. Type a name for the Quick Operation button label in the **Button Text:** box.
6. Click on **Select…** to assign an assay or worklist file to the new button. The Assay/Worklist dialog box will be displayed.
7. Select the file name of the assay or worklist file from the file list box, then click on **OK**. The file name will be displayed next to the button label in the **Buttons:** box.
8. Click on **OK**.

Figure 3-37 Buttons Dialog Box



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To reposition a Quick Operation button:

1. Display the Buttons dialog box (see above). The buttons are listed in the order (left to right) that they are displayed on the Revelation window (Figure 3-3).
2. Click on the button name in the **Buttons:** window and then click on **Move Up** or **Move Down** to move the button name up or down in the listing. The position of the button in the Revelation window will change accordingly.
3. Click on **OK**.

To remove a Quick Operation button:

1. Display the Buttons dialog box (see above).
2. Click on the button name in the **Buttons:** window and then click on **Remove** to remove the button from the listing.
3. Click on **OK**.

To remove all of the Quick Operation buttons:

1. Display the Buttons dialog box (see above).
2. Click on **Clear** to remove all of the buttons from the listing.
3. Click on **OK**.

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Preferences

Use the Preferences tab (Figure 3-38) to select assay preferences.

To select preferences:

1. Click on **Utility** and then click on **Options**.
2. Click on the **Preferences** tab. The Preferences dialog box (Figure 3-38) will be displayed.
3. ***Run Plate Defaults*:**

   Place an **X** in the **Auto save enabled** check box if you want the results from a run to be automatically saved under the plate ID. Otherwise, Revelation will prompt you whether you want the data saved.

   Place an **X** in the **Auto print enabled** check box if you want a dialog box to be automatically displayed prompting for the results of a run to be printed.

   Place an **X** in the **Search for Sample IDs enabled** check box if you want Revelation to search for a Sample ID file and use the sample IDs from that file instead of the template well labels (T1, T2, etc.).

   Note: If Search for Sample IDs is enabled, Revelation will search for a file with the same name as the Plate ID but with an *.sid* extension. If it finds such a file, it will use those Sample IDs for the plate. Otherwise, an error message will be displayed indicating that the file could not be found.

   Place an **X** in the **Auto barcode read enabled** check box if you want the plate barcode to be automatically read by Revelation when the plate is run.

   Place an **X** in the **Ask for lot specific data** check box if you want data that you have entered for the reagent kit to be retrieved when the assay is run.

   Place an **X** in the **Multiple assays** check box if you want more than one assay to be run per plate.

   Place an **X** in the **Orientation Lock** check box if you want replicates for all well types to be filled in the same direction. Then, select the direction in which you want replicates filled (**Columns only** or **Rows only**).

4. ***Default Plate ID*:**

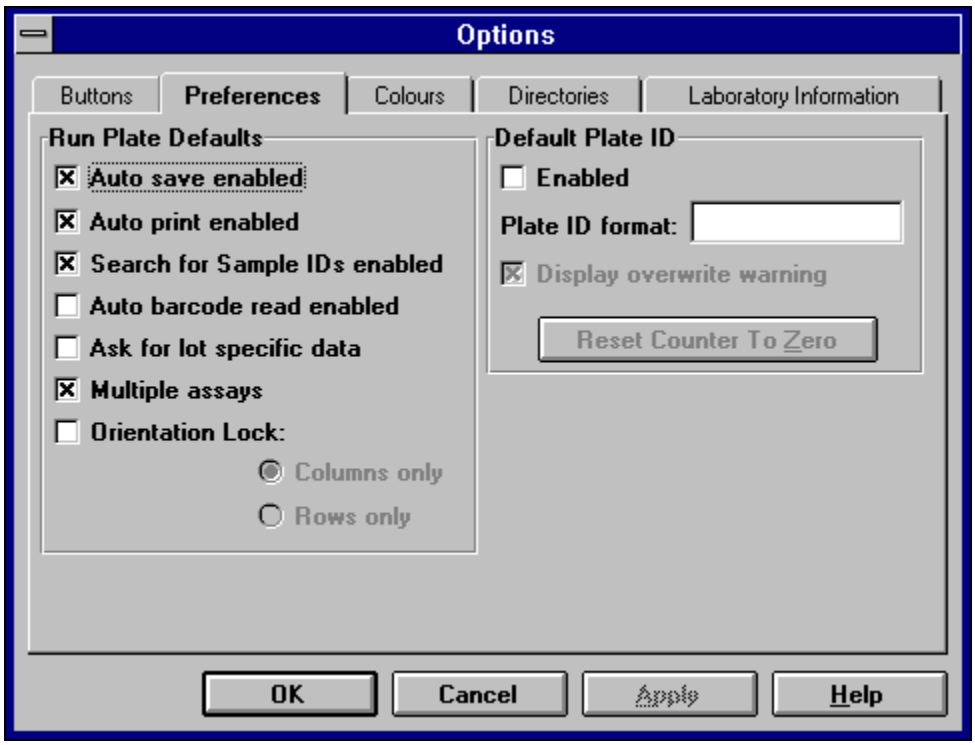
   Place an **X** in the **Enabled** check box if you want an ascending numerical plate ID of up to eight characters to be automatically assigned by Revelation.

   The numerical plate ID can be part of an eight-character alphanumeric ID that you define. For example, you may want the plate IDs to read *run-0001*, *run-0002*, *run-0003*, etc. Type the exact plate ID format that you want in the **Plate ID format:** box. Then, type the numerical format (for example, `0000`)in the **Plate ID format:** box.

   Note: The plate ID must not exceed 8 characters.

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Figure 3-38 Preferences Dialog Box



Place an **x** in the **Display overwrite warning** check box if you want Revelation to warn you if the same plate ID is assigned a second time.

Click on **Reset Counter to Zero** if you want to reset the numeric portion of the plate ID to zero.

> **Example:** If you want plate IDs to read *run-0000*, *run-0001*, *run-0002*, etc., place an **x** in the **Enabled** check box and type `run-0000` in the **Plate ID format:** text box.

5. Click on **OK**.

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Colours

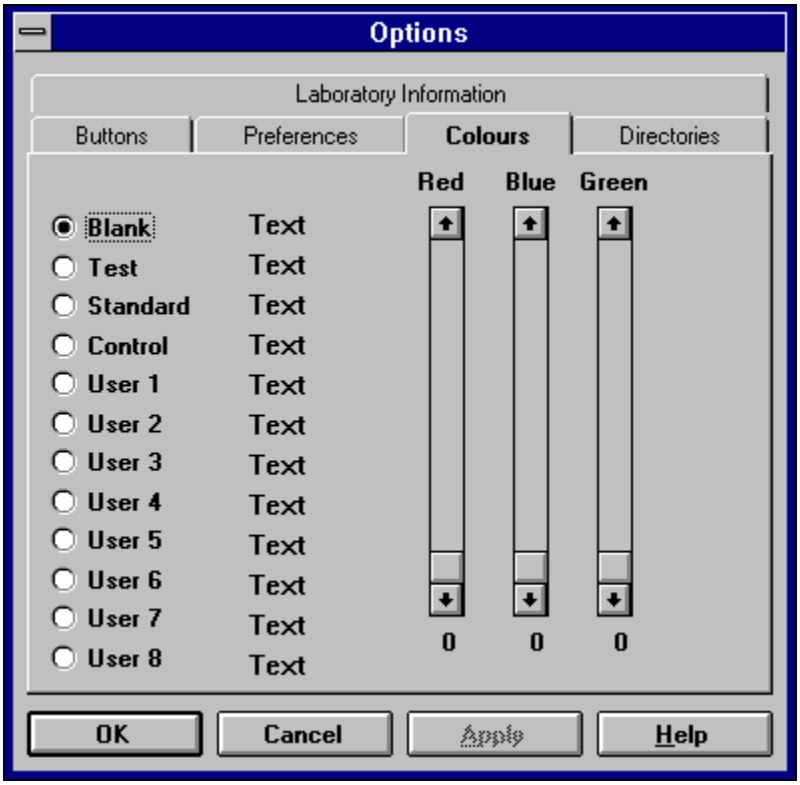
Use the Colours tab (Figure 3-39) to select colours to represent different well types on the template and in the results reports.

Note: Windows normally has use of a limited number of colours, so it will pick the closest colour to the one you have created. If you are using a black and white printer, all colours will be printed as black.

To select colours:

1. Click on **Utility** and then click on **Options**.
2. Click on the **Colours** tab. The Colours dialog box (Figure 3-39) will be displayed.
3. Click on the radio button of the first well type. Then, drag the scroll handles of each colour bar to increase or decrease the red, green and blue components of the colour. The colour you create will be shown in the word "Text" adjacent to that selected well type.
4. Repeat Step **3** for each of the well types that you want to assign colours.
5. Click on **OK**.

Figure 3-39 Colours Dialog Box



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Directories

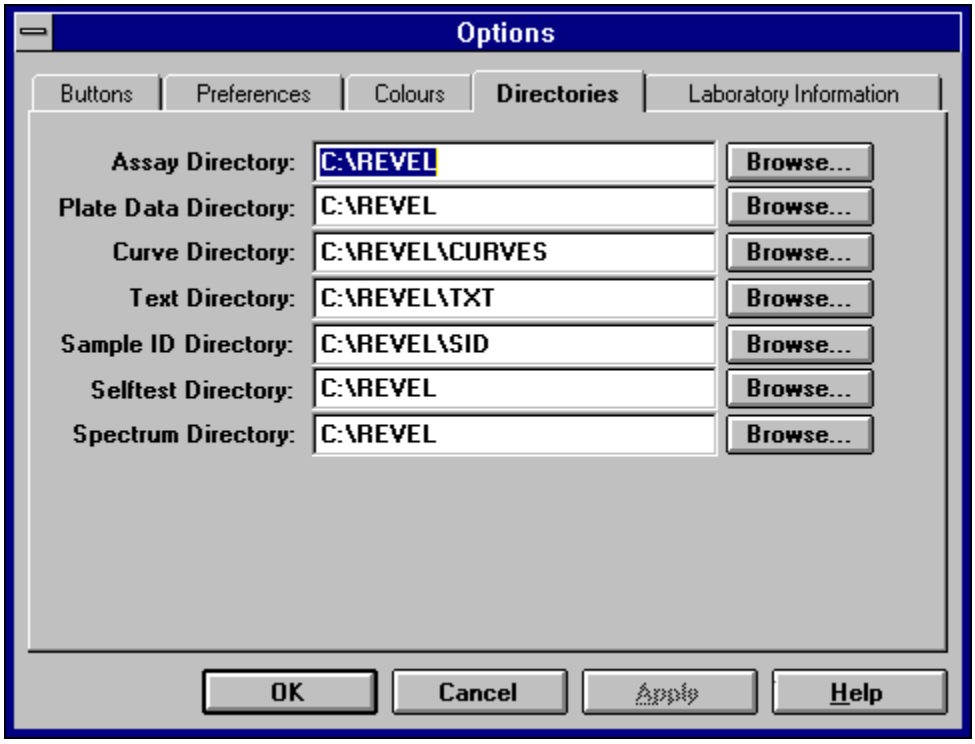
Use the Directories tab (Figure 3-40) to display the sub-directories where the various Revelation file types will be placed.

To display the directories:

1. Click on **Utility** and then click on **Options**.
2. Click on the **Directories** tab. The Directories dialog box (Figure 3-40) will be displayed.
3. If necessary, use the browse buttons to display a particular directory.
4. Click on **OK** when you are finished viewing the directories.

Note: If you choose a directory location which does not exist, you will get a warning message. You can create specific directory locations using the File Manger in Windows® (see your Windows® documentation for additional information).

Figure 3-40 Directories Dialog Box



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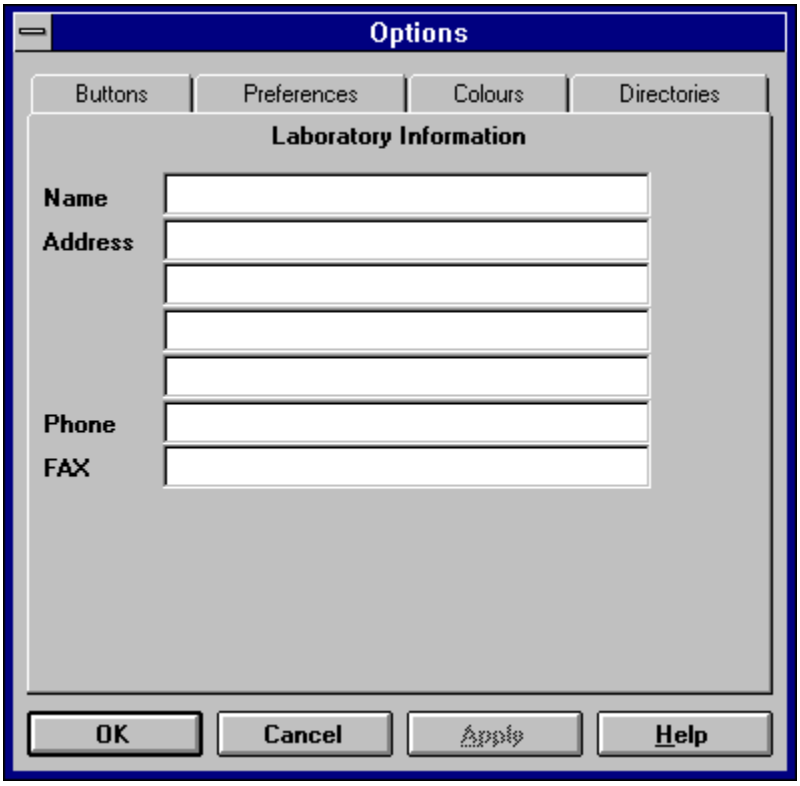
Laboratory Information

Use the Laboratory Information tab (Figure 3-41) to enter the name, address, telephone and fax number of your laboratory. This information will then be printed on the header of each report.

To enter laboratory information:

1. Click on **Utility** and then click on **Options**.
2. Click on the **Laboratory Information** tab. The Laboratory Information dialog box (Figure 3-41) will be displayed.
3. Type the information describing your laboratory.
4. Click on **OK**.

Figure 3-41 Laboratory Information Dialog Box



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Changing the System Password

A system password is used to restrict access to certain dialog boxes and to programs used by service engineers. To assign a new password, follow the procedures described below.

To change the system password:

1. Click on **Utility** and then click on **System Password**. The System Password dialog box (Figure 3-42) will be displayed.
2. Type the current password in the **Enter Password** box. Then, click on **OK**. The New System Password dialog box (Figure 3-43) will be displayed.

> **Note:** If you enter an incorrect password, the system will not respond. If this occurs, delete the password entry (shown as asterisks) by backspacing or highlighting. Then, retype the password.

3. Type the new password in the **Enter Password** box. Re-type the new password in the **Retype Password** box.
4. Click on **OK**.

> **Note:** The default password is **DYNEX**.

Figure 3-42 System Password Dialog Box

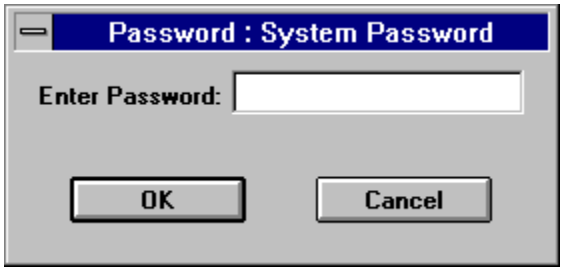


Figure 3-43 New System Password Dialog Box



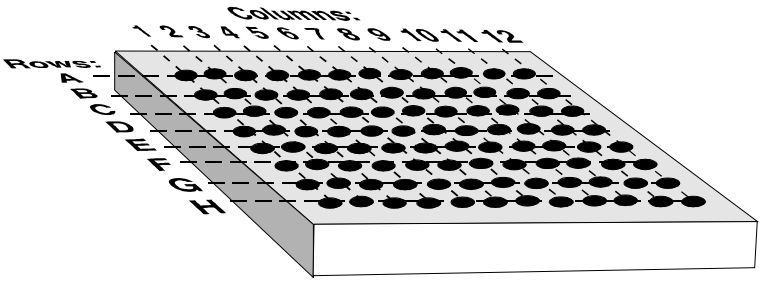
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Naming of Wells

Labeling of Wells on the Plate

The Microplates that you use with the MLX Luminometer contain 96 wells. The wells are arranged in **columns** and **rows**. On the plate, the rows are named **A** though **H** and the columns are named **1** through **12** (Figure 3-44).

Figure 3-44 Naming of Plate Rows and Columns



Well Types

When you define an assay on the MLX, you will assign a particular **well type** to each well that you use on the plate. The well type refers to the purpose of that well in that assay. For example, if you are using nine wells to contain three replicates of three different standards, each of those nine wells will be designated with a well type of **S** (or other symbol that you assign for a standard).

At the time you create an assay, Revelation provides the following default well types:

Well Type	Symbol	Description
Blank	B	Wells used for a blank. Generally, one or two blank wells may be run per plate to measure background luminescence.
Standard	S	Wells that contain a Standard. The Standards are used to calibrate a quantitative assay.
Control	C	Control samples that are used to verify the performance of an assay.
Test	T	All unknown samples.

However, you can define an assay to use different symbols (up to three characters for each symbol) than those shown for these well types. In addition, you can define and name up to eight additional well types.

Note: The procedure for changing the symbol for a well type or for adding new well types is described in the “Template” section of Chapter 4.

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Well Identification

Since different wells of the same well type will probably contain different materials, each well you use must be further identified. This is done using the Template command during Assay Definition (see *Template* on page 99). By default, Revelation identifies wells by sequentially numbering each well type that contains an aliquot of a particular sample.

For example, if you are using nine wells to contain three replicates of three different standards, the three wells containing replicates of the first standard will each be named **S1**, the three wells containing replicates of the second standard will each be named **S2**, and the three wells containing replicates of the third standard will each be named **S3**.

In some dialog boxes, the wells will be identified as

S1 S1 S1
S2 S2 S2
S3 S3 S3
etc.

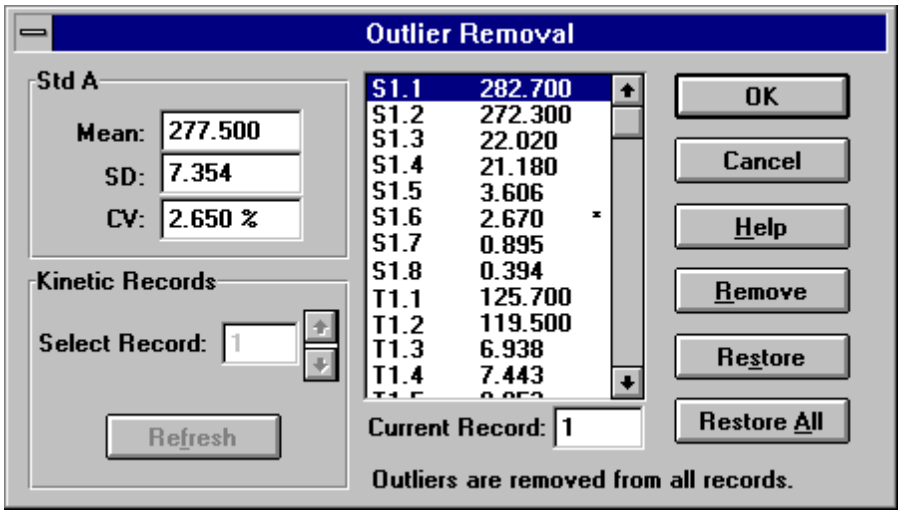
In other dialog boxes (Figure 3-45), each replicate well will be identified as

S1.1 S1.2 S1.3
S2.1 S2.2 S2.2
S3.2 S3.2 S3.3
etc.

If you don't want the wells for a particular well type to be labeled as **1**, **2**, **3**, etc., you can access the Template dialog box (page 100) and click on **Change Label.** Then, you can define new labels such as **A**, **B**, **C**, etc. If there are more than 26 different samples of the well type, the subsequent samples will be labeled **AA**, **BB**, **CC**, etc.

Finally, you can instruct Revelation to search for the Sample ID that corresponds to a particular well identification (page 63). Then, the results report can contain the results listed by Sample ID rather than by well ID.

Figure 3-45 Identification of Replicates



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Assigning Well Types

During definition of the assay, you will use the Template command (page 98) to do the following:

- Assign a well type to each of the wells you use on the plate. An example is shown in Figure 3-46.
- Change the default naming of a well type. Create and name new well types.
- Specify the number of replicates (the number of wells used per sample) for each well type.
- Specify whether replicates are filled in rows (left to right) or in columns (top to bottom).
- Specify whether samples are filled in rows (left to right) or in columns (top to bottom).

Detailed instructions about creating the assay template and defining the assay are provided in Chapter 4.

Figure 3-46 A Typical Template

B1	S1	S2	S3	S4	S5	S6	C1	C2	C3	T1	T2
T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14
T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26
T27	T28	T29	T30	T31	T32	T33	T34	T35	T36	T37	T38
T39	T40	T41	T42	T43	T44	T45	T46	T47	T48	T49	T50
T51	T52	T53	T54	T55	T56	T57	T58	T59	T60	T61	T62
T63	T64	T65	T66	T67	T68	T69	T70	T71	T72	T73	T74
T75	T76	T77	T78	T79	T80	T81	T82	T83	T84	T85	B2

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4 Defining an Assay

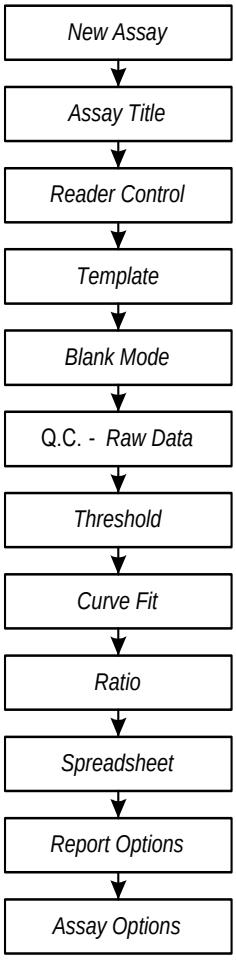
Overview

Once you have configured your MLX, you are ready to define an assay. The detailed procedure for doing this depends upon whether you are defining a glow-endpoint assay, a kinetic assay, a flash assay, or a dual flash assay.

Note: Not all the steps shown are required for a particular assay. For example, if you are defining a qualitative assay, the *Curve Fit* step is not needed.

Since the choices that are available to you in a particular step depend upon the selection you made in a previous step, the steps should be carried out in the sequence shown.

Figure 4-1 Assay Definition Overview



Specify the type of assay (glow-endpoint, kinetic, flash, or dual flash) that you are creating.

Enter the title, author, code and password of the assay.

Specify luminometer parameters such as temperature, shake, gain level, and dispensing options.

Define the positions of the well type samples on the plate. The well type definition includes location, number of replicates, etc.

Select a pre-defined blank mode or define a custom blank mode.

Define the quality control criteria that you want to use to verify that the raw data are acceptable.

(For qualitative or semi- quantitative assays.) Define the threshold values that separate negative, equivocal, and positive samples.

(For quantitative assays.) Define the type of standard curve, the standards used, calculation procedure, and results QC procedure.

(If you want results to be scaled or expressed as a ratio of another value.) Define the ratio equation and the output format.

(If you want to carry out calculations using the results from particular wells.) Define each equation.

Specify the layout of the results report, the data that will be included, and report exporting options.

Specify the order in which calculations should be performed, the summary statistical calculations that should performed, and the well locations for which the Sample ID should be prompted.

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The procedure to define an assay is the same, regardless of whether you are creating a new assay or editing an existing assay.

Creating a New Assay

To create a new assay:

1. Click on **File** and then click on **New Assay**. The Luminometer Assay Type dialog box (Figure 4-2) will be displayed.

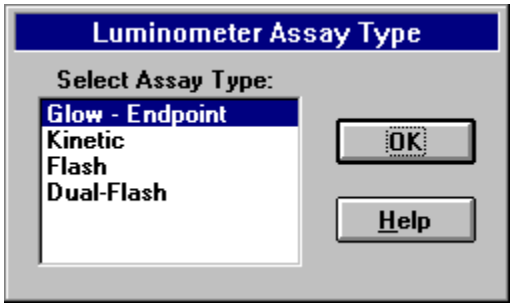
   NOTE: New Assay allows you to create a new assay by editing the default values of a blank assay. **Open** allows you to edit an existing assay.

2. Click on **Glow - Endpoint**, **Kinetic**, **Flash**, or **Dual Flash** to select the type of assay you wish to create. For details on assay types, see Chapter 1. The Assay summary window will be displayed.

   NOTE: The Assay summary window displays all of the assay parameters that have been defined during the assay creation process. As you enter new information, it will be displayed in the Assay summary window.

3. Define the assay using the commands on the Settings menu. The procedure for using each Settings menu command is detailed in the remainder of this chapter.
4. Click on **File** and then click on **Save** to save the assay that you have created.

Figure 4-2 Luminometer Assay Type Dialog Box



Modifying an Existing Assay

To modify an existing assay:

1. Click on **File** and then click on **Open**. The Open dialog box will be displayed.
2. Select the assay file that you want to modify. Then, click on **OK**.
3. If you are prompted for the assay password, enter the password.
4. Modify the assay using the commands and dialog boxes available on the Settings menu. Each Settings menu command is described in the following sections.
5. Click on **File** and then click on **Save** to overwrite the original assay with the modified definition. Or, use **Save As** if you do not want to overwrite the original assay. The redefined assay will be saved under the new name.

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Assay Title

To name the assay:

1. Click on **Settings** and then click on **Assay Title**. The Assay Title dialog box (Figure 4-3) will be displayed.
2. ***Enter Assay Title*:** Type the title (up to 26 characters) that you want to assign to the assay. The title may consist of any characters you can enter using the keyboard.

   NOTE: The title is not the filename under which you save the assay. The filename is entered using the **Save** or **Save As** commands on the **File** menu.

3. ***Written by*:** Type your name, initials or code that identifies you as the author of the assay.
4. ***Enter Password*:** Type a password (up to eight characters) for the assay. The password may consist of any characters you can enter using the keyboard.
5. ***Retype Password*:** Re-type the password to verify that you have typed the password correctly.

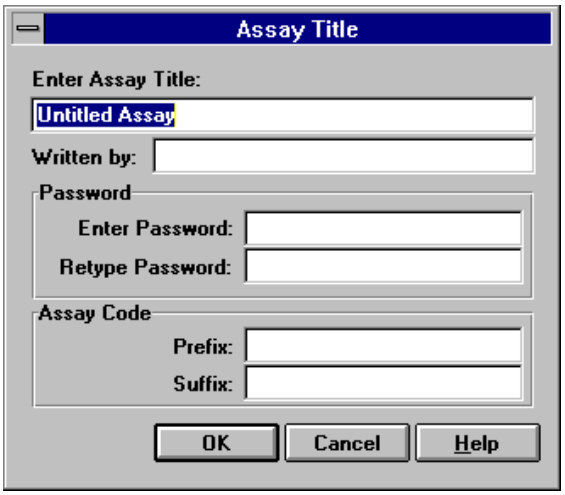
   NOTE: Steps **3** and **4** are optional. If you enter a password, be sure to record it. Although you can view the assay in the future without entering the password, you will not be able to modify it.

6. ***Prefix*:** Type the prefix (up to eight characters) for the assay. The prefix may consist of any characters you can enter using the keyboard.
7. ***Suffix*:** Type the suffix (up to eight characters) for the assay. The suffix may consist of any characters you can enter using the keyboard.

   NOTE: Use the Prefix and/or the Suffix to verify that the appropriate assay is run on a particular plate or group of plates. When a plate is run, Revelation will check the start of the barcode/plate ID against the prefix or the end of the barcode/plate ID against the suffix. If the prefix or suffix is not correct, Revelation will display a warning message.

8. Click on **OK**.

Figure 4-3 Assay Title Dialog Box



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Reader Control: Glow-Endpoint Assays

To specify Reader Control settings for glow-endpoint assays:

1. Click on **Settings** and then click on **Reader Control**. Four tabbed Luminometer Control dialog boxes will be displayed. Select the appropriate tab as outlined below:

Tab	Purpose
Start Mode	Determine when to read the plate relative to the start of the test. The test starts when you click on **Start** in the Run Plate dialog box. See "Start Mode *(Glow-Endpoint)*" on page 77.
Shake	Enable plate shaking and specify the frequency, amplitude, and duration of the shake motion. See "Shake *(Glow-Endpoint)*" on page 78.
Temperature	Enable the heater plate and specify the temperature you wish to maintain. See "Temperature *(Glow-Endpoint)*" on page 79.
Reader Options	Specify gain levels, scaling factors, raw data handling options, and read time per well. See "Reader Options *(Glow-Endpoint)*" on page 80.

2. Instructions for using each dialog box are contained in the following sections.

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Start Mode *(Glow-Endpoint)*

The Start Mode dialog box (Figure 4-4) allows you to specify when Revelation takes the first reading relative to the start of the test.

To specify the start mode:

1. ***Start Mode:*** If you want the first reading to be taken when the test starts, select **Immediate**.

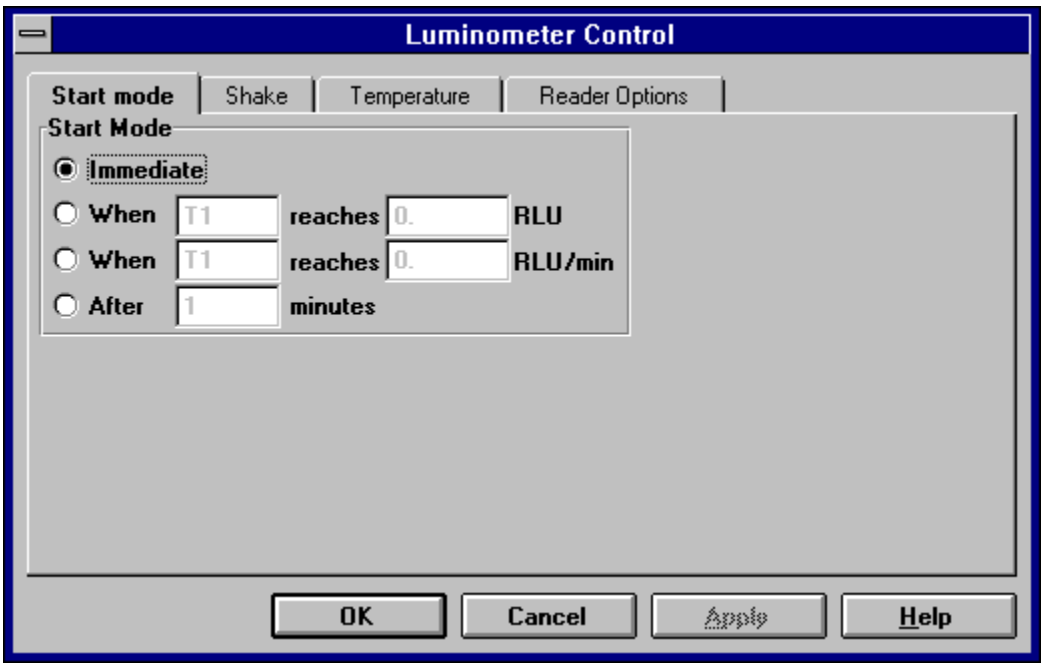
   If you want the first reading to be taken when a specified sample reaches the specified RLU value, select **When…reaches…RLU**. Then, type the well ID of the sample and the RLU value.

   If you want the first reading to be taken when the specified sample reaches the specified RLU/min value, select **When…reaches…RLU/min**. Then, type the well ID of the sample and the RLU/min value.

   If you want the first reading to be taken a specified time after the test starts, select **After…minutes**. Then, type the time delay.

2. Click on **OK**.

Figure 4-4 Start Mode Dialog Box (Glow-Endpoint Assay)



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Shake *(Glow-Endpoint)*

For glow-endpoint assays, the Shake dialog box (Figure 4-5) allows you to specify shaking of the plate once just prior to reading of the plate.

To specify the shake parameters:

1. ***Enable Shaking:*** If you want the plate to be shaken once just prior to reading of the plate, place an **X** in the **Enable Shaking** box.

2. ***Shake Parameters*:** Select the shake intensity (**Low**, **Medium**, or **High**) by clicking on the appropriate radio button.

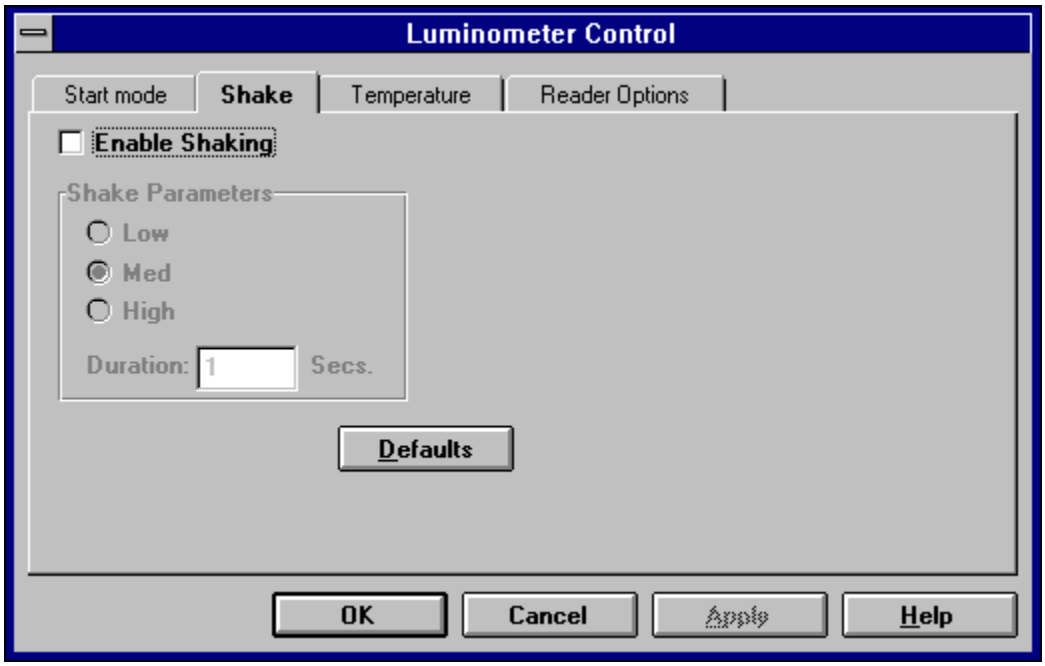
   Then, type the duration of shaking (from **1** to **10** seconds).

   NOTE: The **Shake Parameters** and **Duration** fields are inactive if shaking is not enabled in Step **1**.

3. ***Defaults*:** If you prefer, specify the default shake parameters by clicking on **Defaults**.

4. Click on **OK**.

Figure 4-5 Shake Dialog Box (Glow-Endpoint Assay)



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Temperature *(Glow-Endpoint)*

The Temperature dialog box (Figure 4-6) allows you to enable the heater plate and to specify the temperature you wish to maintain during the assay.

To specify the temperature parameters:

1. ***Enable Heater Plate:*** If you want the heater plate to be enabled during the assay, place an **X** in the **Enable Heater Plate** box.

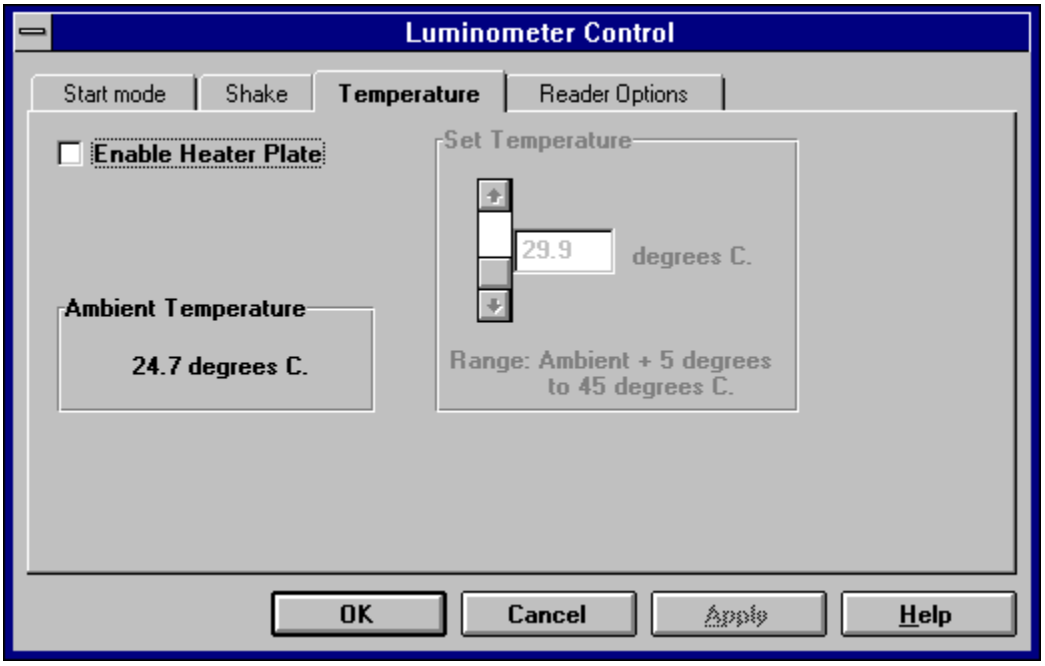
   NOTE: The **Ambient Temperature** field displays the ambient temperature.

2. ***Set Temperature:*** Use the temperature scroll bar to specify the temperature at which the plate is to be maintained during the assay.

   NOTE: You can set the temperature to any value between **5 °C above the ambient temperature** and **45 °C**.
   The **Set Temperature** field is inactive if heating is not enabled in Step **1**.

3. Click on **OK**.

Figure 4-6 Temperature Dialog Box (Glow-Endpoint Assay)



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Reader Options *(Glow-Endpoint)*

The Reader Options dialog box (Figure 4-7) allows you to specify gain levels, scaling factors, raw data handling options, and read time per well for the assay.

To specify reader options:

1. ***Gain Level:*** To match the intensity of emitted light to the full scale range of the detector, select the appropriate gain level (in RLU).

   Or, if you prefer, select **Auto Gain**.

2. ***Scaling Factor:*** Select the factor (from 1 to 10,000) that results will be multiplied by.

3. ***Raw Data Handling:*** Select between **Average all Readings** and **Sum all Readings**.

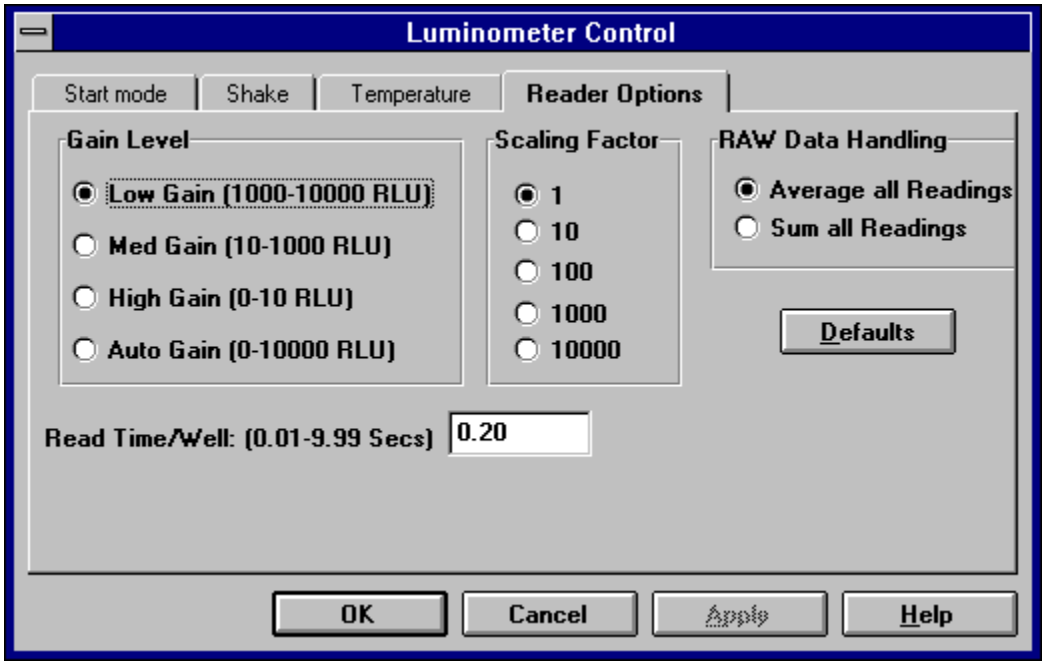
4. ***Read Time/Well:*** Enter the time (in seconds) of each well reading. You can type any value between **0.01** and **9.99** seconds.

   NOTE: If you enter a value of **1.01** seconds or greater, data will be displayed in tabular form while the plate is being read.

5. ***Defaults*:** If you prefer, specify the default read parameters by clicking on **Defaults**.

6. Click on **OK**.

Figure 4-7 Reader Options Dialog Box (Glow-Endpoint Assay)



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Reader Control: Kinetic Assays

To specify Reader Control settings for kinetic assays:

1. Click on **Settings** and then click on **Reader Control**. Eight tabbed Luminometer Control dialog boxes will be displayed. Select the appropriate tab as outlined below:

Tab	Purpose
Calculation Mode	Select a Calculation Mode, specify which readings to include, choose when to subtract blank values, and select a particular well to monitor during the reading process. See "Calculation Mode *(Kinetic)* " on page 82.
Kinetic Time Interval	Specify a fixed or variable time interval between readings, and the length of time between fixed readings (if "fixed" is selected). See "Kinetic Time Interval *(Kinetic)*" on page 88.
Kinetic Graphs	Specify the wells or combination of wells that will be included in the Kinetic Graphs that are contained in the results report. See "Kinetic Graphs *(Kinetic)*" on page 89.
Start/Stop Mode	Specify when the first and last readings are taken relative to the start of the test. The test starts when you click on **Start** in the Run Plate dialog box. For further information, see "Start/Stop Mode *(Kinetic)*" on page 83.
Shake	Enable plate shaking and specify the frequency, amplitude, and duration of the shake motion. See "Shake *(Kinetic)*" on page 85.
Temperature	Enable the heater plate and specify the temperature you wish to maintain. See "Temperature *(Kinetic)*" on page 86.
Reader Options	Specify gain levels, scaling factors, raw data handling options, and read time per well. For further information, see "Reader Options *(Kinetic)*" on page 87.
Dispensing Options	Specify the volume of liquid to be dispensed by each dispenser and the timing of the dispense. For further information, see "Dispensing Options *(Kinetic)*" on page 90.

2. Instructions for using each dialog box are contained in the following sections.

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Calculation Mode *(Kinetic)*

The Calculation Mode dialog box (Figure 4-8) allows you to select a calculation mode, specify which readings to use, choose when to subtract blank values, and select a particular well to monitor during the reading process.

To specify the calculation mode:

1. ***Calculation Mode:*** The Calculation Modes that you can choose from are described below. Select the Calculation Mode you want by clicking on the appropriate radio button.

Calculation Mode	Description
RLU/min (total)	Total rate of change (in RLU/min) over the period in which measurements will be taken.
RLU/min (Vmax)	The maximum rate of change (in RLU/min) that is to be measured.
RLU/min (Linear Regression)	Rate of change (in RLU/min) over the period in which measurements are taken, calculated using linear regression of the readings.
Time to Vmax	Time for the RLU/min reading to reach the maximum rate.
Time to defined RLU/min	Time for the RLU/min reading to reach a specified RLU/min value.

2. ***Moving Average:*** Type the number of individual readings that should be used to calculate the Vmax measurement.

3. ***Use Readings:*** If you want to perform calculations over the entire range of readings, select **All**. If you want to exclude readings at the beginning and/or the end of the test from the calculation, select **Range** and type the first and last reading that is to be included.

   NOTE: The calculation will be performed on the range of readings you specify. However, all readings will be included in the Kinetic Graphs.

4. ***Blank Subtraction:*** Select whether the blank value(s) should be subtracted before (**Intrarecord**) or after (**Post Kinetic Calculations**) the calculation has been performed.

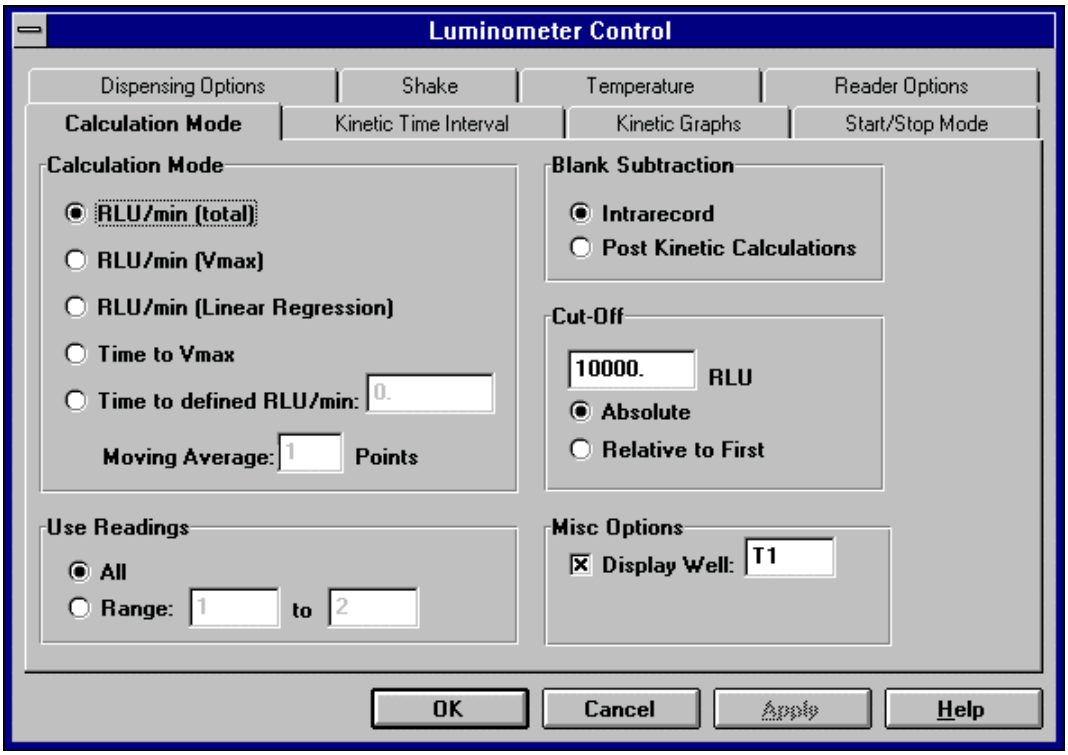
5. ***Cut-Off:*** If you want to specify an RLU value above which data will not be processed, type the RLU value in the **RLU** box. Then, select **Absolute** if the value you entered is the absolute RLU limit or **Relative to First** if the limit is relative to the first RLU reading.

6. ***Misc Options:*** If you want to display readings for a particular well as the assay is run, place an **x** in the **Display Well:** box. Then, type the well ID for which readings will be displayed.

7. Click on **OK**.

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Figure 4-8 Calculation Mode Dialog Box (Kinetic Assay)



Start/Stop Mode *(Kinetic)*

The Start/Stop Mode dialog box (Figure 4-9) allows you to determine when Revelation takes the first and last readings relative to the start of the test. The test starts when you click on **Start** in the Run Plate dialog box.

To specify the start/stop mode:

1. ***Start Mode:*** If you want the first reading to be taken when the test starts, select **Immediate**.

   If you want the first reading to be taken when the specified sample reaches the specified RLU value, select **When…reaches…RLU**. Then, type the sample and the RLU value.

   If you want the first reading to be taken when the specified sample reaches the specified RLU/min value, select **When…reaches…RLU/min**. Then, type the sample and the RLU/min value.

   If you want the first reading to be taken a specified time after the test starts, select **After…minutes** radio button. Then, type the time delay.

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2. ***Stop Mode:*** If you want Revelation to stop reading after a specified number of readings, select **After…Reading(s)**. Then, type the number of readings.

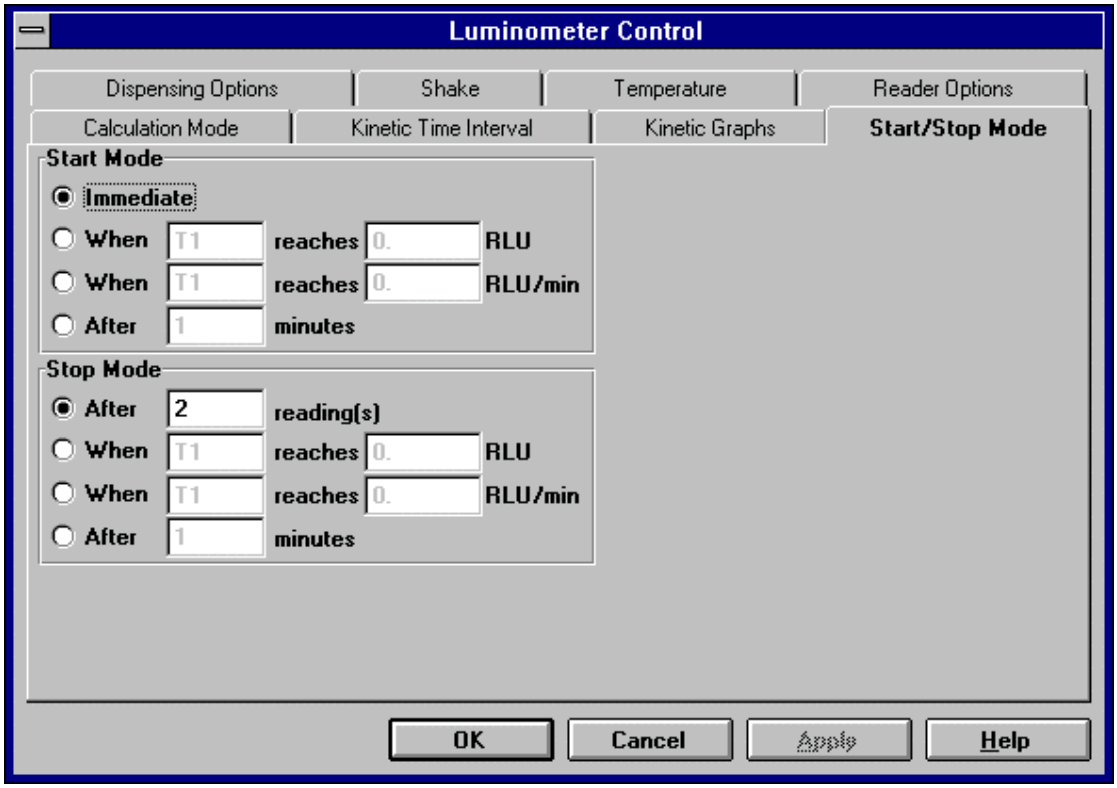
   If you want the system to stop reading when the specified sample reaches the specified RLU value, select **When…reaches…RLU**. Then, type the sample and the RLU value.

   If you want the system to stop reading when the specified sample reaches the specified RLU/min value, select **When…reaches…RLU/min**. Then, type the sample and the RLU/min value.

   If you want the system to stop reading a specified time after the test starts, select **After…minutes**. Then, type the time.

3. Click on **OK**.

Figure 4-9 Start/Stop Mode Dialog Box (Kinetic Assay)



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Shake (Kinetic)

For a kinetic assay, the Shake dialog box (Figure 4-10) allows you to specify shaking of the plate whenever reagent is dispensed from any of the three injectors.

To specify the shake parameters:

1. ***Enable Shaking:*** If you want the plate to be shaken whenever reagent is dispensed from any of the dispensers, place an **x** in the **Enable Shaking** box.

2. ***Shake Parameters*:** Select the shake intensity (**Low**, **Medium**, or **High**) by clicking on the appropriate radio button.

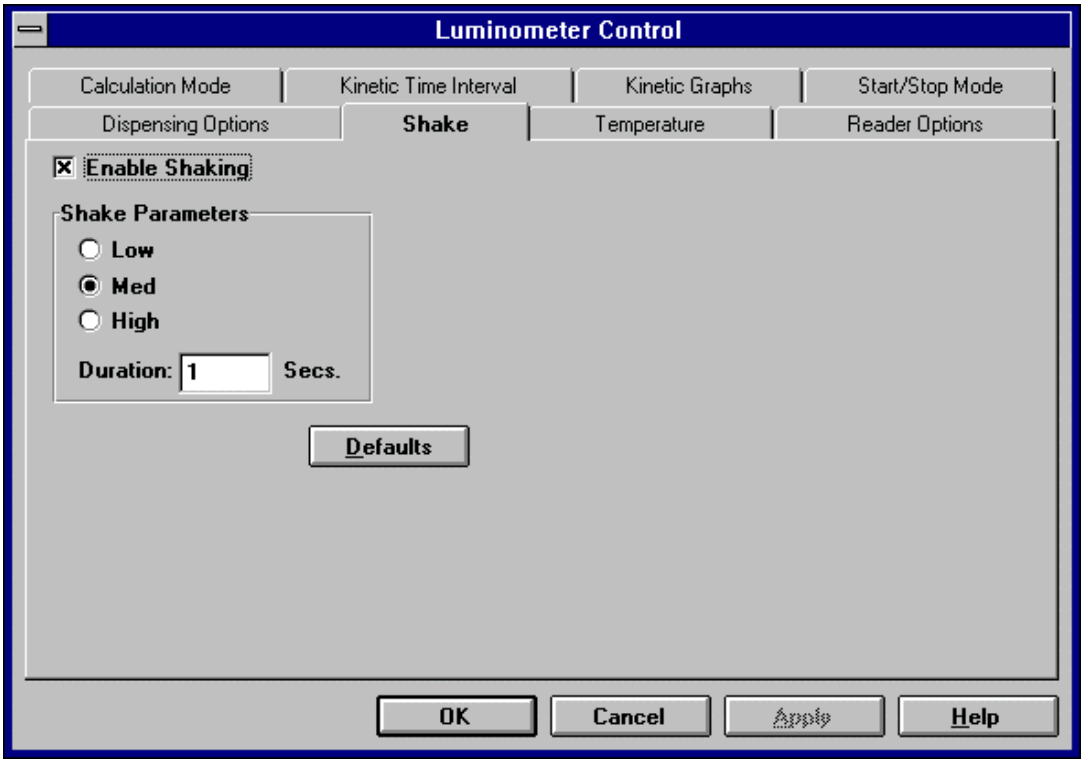
   Then, type the duration of shaking (from **1** to **10** seconds).

   NOTE: The **Shake Parameters** and **Duration** fields are inactive if shaking is not enabled in Step **1**.

3. ***Defaults*:** If you prefer, specify the default shake parameters by clicking on **Default**.

4. Click on **OK**.

Figure 4-10 Shake Dialog Box (Kinetic Assay)



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Temperature (Kinetic)

The Temperature dialog box (Figure 4-11) allows you to enable the heater plate and to specify the temperature you wish to maintain during the assay.

To specify the temperature parameters:

1. ***Enable Heater Plate:*** If you want the heater plate to be enabled during the assay, place an **X** in the **Enable Heater Plate** box.

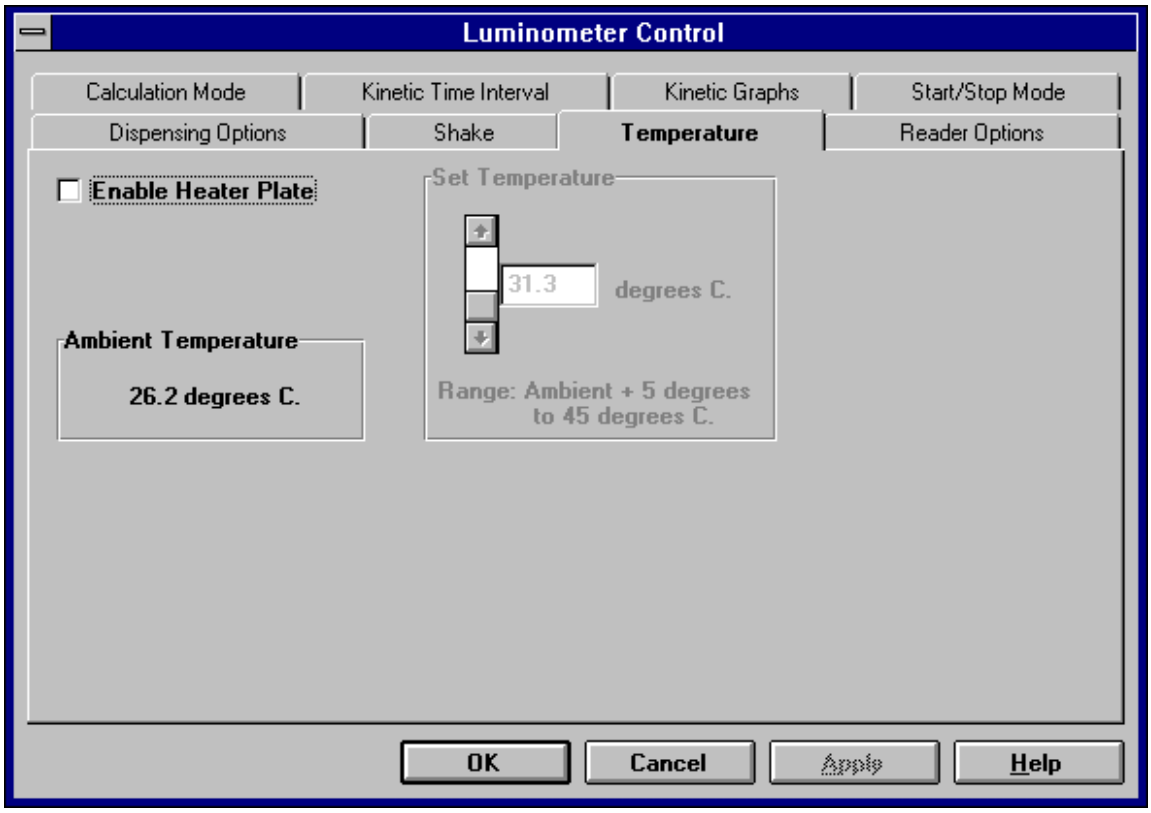
   NOTE: The **Ambient Temperature** field displays the ambient temperature.

2. ***Set Temperature:*** Use the temperature scroll bar to specify the temperature at which the plate is to be maintained during the assay.

   NOTE: You can set the temperature to any value between **5 °C above the ambient temperature** and **45 °C**.
   The **Set Temperature** field is inactive if heating is not enabled in Step **1**.

3. Click on **OK**.

Figure 4-11 Temperature Dialog Box (Kinetic Assay)



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Reader Options *(Kinetic)*

The Reader Options dialog box (Figure 4-12) allows you to specify gain levels, scaling factors, raw data handling options, and read time per well for the assay.

To specify reader options:

1. ***Gain Level:*** To match the intensity of emitted light to the full scale range of the detector, select the gain level (in RLU) by clicking on the appropriate radio button.

   If you prefer, select the **Auto Gain** radio button.

2. ***Scaling Factor:*** Select the factor (from 1 to 10,000) that results will be multiplied by.

3. ***Raw Data Handling:*** Select between **Average all Readings** and **Sum all Readings**.

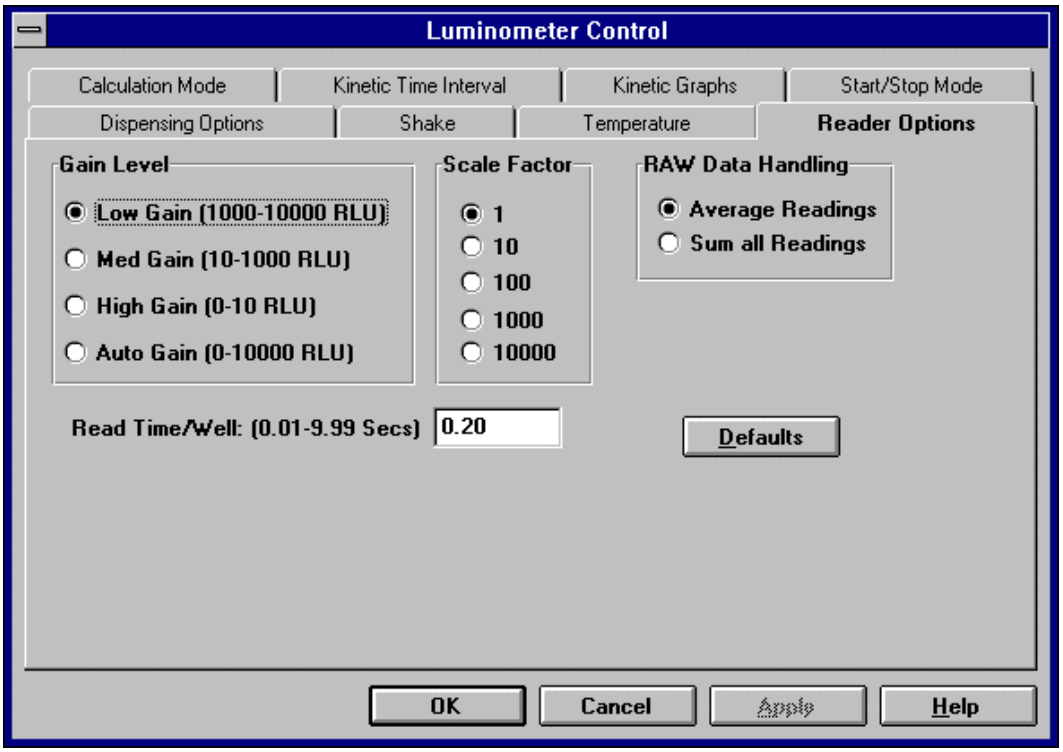
4. ***Read Time/Well:*** Type the number of seconds for which each well should be read.

   NOTE: You can enter any value between **0.01** and **9.99** seconds.

5. ***Defaults*****:** If you prefer, specify the default read parameters by clicking on **Defaults**.

6. Click on **OK**.

Figure 4-12 Reader Options Dialog Box (Kinetic Assay)



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Kinetic Time Interval *(Kinetic)*

The Kinetic Time Interval dialog box (Figure 4-13) will be activated only if you specified that readings stop after a specified number of readings (see Start/Stop Mode on page 84).

To specify kinetic time interval:

1. ***Time Interval:*** Select **Variable** (a different time between each reading) or **Fixed** (the same time between all readings) by clicking on the appropriate radio button.
2. ***Fixed Time Interval:*** Type the time (in seconds) between each reading.

   NOTE: This field is inactive if you selected **Variable** in Step **1**.

3. ***Variable Time Interval:*** This field displays the time (as **hours:min.sec**) at which each reading is taken. The first reading is always taken at **00** hours, **00** minutes, and **00** seconds after start of the assay. If the second reading is taken at **00** hours, **01** minutes, and **00** seconds after start of the assay, this will be displayed as **00:01.00**.

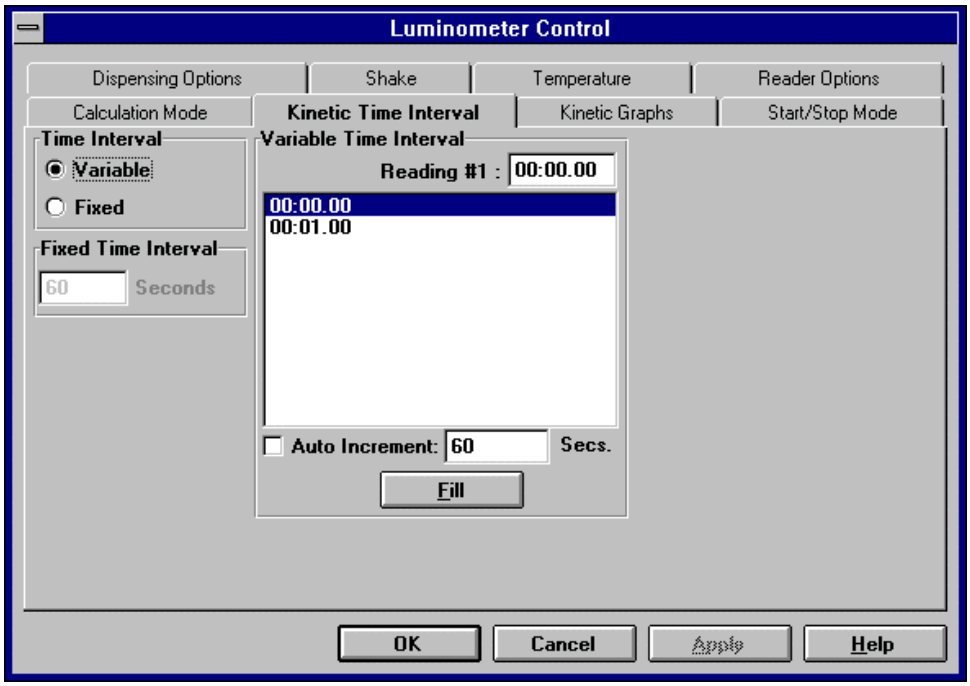
   NOTE: This field is inactive if you selected **Fixed** in Step **1**.

   To change the time at which a particular reading is taken, click on that reading time. The reading time you selected will be displayed in the box above. Then, edit the reading in the box by highlighting and retyping the numerals you want to change. Repeat the procedure for any other readings you want to edit.

   To add a fixed time to each of the current reading times, place an **X** in the **Auto Increment** box and type the time (in seconds) that you want to add to each reading. Then, click on **Fill**. The displayed readings will be updated.

4. Click on **OK**.

Figure 4-13 Kinetic Time Interval Dialog Box (Kinetic Assay)



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Kinetic Graphs *(Kinetic)*

The Kinetic Graphs dialog box (Figure 4-14) allows you to designate wells to be included in the Kinetic Graphs portion of the results report and to specify whether results from designated wells are shown together in a single plot or shown in separate plots.

To designate wells to be plotted:

1. ***Mode:*** Use **Location** to designate individual wells on the basis of their location on the plate. Use **Replicate** to automatically select all wells having the same sample type when any one of the wells is selected.

   NOTE: Both modes can be used. For example, all of the positive controls can be selected by using the **Replicate** mode and then clicking on just one of the positive control well locations on the diagram. Then, specific wells can be selected by using the **Location** mode and then clicking on each of the additional wells to be included.

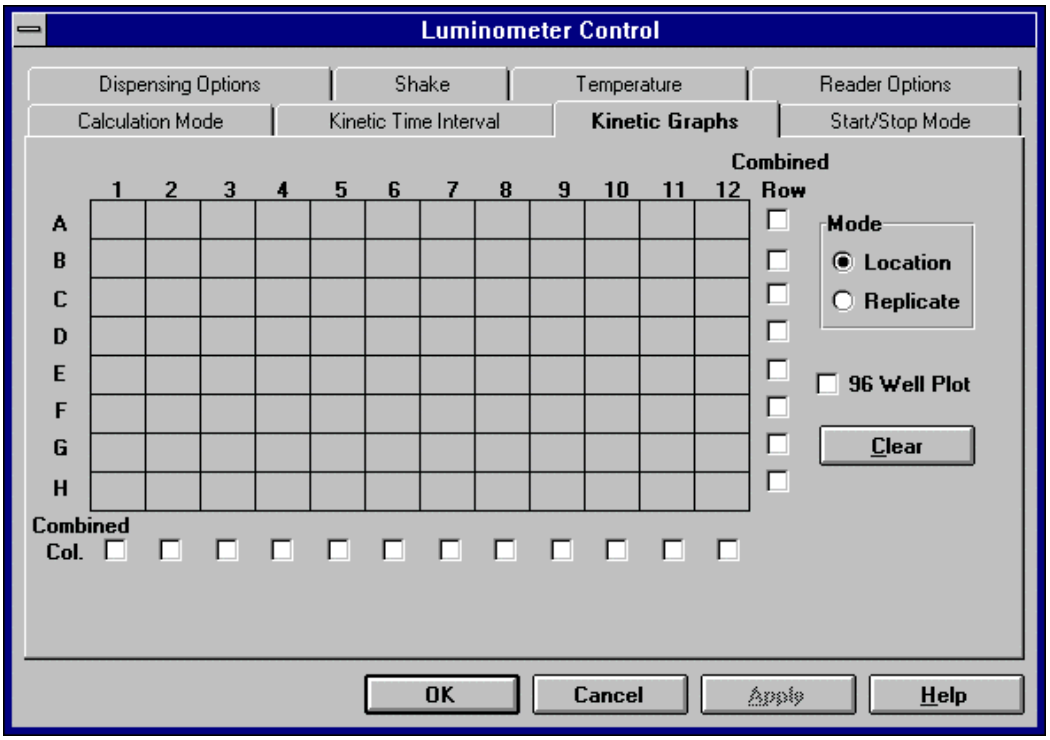
2. ***Well Selection:*** To display the results from an individual well on a separate plot, create a check mark for that well by clicking in the corresponding section of the plate diagram. To display the results from all of the wells in a column or a row in a single plot, place an **X** in the corresponding **Combined Col.** or **Combined Row** check box.

   To display the results from all 96 wells on separate plots, place an **X** in the **96 Well Plot** check box.

   To deselect all of the wells, click on **Clear**.

3. Click on **OK**.

Figure 4-14 Kinetic Graphs Dialog Box (Kinetic Assay)



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Dispensing Options *(Kinetic)*

Reading of the plate for a kinetic assay occurs at least twice. Each reading of the plate is referred to as a **cycle**. If reagent is to be dispensed during a kinetic assay, the volume of reagent that is dispensed and the reading cycle after which reagent is dispensed are specified. Any of the dispensers can be used during a kinetic assay.

Note: To dispense a reagent before any kinetic readings, set the cycle to zero. Setting the dispenser volume to zero disables dispensing for that dispenser.

For example, if dispensing of 50 microliters of reagent from Dispenser A is specified to occur after the fifth reading cycle and the reading intervals are two minutes, reagent will be dispensed into each well at the end of the fifth cycle and the two minute timing will begin.

Refer to page 99 for additional details concerning use of the dispensers.

The Dispensing Options dialog box (Figure 4-15) for kinetic assays allows you to specify the **Volume** of reagent dispensed and the **Cycle** after which reagent is dispensed.

To specify dispensing options:

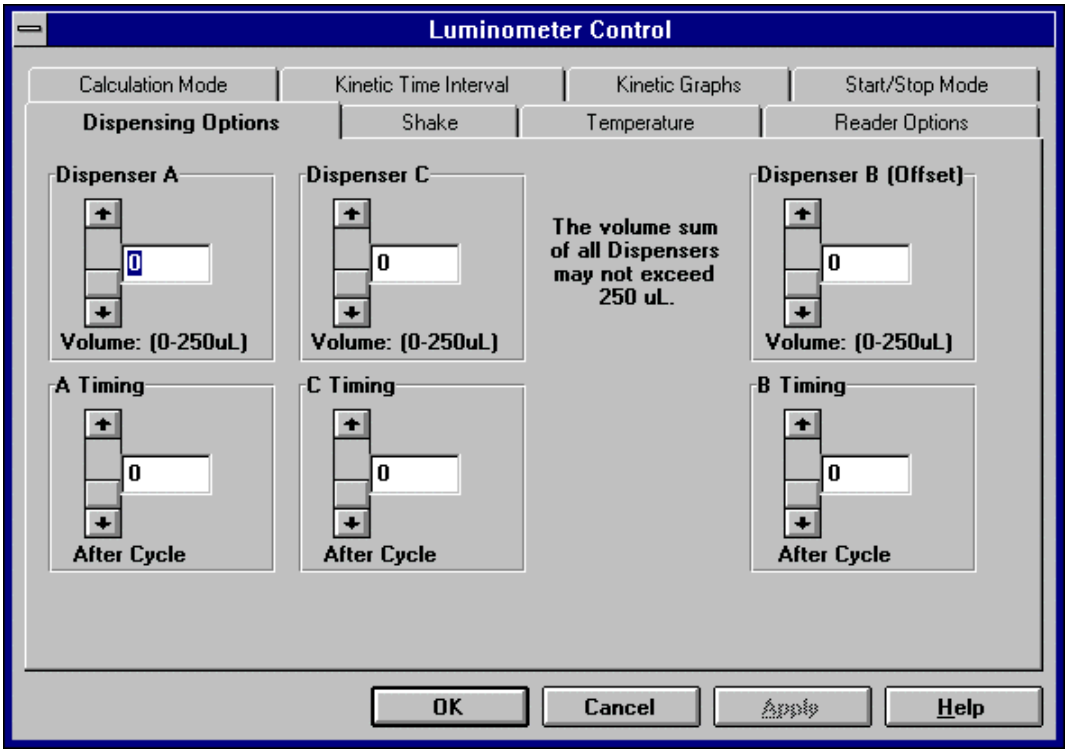
1. ***Dispenser A:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser A.
2. ***A Timing:*** Use the scroll bar to specify the kinetic reading cycle after which reagent in Dispenser A is dispensed and data collected.
3. ***Dispenser C:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser C.
4. ***C Timing:*** Use the scroll bar to specify the kinetic reading cycle after which reagent in Dispenser C is dispensed and data collected.
5. ***Dispenser B (Offset):*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser B.
6. ***B Timing:*** Use the scroll bar to specify the kinetic reading cycle after which reagent in Dispenser B is dispensed and data collected.

   NOTE: The total volume dispensed by the three dispensers may not exceed 250 µL.

7. Click on **OK**.

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Figure 4-15 Dispensing Options Dialog Box (Kinetic Assay)



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Reader Control: Flash Assays

To specify Reader Control settings for flash assays:

1. Click on **Settings** and then click on **Reader Control**. Eight tabbed Luminometer Control dialog boxes will be displayed. Select the appropriate tab as outlined below:

Tab	Purpose
Calculation Mode	Select a Calculation Mode, specify the Total Read Time, the Start and Stop Integration Times, and the Before and After Peak Times. See "Calculation Mode *(Flash)*" on page 93.
Start Mode	Specify when the first flash readings are taken relative to the start of the test. The test starts when you click on **Start** in the Run Plate dialog box. For further information, see "Start Mode *(Flash)*" on page 95.
Dispensing Options	Specify the volume of liquid to be dispensed by each dispenser and the time delays that are required between dispenses. See "Dispensing Options *(Flash)*" on page 99.
Report Data	Select the data to be included in the result reports. See "Report Data *(Flash)*" on page 103.
Data Graphs	Specify whether the plotted readings for each well are displayed after each well is read and the wells or combination of wells that are to be included in the graphs that are part of the results report. See "Data Graphs *(Flash)*" on page 105.
Shake	Enable plate shaking and specify the frequency, amplitude, and duration of the shake motion. See "Shake *(Flash)*" on page 96.
Temperature	Enable the heater plate and specify the temperature you wish to maintain. See "Temperature *(Flash)*" on page 97.
Reader Options	Specify gain levels and scaling factors. See "Reader Options *(Flash)*" on page 98.

2. Instructions for using each dialog box are contained in the following sections.

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Calculation Mode *(Flash)*

The Calculation Mode dialog box (Figure 4-16) allows you to select a Calculation Mode and specify the Total Read Time, the Start and Stop Integration Times, and the Before and After Peak Times. Refer to page 12 in Chapter 1 for definition of these terms.

To specify the calculation mode:

1. ***Calculation Mode:*** The Calculation Modes that you can choose from are described below. Select the Calculation Mode you want by clicking on the appropriate radio button.

Calculation Mode	Description
Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Integrate Window.
Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Integrate Window.
Peak during Delay (in RLU)	Value of the maximum reading recorded prior to the Start Integration Time.
Time of Peak during Delay (in Secs)	Time that the maximum reading was recorded prior to the Start Integration Time.
Peak during Integrate (in RLU)	Value of the maximum reading recorded during the specified Integrate Window.
Time of Peak during Integrate (in Secs)	Time that the maximum reading was recorded during the specified Integrate Window.
Before Peak Integral (in RLU)	Sum of the values (in RLU) of all the readings recorded during the specified Before Peak Window.
After Peak Integral (in RLU)	Sum of the values (in RLU) of all the readings recorded during the specified After Peak Window.

2. ***Before/After Peak Times:*** Type a value for **Before Peak Window**. Then, type a value for **After Peak Window**.

   NOTE: The sum of **Before Peak Window** and **After Peak Window** may not exceed the **smaller** of 20 seconds or the Integrate Window Time.

3. ***Read Time/Integration Times:*** Use the scrollbar to set the **Total Read Time**. The Total Read Time above the scrollbar and on the diagram changes accordingly.

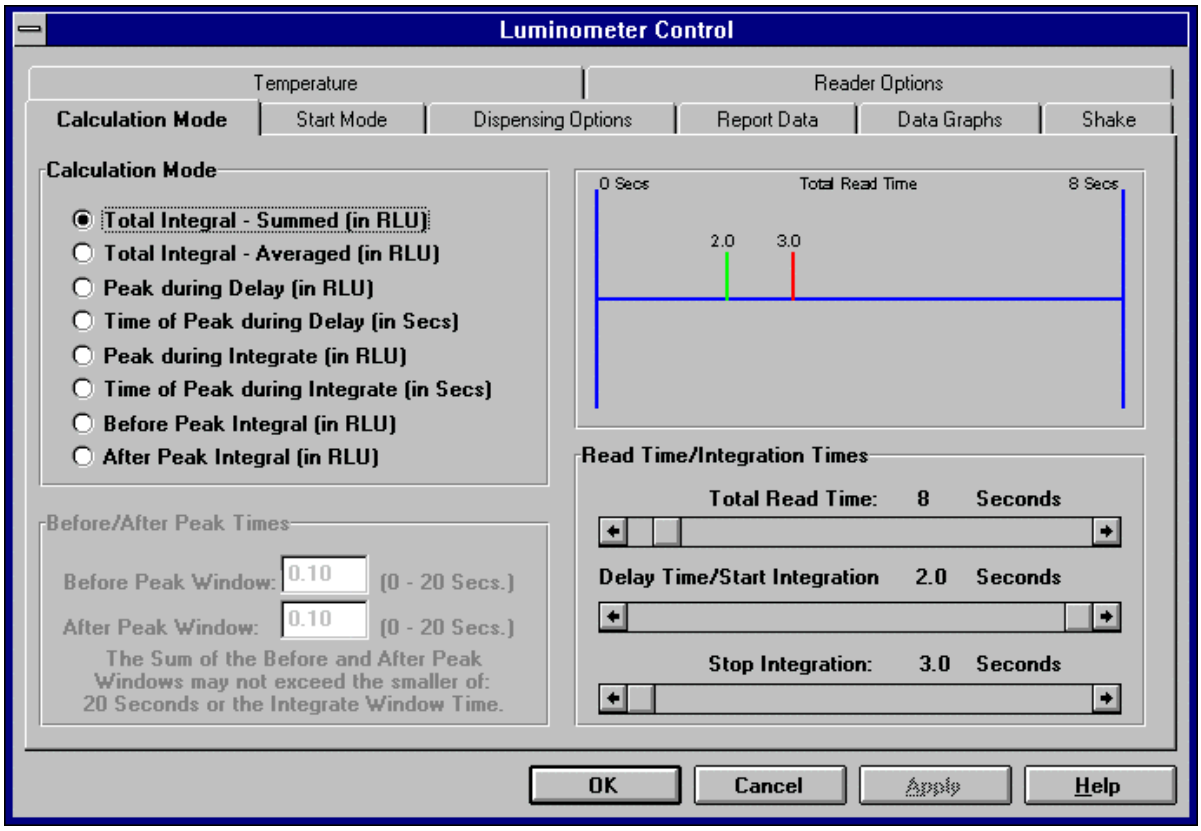
   Use the scrollbar to set the **Delay Time/ Start Integration**. The Delay Time above the bar and on the diagram changes accordingly. Use the scrollbar to set the **Stop Integration**. The Stop Integration Time displayed above the bar and on the diagram changes accordingly.

   NOTE: The **Start** and the **Stop** times must be at least **1.0** second. The minimum integration time (from Start to Stop) is **1.0** second.

4. Click on **OK**.

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Figure 4-16 Calculation Mode Dialog Box (Flash Assay)



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Start Mode *(Flash)*

The Start Mode dialog box (Figure 4-17) allows you to specify when Revelation takes the first reading relative to the start of the test. If you specify a delay between the start of the test and the first reading, the plate carrier retracts and the plate is incubated in the dark until the first reading is taken.

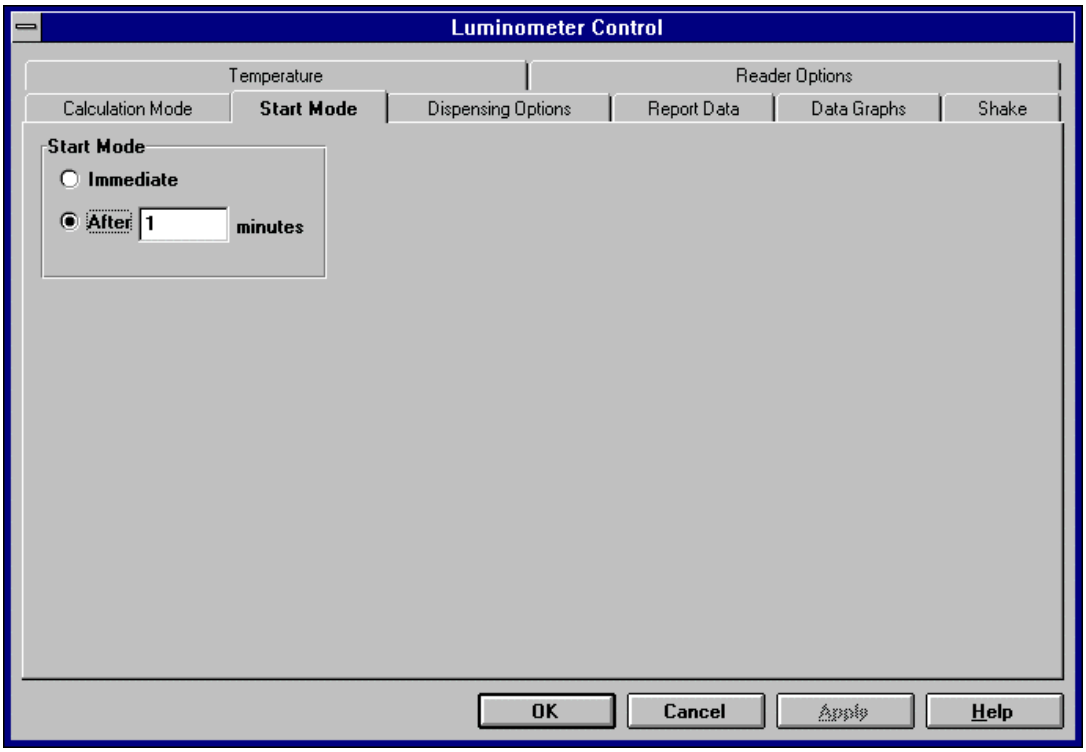
To specify the start mode:

1. ***Start Mode:*** If you want the first reading to be taken when the test starts, select **Immediate**.

   If you want the first reading to be taken a specified time after the test starts, select **After…minutes**. Then, type the time delay.

2. Click on **OK**.

Figure 4-17 Start Mode Dialog Box (Flash Assay)



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Shake *(Flash)*

For flash assays, the Shake dialog box (Figure 4-18) allows you to specify shaking of the plate while reagents are being dispensed into each well.

To specify the shake parameters:

1. ***Enable Shaking:*** If you want the plate to be shaken during dispensing of reagent from Dispenser A (just prior to reading), place an **x** in the **Enable Shaking** box.

   NOTE: If shaking is enabled for a flash assay, the plate will also be shaken during dispensing of reagent from Dispenser B (Offset).

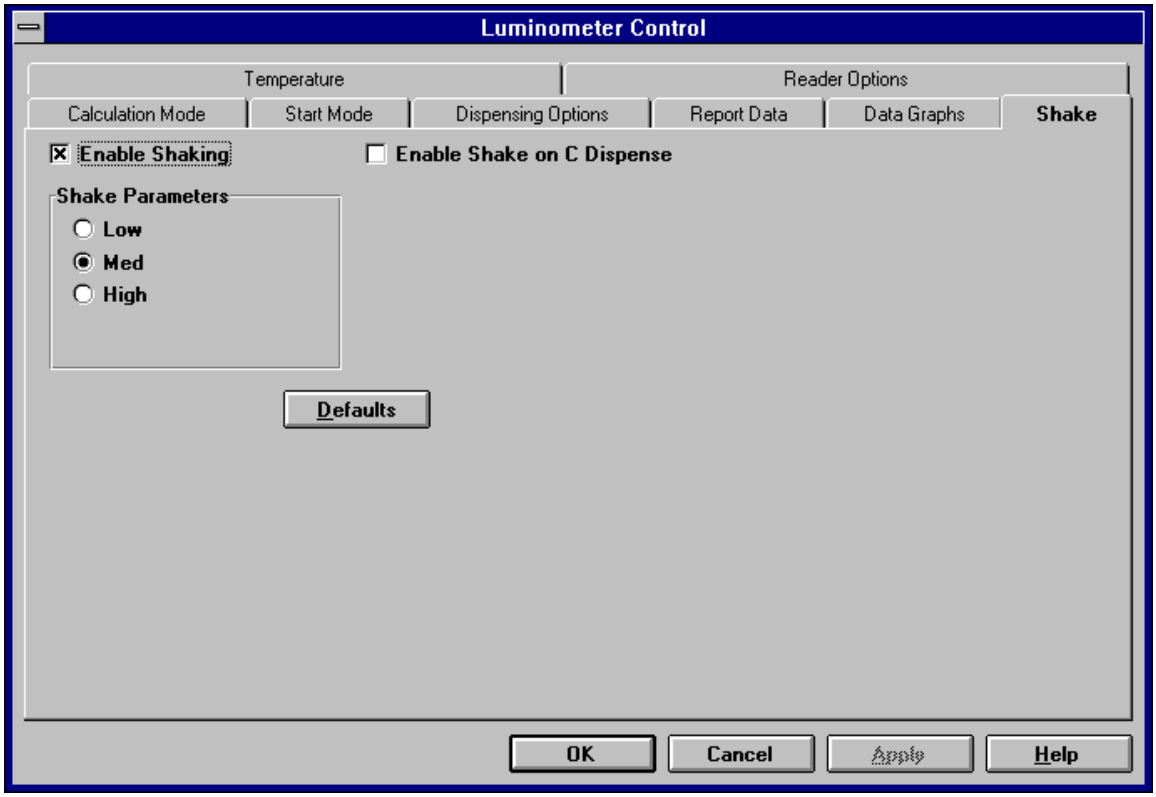
2. ***Shake Parameters*:** Select the shake intensity (**Low**, **Medium**, or **High**) by clicking on the appropriate radio button.

3. If you specify an A to C delay for dispensing and you also want the plate to be shaken during dispensing of reagent from Dispenser C, place an **x** in the **Enable Shake on C Dispense** box.

4. ***Defaults*:** If you prefer, specify the default shake parameters by clicking on **Default**.

5. Click on **OK**.

Figure 4-18 Shake Dialog Box (Flash Assay)



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Temperature *(Flash)*

The Temperature dialog box (Figure 4-19) allows you to enable the heater plate and to specify the temperature you wish to maintain during the assay.

To specify the temperature parameters:

1. ***Enable Heater Plate:*** If you want the heater plate to be enabled during the assay, place an **X** in the **Enable Heater Plate** box.

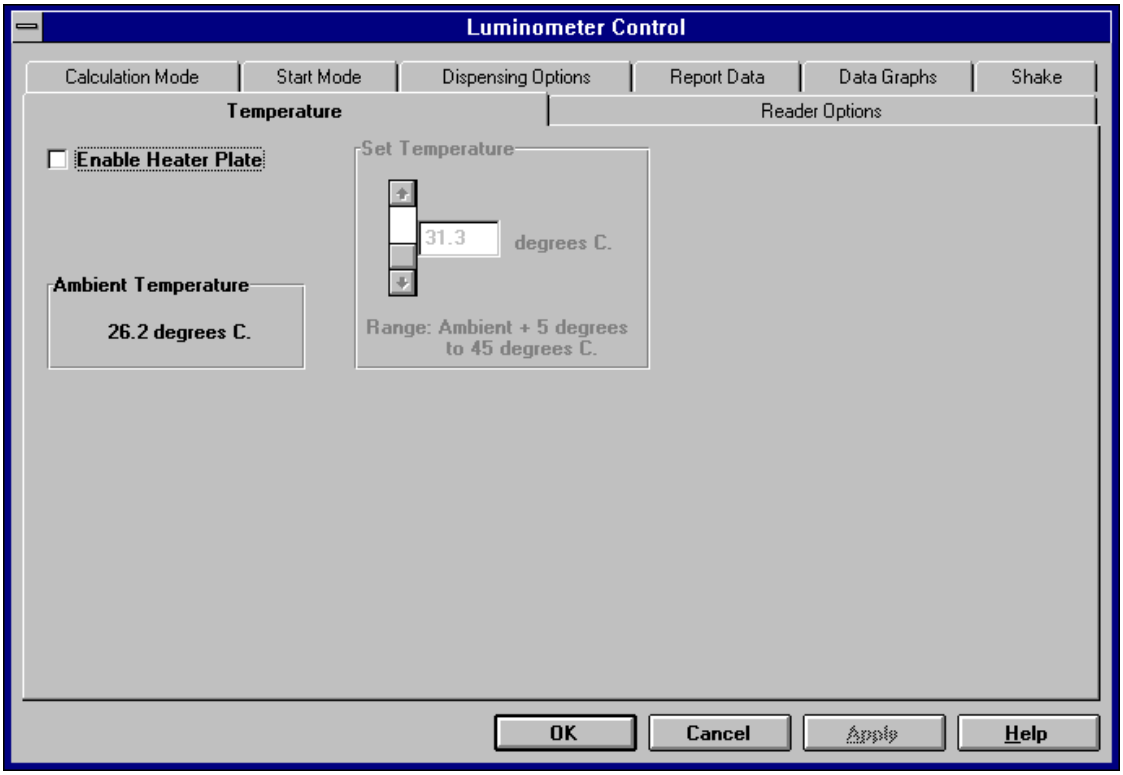
   NOTE: The **Ambient Temperature** field displays the ambient temperature.

2. ***Set Temperature:*** Use the temperature scroll bar to specify the temperature at which the plate is to be maintained during the assay.

   NOTE: You can set the temperature to any value between **5 °C above the ambient temperature** and **45 °C**.
   The **Set Temperature** field is inactive if heating is not enabled in Step **1**.

3. Click on **OK**.

Figure 4-19 Temperature Dialog Box (Flash Assay)



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Reader Options *(Flash)*

The Reader Options dialog box (Figure 4-20) allows you to specify gain levels and scaling factors for the assay.

To specify reader options:

1. ***Gain Level:*** To match the intensity of emitted light to the full scale range of the detector, select the gain level (in RLU) by clicking on the appropriate radio button.

   If you prefer, select the **Auto Gain** radio button.

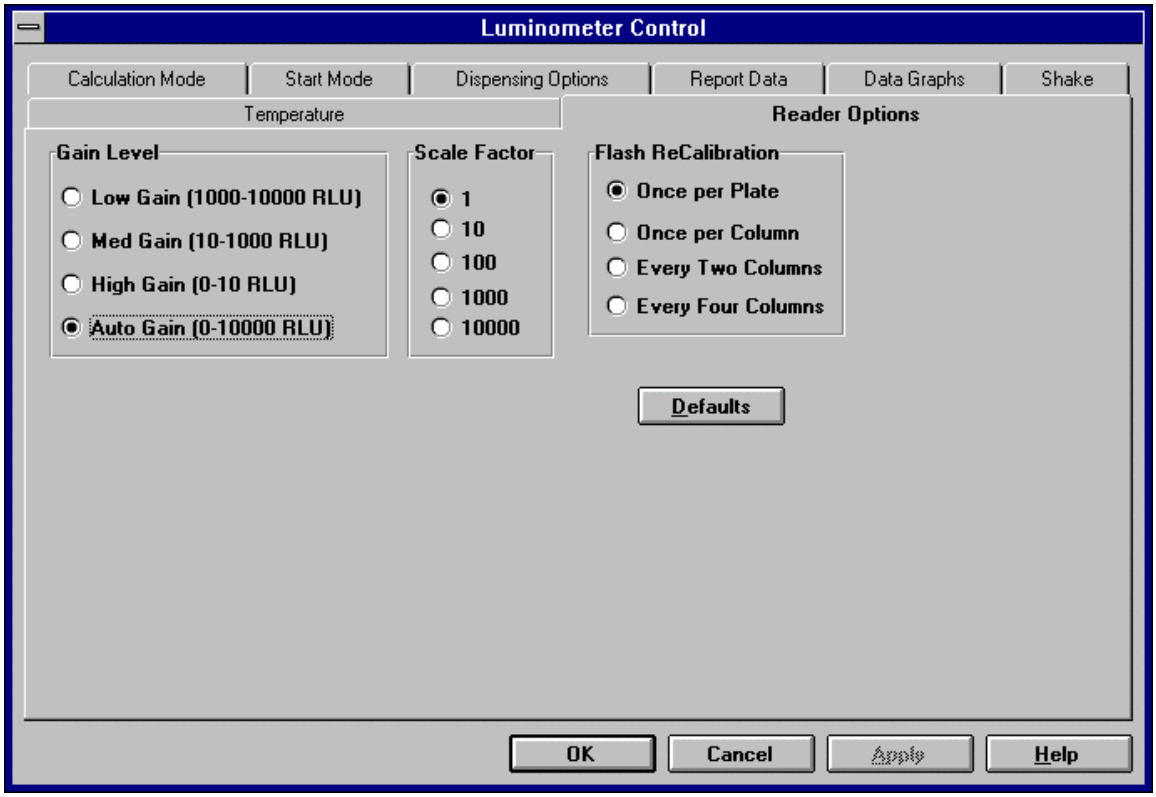
2. ***Scaling Factor:*** Select the factor (from 1 to 10,000) that results will be multiplied by.

3. ***Flash Recalibration:*** Select the frequency (**Once per Plate**, **Once per Column**, **Every Two Columns**, or **Every Four Columns**) of flash recalibration.

4. ***Defaults*:** If you prefer, specify the default read parameters by clicking on **Defaults**.

5. Click on **OK**.

Figure 4-20 Reader Options Dialog Box (Flash Assay)



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Dispensing Options *(Flash)*

The use of the dispensers for flash assays is outlined below:

Dispenser	Purpose
A	Triggers the flash reaction. Reading of the well always starts when reagent is dispensed into the well from Injector A.
B (Offset)	Dispenses reagent into the well before the reaction is triggered and reading starts. This may be a reagent to prepare the sample for reading (to clarify the sample, for example). Specified by "Delay Before Read."
C	Dispenses reagent into the well at the same time or later than Dispenser A. Specified by "A to C Delay."

The Offset (B) injector is mounted so that it is positioned one well ahead of injector A and injector C. During a flash assay, the Offset (B) reagent is always dispensed into a well first. Then the plate carrier advances one well position, causing that well to be positioned under injector A and injector C and the next well to be positioned under the Offset (B) injector. The well remains under injector A and injector C until both reagents have been dispensed and the readings have been taken for the **Total Read Time** specified in the Assay Settings.

The length of time that a well remains under the dispenser/reader head is determined by the following:

- **Delay Before Read** (Dispensing Options, page 99).
- **A to C Delay** (Dispensing Options, page 99).
- **Total Read Time** (Calculation Mode, page 93)

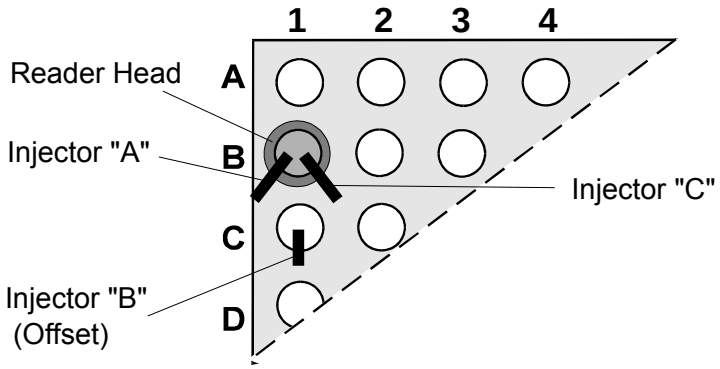


Figure 4-21. Relative Positions of Injectors and Reader Head

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Examples of how the assay cycle is affected by settings of **Delay Before Read**, **A to C Delay**, and **Total Read Time** are shown below.

Effect of Different Assay Settings

Delay Before Read	A to C Delay	Total Read Time	Description
3 sec	0 sec	3 sec	First Cycle: Dispense B into the first well. Advance to next well after 3 seconds. Remaining Cycles: Dispense A and C into the current well at the same time. Read for 3 seconds and advance to the next well. Dispense B into the next well at the same time that A and C are dispensed into the current well. Cycle is repeated every 3 seconds.
1 sec	3 sec	3 sec	First Cycle: Dispense B into the first well. Advance to next well after 1 second. Remaining Cycles: Dispense A into the current well. Read for 3 seconds and dispense C. Then, advance to the next well. Dispense B into the next well two seconds after A and C are dispensed into the current well. Cycle is repeated every 3 seconds.
3 sec	3 sec	3 sec	First Cycle: Dispense B into the first well. Advance to next well after 3 seconds. Remaining Cycles: Dispense A into the current well. Read for 3 seconds and dispense C. Then, advance to the next well. Dispense B into the next well at the same time that A is dispensed into the current well. Cycle is repeated every 3 seconds.
3 sec	3 sec	5 sec	First Cycle: Dispense B into the first well. Advance to next well after 3 seconds. Remaining Cycles: Dispense A into the current well. Read for 3 seconds and dispense C. Read for two more seconds. Then, advance to the next well. Dispense B into the next well 2 seconds after A is dispensed into the current well. Cycle is repeated every 5 seconds.

Effect of Different Assay Settings (Continued)

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10 sec	3 sec	3 sec	First Cycle: Dispense B into the first well. Advance to next well after 7 seconds. Remaining Cycles: Dispense A into the current well. Read for 3 seconds and dispense C. Wait 7 seconds and advance to the next well. Dispense B into the next well at the same time that A is dispensed into the current well. Cycle is repeated every 10 seconds.

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The Dispensing Options dialog box (Figure 4-22) allows you to specify the **Delay Before Read** time the **A to C Delay** time, and the volume of liquid dispensed by each dispenser.

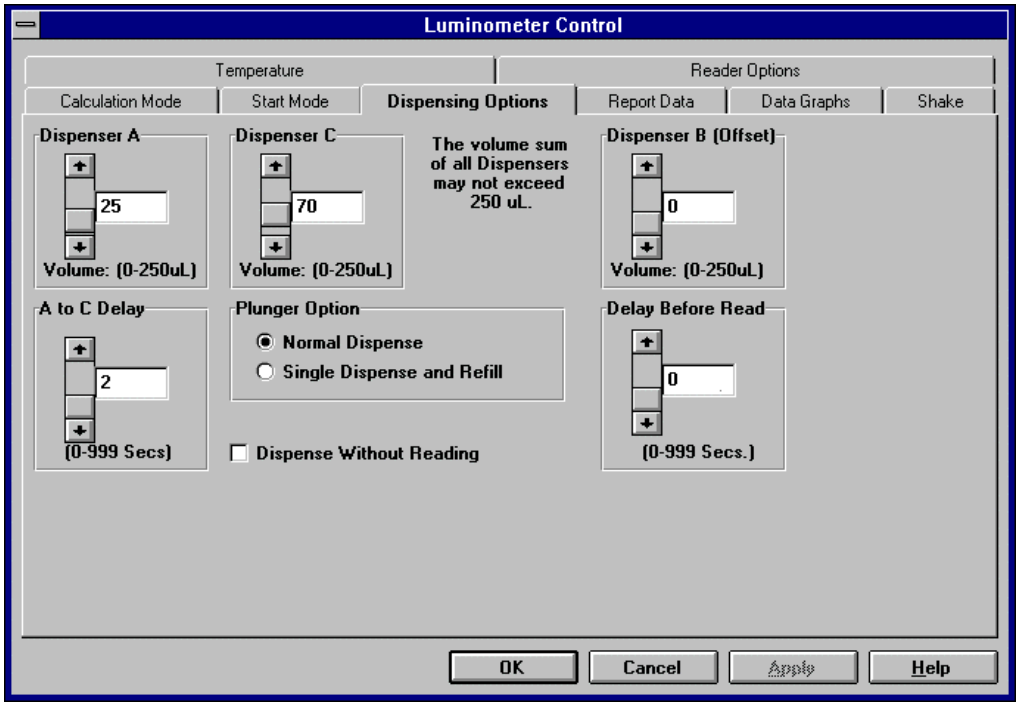
To specify dispenser options:

1. ***Dispenser A:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser A.
2. ***Dispenser C:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser C.
3. ***Dispenser B (Offset):*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser B (Offset).

   NOTE: The total volume dispensed by the three dispensers may not exceed 250 µL.

4. ***A to C Delay:*** Use the scroll bar to specify the time delay (0 to 999 seconds) between the completion of dispensing A and the activation of Dispenser C.
5. ***Plunger Option:*** Select **Normal Dispense** if you want the dispenser syringes to refill only when required. Select **Single Dispense and Refill** if you want the dispenser syringes to refill after each dispense.
6. ***Delay Before Read:*** Use the scroll bar to specify the time delay (0 to 999 seconds) between dispensing of B (Offset) and the start of reading and/or dispensing of A.
7. ***Dispense Without Reading:*** If you want to dispense without reading, place an **X** in this box.
8. Click on **OK**.

Figure 4-22 Dispensing Options Dialog Box



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Report Data *(Flash)*

The Report Data dialog box (Figure 4-23) allows you to select the data that will be included in the results reports.

To specify the report data:

1. ***Include Data in Report:*** The data types that you can choose from are described below. Select the data type you want by clicking on the appropriate radio button.

Data Type	Description
Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Integrate Window.
Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Integrate Window.
Peak during Delay (in RLU)	Value of the maximum reading recorded prior to the Start Integration Time.
Time of Peak during Delay (in Secs)	Time that the maximum reading was recorded prior to the Start Integration Time.
Peak during Integrate (in RLU)	Value of the maximum reading recorded during the specified Integrate Window.
Time of Peak during Integrate (in Secs)	Time that the maximum reading was recorded during the specified Integrate Window.
Before Peak Integral (in RLU)	Sum of the values (in RLU) of all the readings recorded during the specified Before Peak Window.
After Peak Integral (in RLU)	Sum of the values (in RLU) of all the readings recorded during the specified After Peak Window.

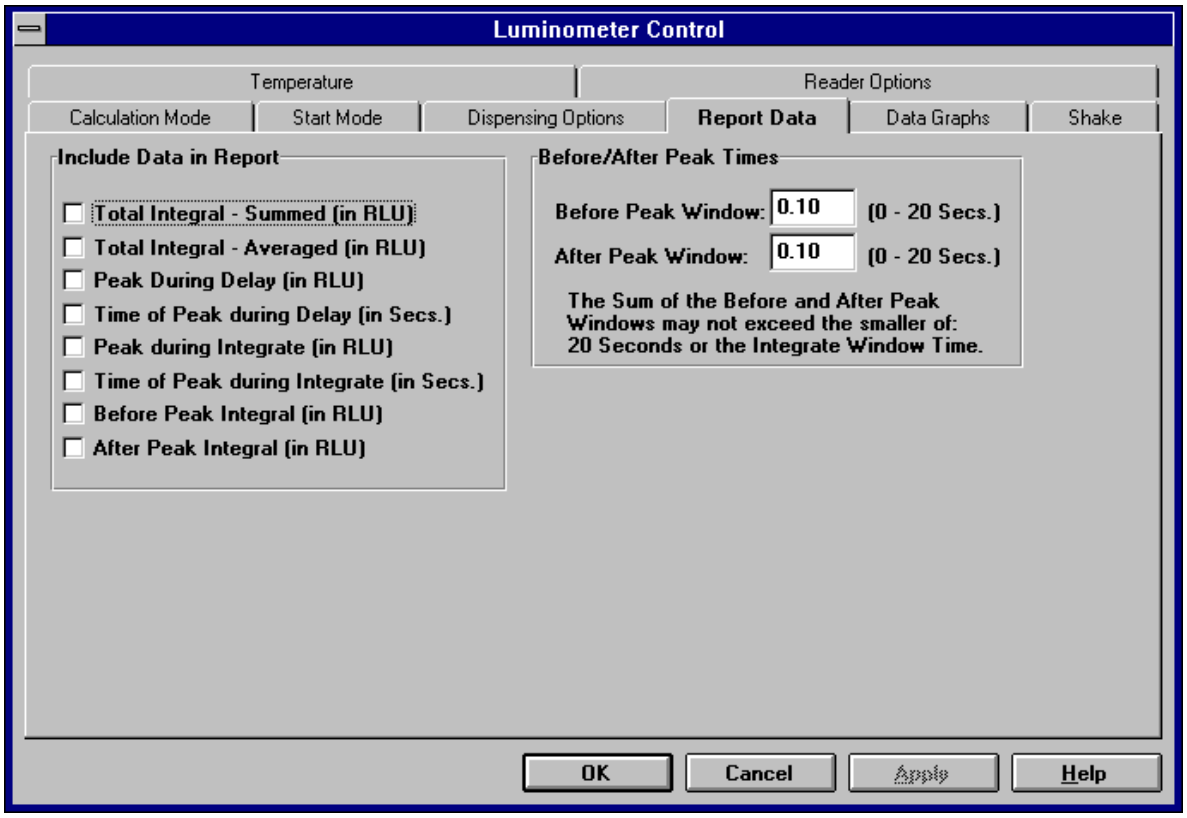
2. ***Before/After Peak Times:*** Type a value for **Before Peak Window**. Then, type a value for **After Peak Window**.

   NOTE: The sum of **Before Peak Window** and **After Peak Window** may not exceed the **smaller** of 20 seconds or the Integrate Window Time.

3. Click on **OK**.

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Figure 4-23 Report Data Dialog Box (Flash Assay)



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Data Graphs *(Flash)*

The Data Graphs dialog box (Figure 4-14) allows you to specify which wells or combination of wells are included in the graphs that are part of the results report and to specify whether the plotted readings for each well are displayed after each well is read.

To specify wells to include:

1. ***Well Selection:*** To select an individual well, click in the corresponding section of the plate diagram. To select an entire column or an entire row, place an **x** in the corresponding **Whole Col.** or **Whole Row** check box.

   To select all of the wells, place an **x** in the **Plot all 96 Wells** check box.

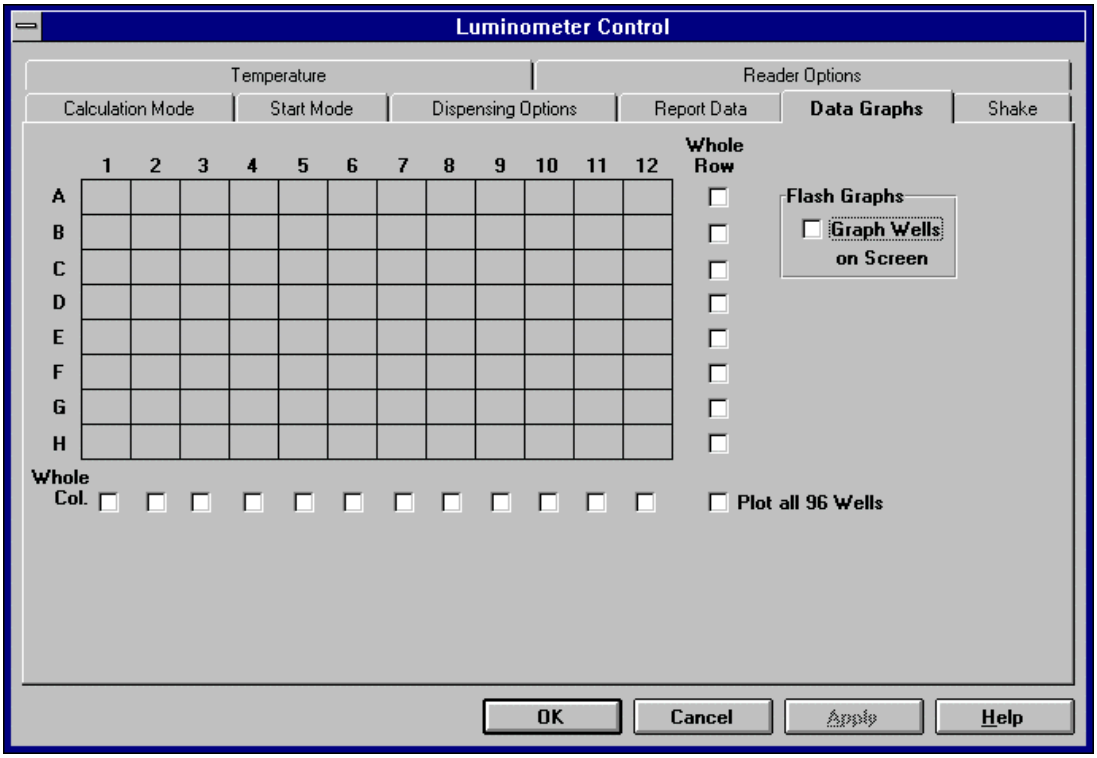
   To deselect an individual well, click in the corresponding section of the plate diagram.

2. ***Flash Graphs:*** To display the plotted readings for each well after the well is read, place an **x** in the **Graph Wells on Screen** check box..

   NOTE: If you select this option, the plotted results will be displayed after reading of the well is completed. Reading of the next well will not commence until you click on **Continue**.

3. Click on **OK**.

Figure 4-24 Data Graphs Dialog Box (Flash Assay)



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Reader Control: Dual Flash Assays

To specify Reader Control settings for dual flash assays:

1. Click on **Settings** and then click on **Reader Control**. Eight tabbed Luminometer Control dialog boxes will be displayed. Select the appropriate tab as outlined below:

Tab	Purpose
Calculation Mode	Select a Calculation Mode, specify the Total Read Time, and specify the First and Second Integration Times. See "Calculation Mode *(Dual Flash)*" on page 107.
Start Mode	Specify when the first flash readings are taken relative to the start of the test. The test starts when you click on **Start** in the Run Plate dialog box. For further information, see "Start Mode *(Dual Flash)*" on page 109.
Dispensing Options	Specify the volume of liquid to be dispensed by each dispenser and the time delays that are required between dispenses. See "Dispensing Options *(Dual Flash)*" on page 113.
Report Data	Select the data to be included in the result reports. See "Report Data *(Dual Flash)*" on page 115.
Data Graphs	Specify whether the plotted readings for each well are displayed after each well is read and the wells or combination of wells that are to be included in the graphs that are part of the results report. See "Data Graphs *(Dual Flash)*" on page 117.
Shake	Enable plate shaking and specify the frequency, amplitude, and duration of the shake motion. See "Shake *(Dual Flash)*" on page 110.
Temperature	Enable the heater plate and specify the temperature you wish to maintain. See "Temperature *(Dual Flash)*" on page 111.
Reader Options	Specify gain levels and scaling factors. See "Reader Options *(Dual Flash)*" on page 112.

2. Instructions for using each dialog box are contained in the following sections.

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Calculation Mode *(Dual Flash)*

The Calculation Mode dialog box (Figure 4-25) allows you to select a Calculation Mode and specify the Total Read Time and the First and Second Integration Times. Refer to page 12 in Chapter 1 for definition of these terms.

To specify the calculation mode:

1. ***Calculation Mode:*** The Calculation Modes that you can choose from are described below. Select the Calculation Mode you want by clicking on the appropriate radio button.

Calculation Mode	Description
First Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified First Integrate Window.
First Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified First Integrate Window.
Second Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Second Integrate Window.
Second Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Second Integrate Window.
Ratio of First to Second Total Integral	Value of the First Total Integral divided by the value of the Second Total Integral.
Ratio of Second to First Total Integral	Value of the Second Total Integral divided by the value of the First Total Integral.
Ratio Computation	Allows you to select whether the summed RLU values or the average RLU values are used for calculating the ratio.

2. ***Total Read Time:*** Use the scrollbar to set the **Total Read Time**. The Total Read Time above the scrollbar and on the diagram changes accordingly.
3. ***Integration Times:*** Use the scrollbar to set the **Start Time** and the **Stop Time** for the first integration window. The Total Read Time above the scrollbar and on the diagram changes accordingly.

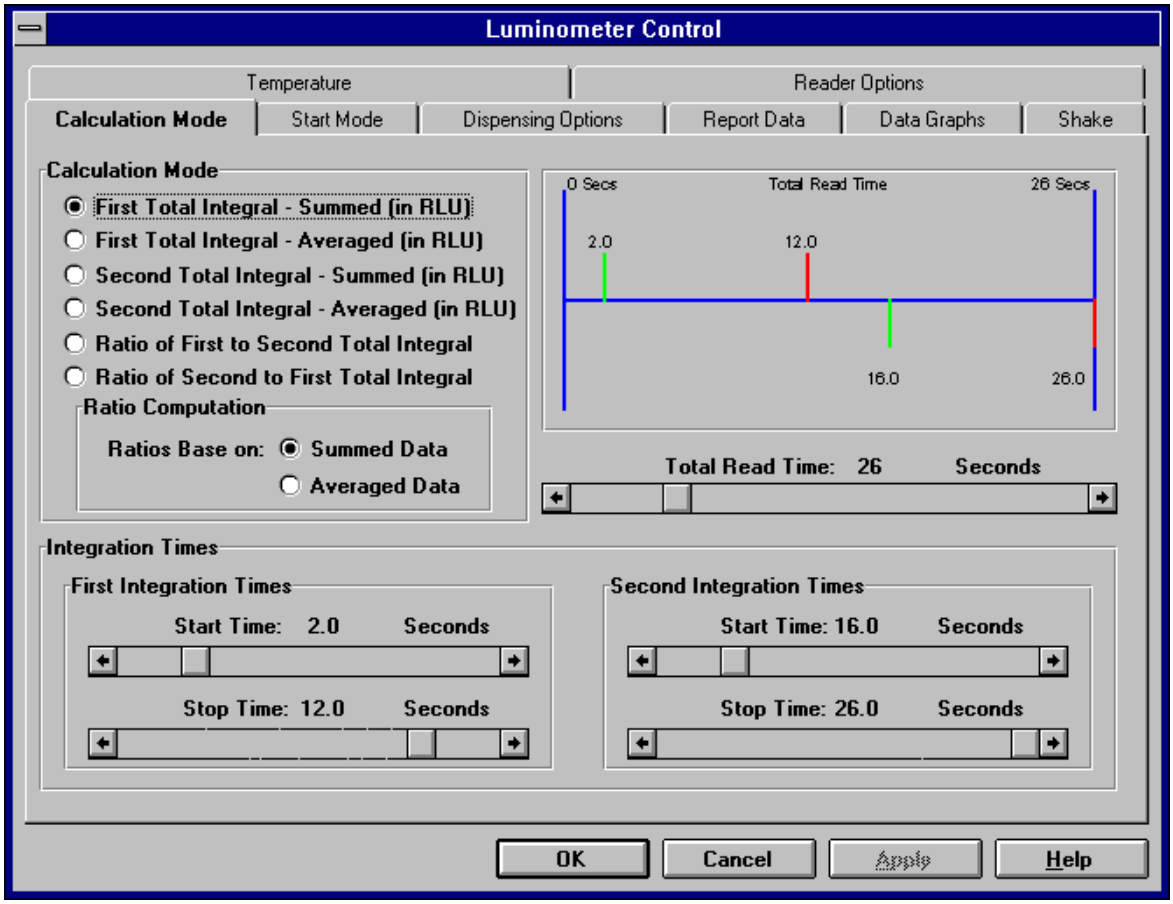
   Use the scrollbar to set the **Start Time** and the **Stop Time** for the second integration window. The Total Read Time above the scrollbar and on the diagram again changes accordingly.

   NOTE: The **Start Time** and the **Stop Time** of both integration windows must be at least **1.0** second. The **Start Time** of the **Second Integration Time** must exceed the **Stop Time** of the **First Integration Time** by at least **2.0** seconds.

4. Click on **OK**.

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Figure 4-25 Calculation Mode Dialog Box (Dual Flash Assay)



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Start Mode *(Dual Flash)*

The Start Mode dialog box (Figure 4-26) allows you to specify when Revelation takes the first reading relative to the start of the test. If you specify a delay between the start of the test and the first reading, the plate carrier retracts and the plate is incubated in the dark until the first reading is taken

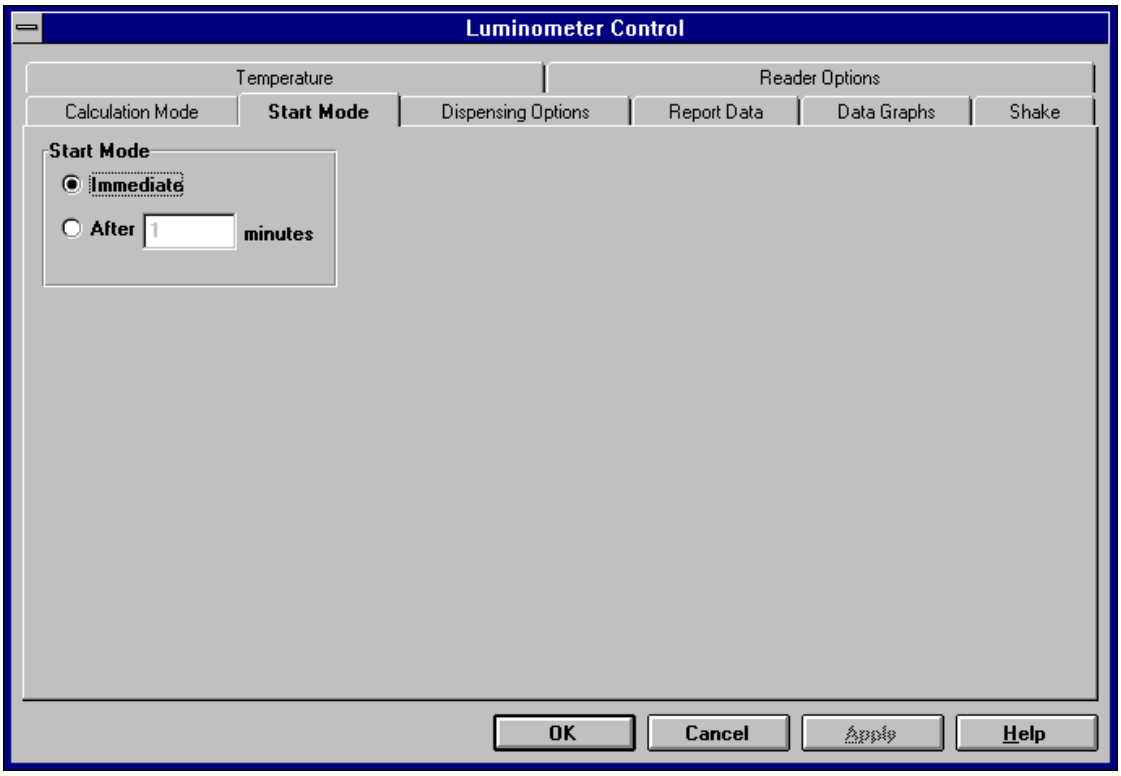
To specify the start mode:

1. ***Start Mode:*** If you want the first reading to be taken when the test starts, select **Immediate**.

   If you want the first reading to be taken a specified time after the test starts, select **After…minutes**. Then, type the time delay.

2. Click on **OK**.

Figure 4-26 Start Mode Dialog Box (Dual Flash Assay)



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Shake *(Dual Flash)*

The Shake dialog box (Figure 4-27) allows you to specify when shaking of the plate should occur during a dual flash assay, to specify the duration of shaking, and to specify the intensity of shaking.

To specify the shake parameters:

1. ***Shake Parameters*:** Select the shake intensity (**Low**, **Medium**, or **High**) by clicking on the appropriate radio button.

   Specify the **Duration** of shaking by typing the duration time (**1** to **10** seconds).

   NOTE: Shaking Duration applies to all of the Dispensers specified in Shake Control (see next step). Shaking always stops at the end of each integration time.

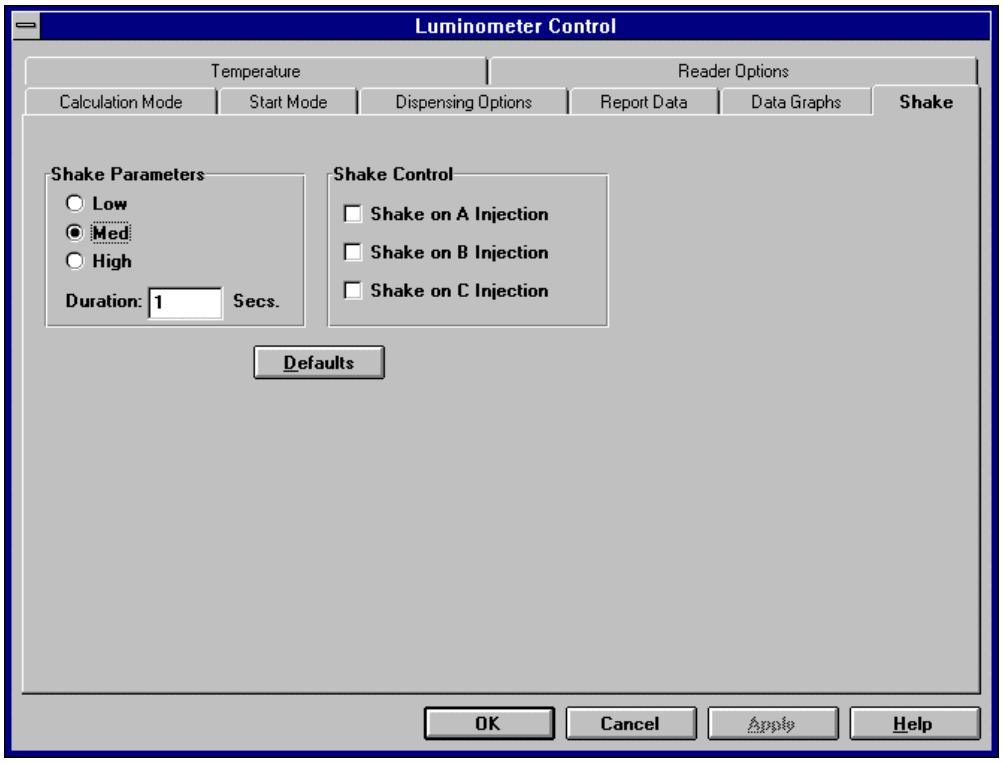
2. ***Shake Control*:** Specify shaking to occur **on A Injection**, **on B Injection**, and/or **on C Injection** by placing an **x** in one or more of the boxes.

   NOTE: If shaking is specified for C injection during the same integration time in which A is dispensed, shaking will also occur during A injection. Shaking does not occur during on B injection if B is dispensed during a reading.

3. ***Defaults*:** If you prefer, specify the default shake parameters by clicking on **Defaults**.

4. Click on **OK**.

Figure 4-27 Shake Dialog Box (Dual Flash Assay)



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Temperature *(Dual Flash)*

The Temperature dialog box (Figure 4-28) allows you to enable the heater plate and to specify the temperature you wish to maintain during the assay.

To specify the temperature parameters:

1. ***Enable Heater Plate:*** If you want the heater plate to be enabled during the assay, place an **X** in the **Enable Heater Plate** box.

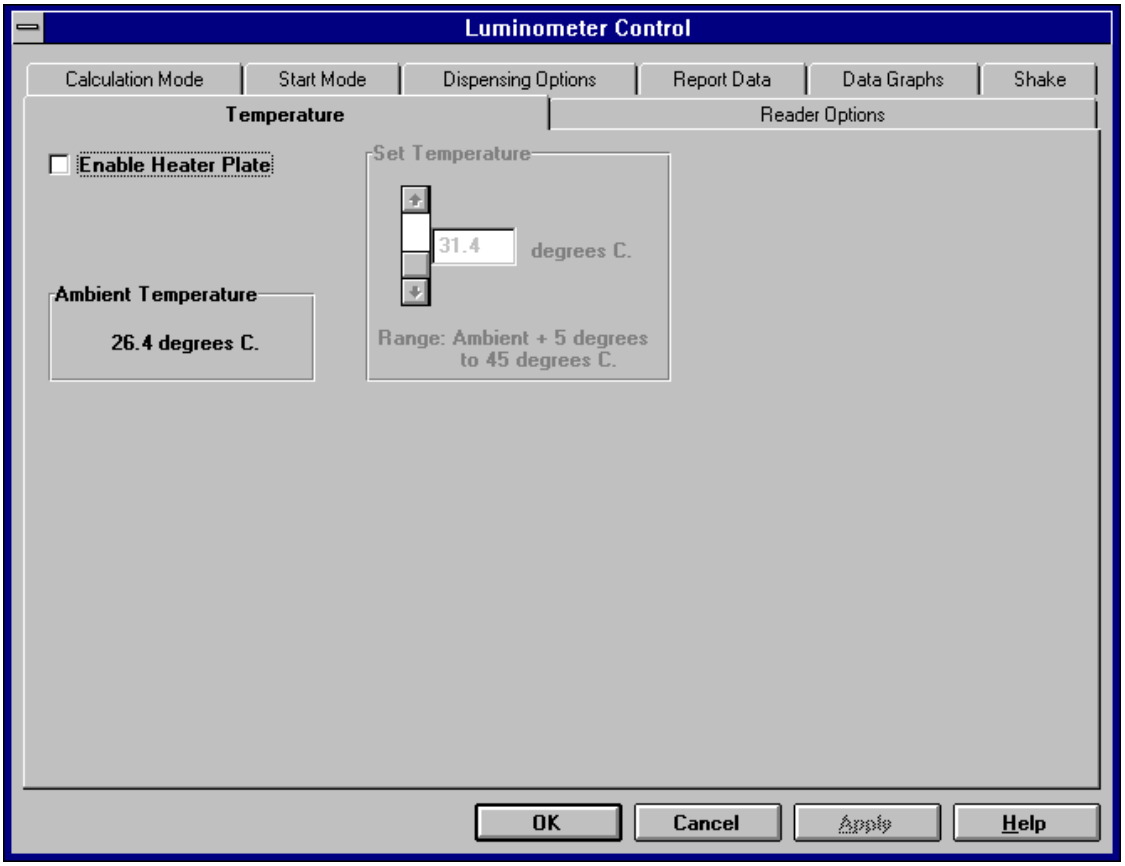
   NOTE: The **Ambient Temperature** field displays the ambient temperature.

2. ***Set Temperature:*** Use the temperature scroll bar to specify the temperature at which the plate is to be maintained during the assay.

   NOTE: You can set the temperature to any value between **5 °C above the ambient temperature** and **45 °C**.
   The **Set Temperature** field is inactive if heating is not enabled in Step **1**.

3. Click on **OK**.

Figure 4-28 Temperature Dialog Box (Dual Flash Assay)



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Reader Options *(Dual Flash)*

The Reader Options dialog box (Figure 4-29) allows you to specify gain levels and scaling factors for the assay.

To specify reader options:

1. ***Gain Level:*** To match the intensity of emitted light to the full scale range of the detector, select the gain level (in RLU) by clicking on the appropriate radio button.

   If you prefer, select the **Auto Gain** radio button.

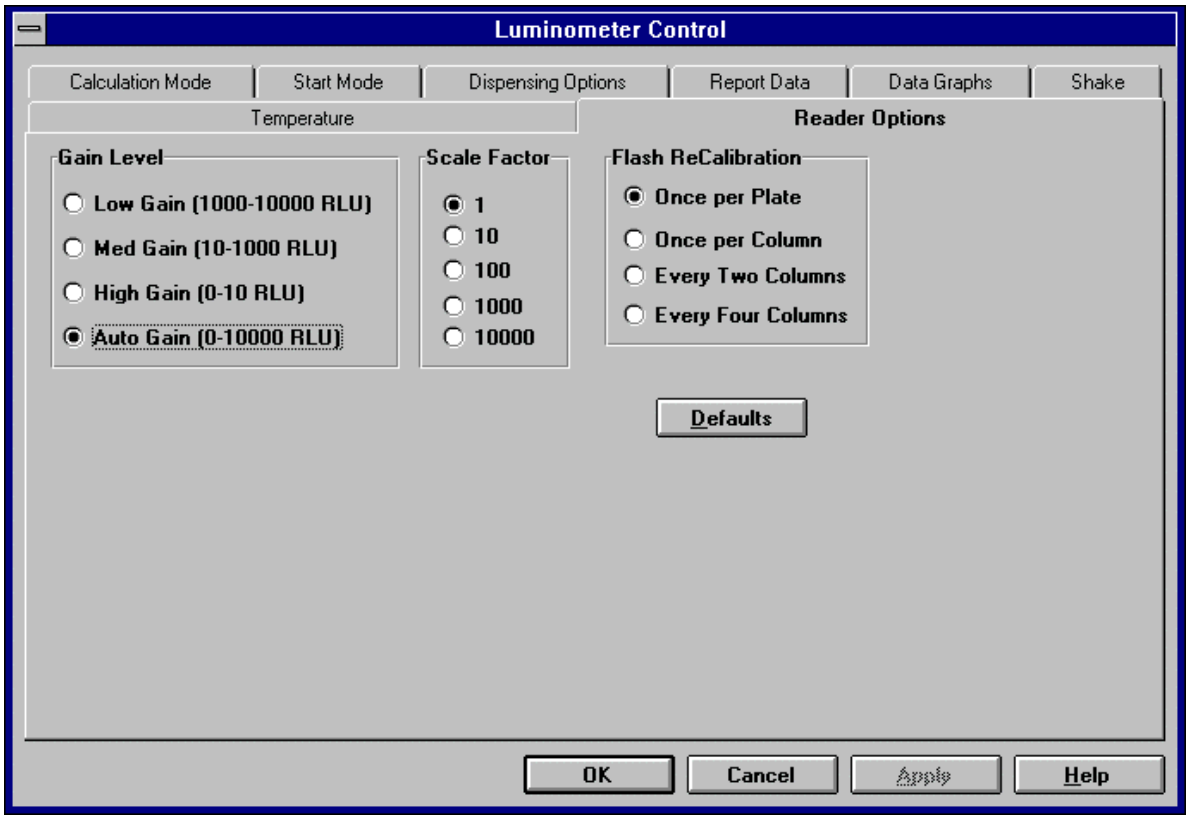
2. ***Scaling Factor:*** Select the factor (from 1 to 10,000) that results will be multiplied by.

3. ***Flash Recalibration:*** Select the frequency (**Once per Plate**, **Once per Column**, **Every Two Columns**, or **Every Four Columns**) of flash recalibration.

4. ***Defaults*:** If you prefer, specify the default read parameters by clicking on **Defaults**.

5. Click on **OK**.

Figure 4-29 Reader Options Dialog Box (Dual Flash Assay)



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Dispensing Options *(Dual Flash)*

The use of the dispensers in dual flash assays is outlined below:

Dispenser	Purpose
A	Triggers the flash reaction. Reading of the well always starts when reagent is dispensed into the well from Injector A.
B (Offset)	Dispenses reagent into the well before the reaction is triggered and reading starts. This may be a reagent to prepare the sample for reading (to clarify the sample, for example). Specified by "Delay Before Read."
C	Dispenses reagent into the well at the same time or later than Dispenser A. Specified by "A to C Delay."

Refer to page 99 for additional details concerning use of the dispensers.

The Dispensing Options dialog box (Figure 4-30) allows you to specify the **Delay Before Read** time the **A to C Delay** time, and the volume of liquid dispensed by each dispenser.

To specify dispenser options:

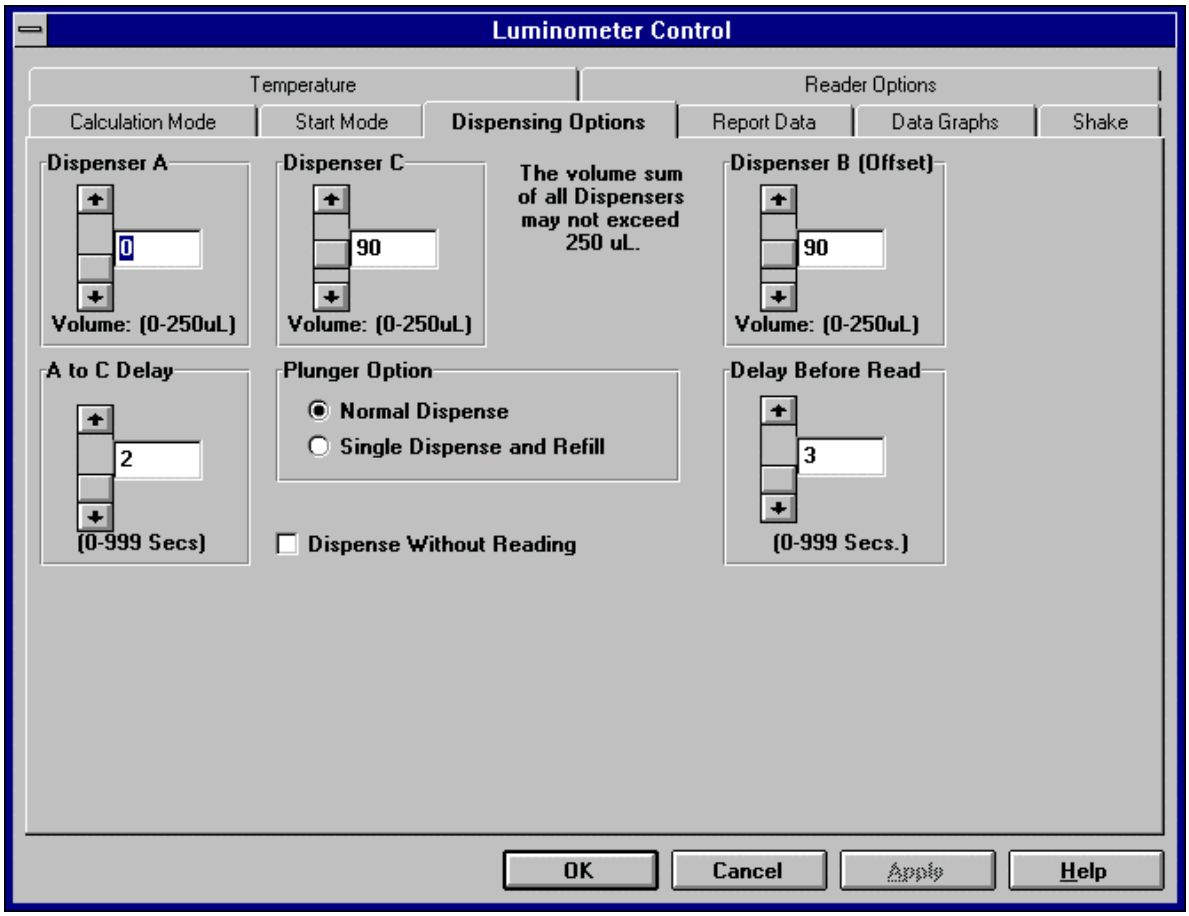
1. ***Dispenser A:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser A.
2. ***Dispenser C:*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser C.
3. ***Dispenser B (Offset):*** Use the scroll bar to specify the volume (in µL) to be dispensed by Dispenser B (Offset).

   NOTE: The total volume dispensed by the three dispensers may not exceed 250 µL.

4. ***A to C Delay:*** Use the scroll bar to specify the time delay (0 to 999 seconds) between the completion of dispensing A and the activation of Dispenser C.
5. ***Plunger Option:*** Select **Normal Dispense** if you want the dispenser syringes to refill only when required. Select **Single Dispense and Refill** if you want the dispenser syringes to refill after each dispense.
6. ***Delay Before Read:*** Use the scroll bar to specify the time delay (0 to 999 seconds) between dispensing of B (Offset) and the start of reading and/or dispensing of A.
7. ***Dispense Without Reading:*** If you want to dispense without reading, place an **X** in this box.
8. Click on **OK**.

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Figure 4-30 Dispensing Options Dialog Box (Dual Flash Assay)



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Report Data *(Dual Flash)*

The Report Data dialog box (Figure 4-31) allows you to select the data that will be included in the results reports.

To specify the report data:

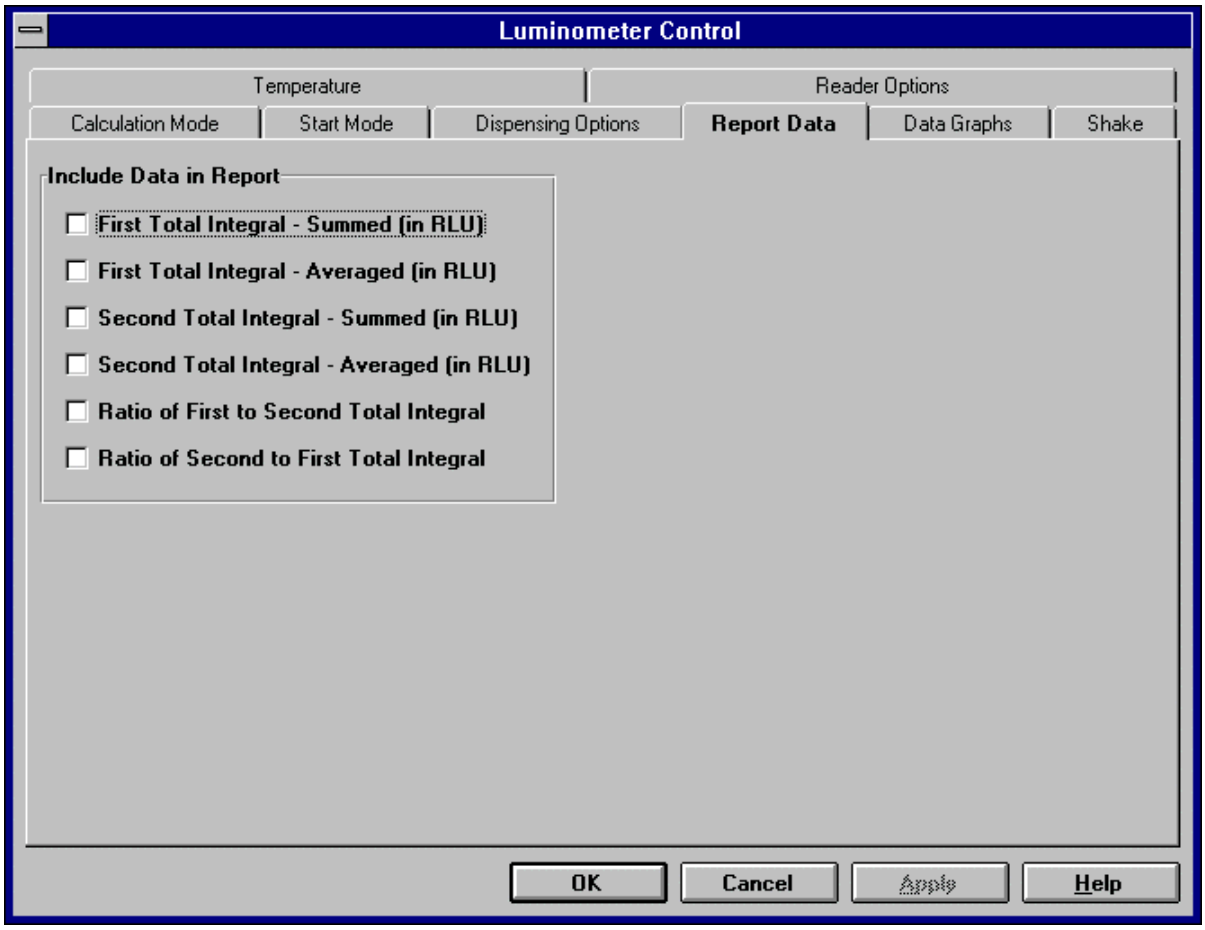
1. ***Include Data in Report:*** The data types that you can choose from are described below. Select the data type you want by clicking on the appropriate radio button.

Data Type	Description
First Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified First Integrate Window.
First Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified First Integrate Window.
Second Total Integral - Summed (in RLU)	Sum of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Second Integrate Window.
Second Total Integral - Averaged (in RLU)	Average of the values (in RLU) of all the readings (100 readings per second) recorded during the specified Second Integrate Window.
Ratio of First to Second Total Integral	Value of the First Total Integral divided by the value of the Second Total Integral.
Ratio of Second to First Total Integral	Value of the Second Total Integral divided by the value of the First Total Integral.

3. Click on **OK**.

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Figure 4-31 Report Data Dialog Box (Dual Flash Assay)



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Data Graphs *(Dual Flash)*

The Data Graphs dialog box (Figure 4-32) allows you to specify which wells or combination of wells are included in the graphs that are part of the results report and to specify whether the plotted readings for each well are displayed after each well is read.

To specify wells to include:

1. ***Well Selection:*** To select an individual well, click in the corresponding section of the plate diagram. To select an entire column or an entire row, place an **x** in the corresponding **Whole Col.** or **Whole Row** check box.

   To select all of the wells, place an **x** in the **Plot all 96 Wells** check box.

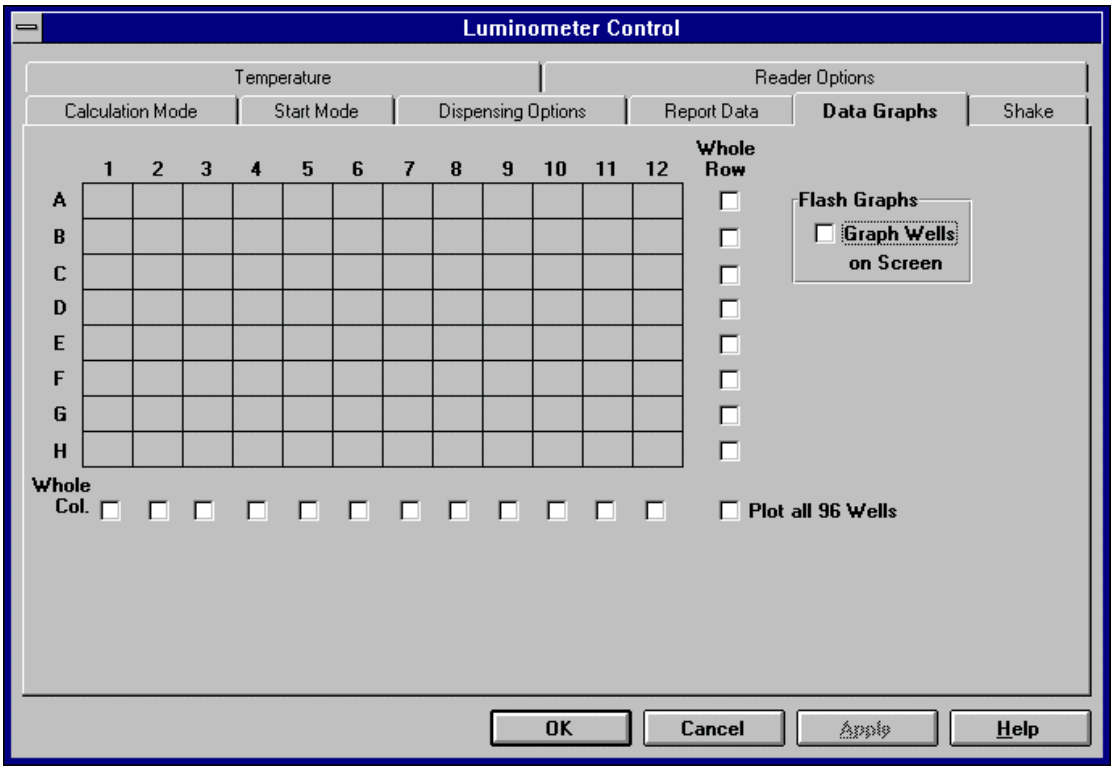
   To deselect an individual well, click in the corresponding section of the plate diagram.

2. ***Flash Graphs:*** To display the plotted readings for each well after the well is read, place an **x** in the **Graph Wells on Screen** check box.

   NOTE: If you select this option, the plotted results will be displayed after reading of the well is completed. Reading of the next well will not commence until you click on **Continue**.

3. Click on **OK**.

Figure 4-32 Data Graphs Dialog Box (Dual Flash Assay)



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Template

The Template command displays the Template dialog box (Figure 4-33), which allows you to assign well types to well positions, rename well types, create and name new well types, and specify the number of replicates, the orientation, and the fill direction.

Note: A particular well type corresponds to a particular sample type. An assay for a particular well type can be copied to multiple wells for a specific sample, resulting in replicate determinations. Or, if you wish to perform a sample dilution on the plate, you can allocate wells to create a dilution block, which can then be copied like any other sample.

To define the template:

1. ***Well Type:*** Select the first well type that you want to assign to the template. The default well types are described below:

Well Type	Symbol	Description
Blank	B	Wells used for a blank. Generally, one or two blank wells may be run per plate to subtract background luminescence.
Standard	S	Wells that contain a Standard. The Standards are used to calibrate a quantitative assay.
Control	C	Control samples that are used to verify the performance of an assay.
Test	T	All unknown samples.
Delete	---	Removes from the diagram the well positions that you will not use.

NOTE: You can define additional well types by selecting any of the user definable types (#1 through #8). Then, click on **Change Label** as outlined below.

2. ***Replicates:*** Using the arrow buttons, select the number of replicates that are to be assigned for that sample type.

NOTE: Up to 96 replicates can be assigned.

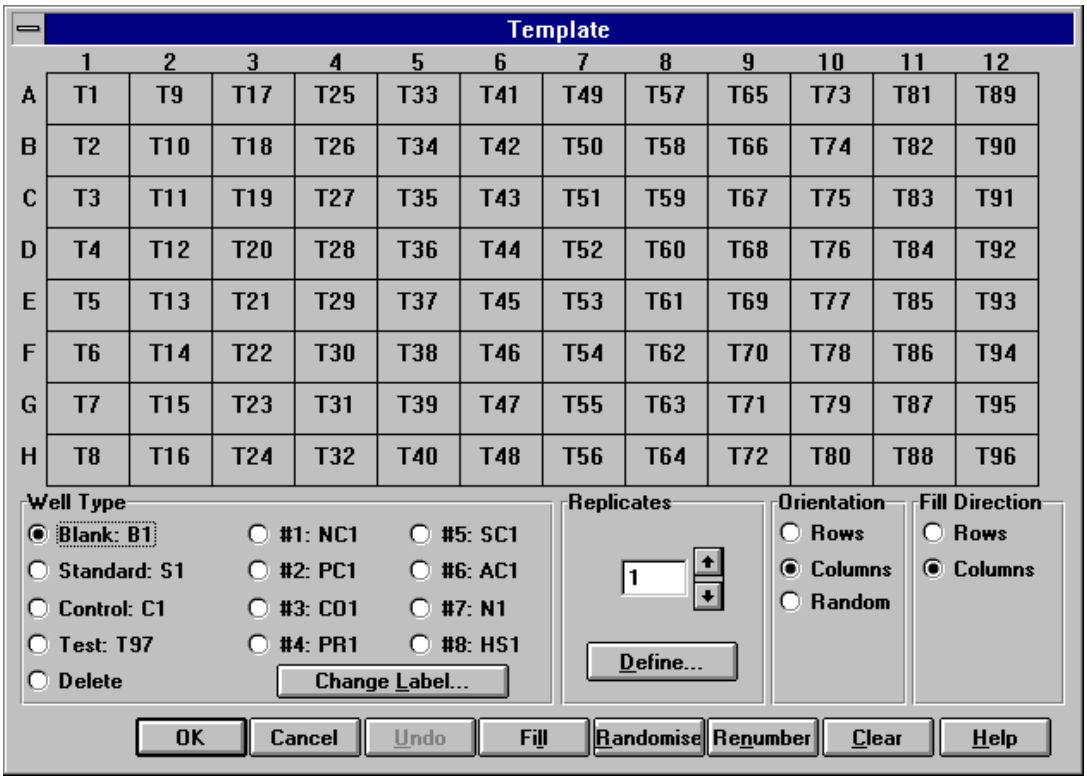
3. ***Orientation:*** Select **Rows** if you want replicate wells to be automatically assigned by row (from left to right). Select **Columns** if you want replicate wells to be automatically assigned by column (from top to bottom). Select **Random** if you want replicate wells to be automatically assigned to random positions.

NOTE: If you select **Columns**, eight is the maximum number of replicates you can assign.

4. ***Fill Direction:*** Select **Rows** if you want the replicate array you defined above to be automatically assigned by row (from left to right). Select **Columns** if you want the replicate array to be automatically assigned by column (from top to bottom).
5. After you have assigned any well types to specific positions as outlined above, you can automatically fill the remaining positions with a specified well type. To do this, select the well type, the replicate number, the orientation, the direction of the samples, and then click on **Fill**.

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Figure 4-33 Template Dialog Box



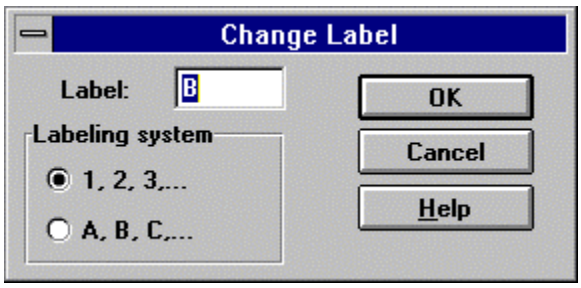
6. Use the Operation Buttons to further modify the template if necessary. The Operation Buttons that you will use for this are described below:

Button	Description
Change Label	Click on **Change Label** after you have highlighted the Well Type you want to rename. The Change Label dialog box (Figure 4-34) will be displayed. Type the new label (up to 3 characters) you assign to that type, select whether you want numerals or letters to designate different samples of that type, and then click on **OK**.
Undo	Undo the last action.
Randomize	Shuffle all the well type sample replicates to new, random locations.
Renumber	Renumber all well type samples into unbroken sequences. This is useful if the plate has been edited and some samples have been deleted leaving gaps in the number sequence.
Clear	Clear all the existing well assignments, so that you can redefine the template from the beginning.
Define	Define a series of wells that will be used for testing a serial dilution of a sample for a titer test. The procedure for doing this is detailed on the following page.

7. Click on **OK**.

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Figure 4-34 Change Label Dialog Box



Define

The Define dialog box (Figure 4-35) is used for serial dilutions. This dialog box allows you to place more than one well in a sample to form a block of wells (which can also be copied into replicates if necessary). This is useful if you are performing serial dilutions on the plate as part of a titer test.

For example, you may want to perform a test on a series of dilutions of unknown samples and on a different series of dilutions of a positive control:

- Undiluted unknown
- 1:2 dilution of unknown
- 1:4 dilution of unknown
- 1:8 dilution of unknown
- 1:16 dilution of unknown
- Undiluted control
- 1:8 dilution of control

The Define dialog box allows you to assign wells on the plate to serial dilutions for the sample types that you designate.

To define a template for serial dilution tests:

1. ***Sample Orientation:*** Select the direction on the plate that the serial dilutions are arranged. In the example above, the serial dilutions are arranged in columns.

2. ***Type:*** Select the first well type that you want to assign to the template.

> **Note:** The well type **Empty** designates a well in which undiluted sample was placed for subsequent dilution. These wells are not tested.

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3. ***Wells per Sample:*** Designate the number of wells that are allocated to the serial dilutions for the well type you designated in the previous step. For example, if the unknowns are being diluted 1:2 through 1:16 as outlined above, four wells should be designated. Include any samples that you are including in the dilution block as a control.

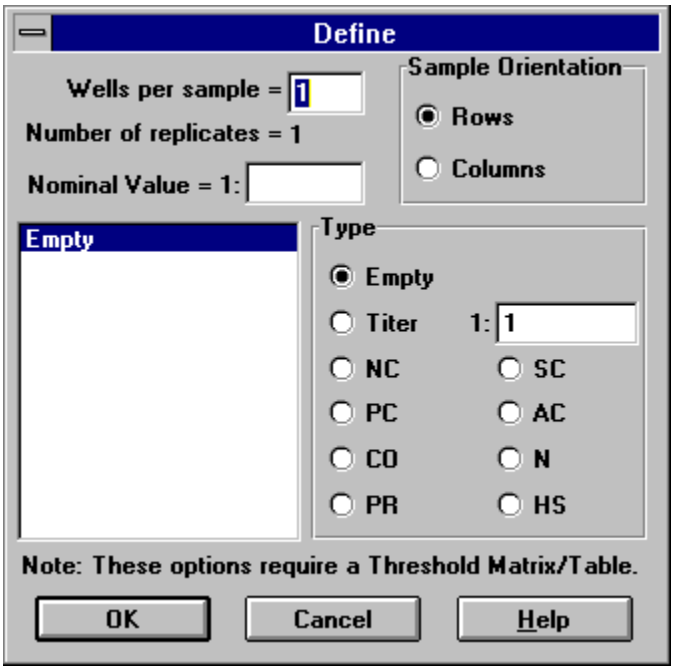
> **Note:** The number of replicates for each sample is established in the Template dialog box, as outlined in the previous procedure. The number of replicates is displayed here for information only.

4. ***Nominal Value:*** Enter the nominal value to be used by the MLX to check the results. The nominal value is the inverse of the dilution factor at which you expect the test results to switch from negative to positive (or vice versa).

> **Note:** The nominal value has a tolerance of a factor of two. For example, if you define a dilution block that contains five wells that are assigned dilutions of 1:1, 1:4, 1:16, 1:64, 1:256 and you expect the reaction to become positive at a dilution 1:16, the 1:16 dilution is the nominal value.
>
> When the results are processed, the MLX will check that the result which changed from negative to positive occurred within a factor of two of the 1:16 nominal value (1:8 to 1:32). If the change occurred outside this tolerance, the system will display a warning and ask if you want to discard the results

5. Click on **OK**.

Figure 4-35 Define Dialog Box



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Blank Mode

For certain applications, you may want to perform blank corrections. For example, you may wish to subtract the background luminescence of a reagent solution from the result. By selecting the appropriate Blank Mode setting, you can instruct the MLX to read the RLU value of a reagent blank sample and subtract this from all other RLU readings.

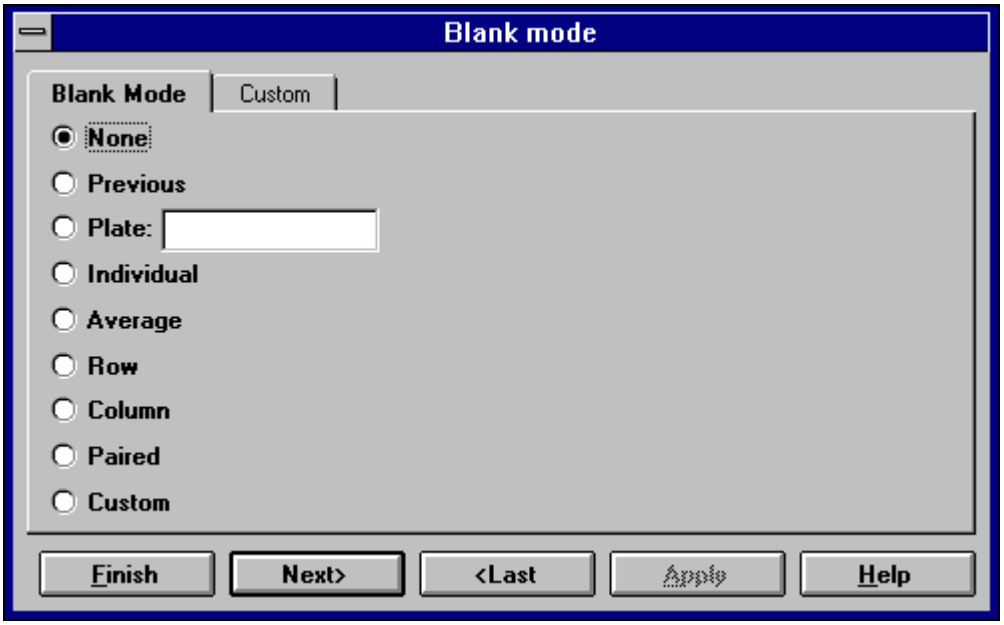
If you specify any blank mode other than **None** (see below), you must define at least one well on the template as a blank.

To specify the blank mode:

1. Click on **Settings** and then click on **Blank Mode**. The Blank Mode dialog box, containing the Blank Mode tab and the Custom tab will be displayed (Figure 4-36). Both tabs are described below:

Tab	Purpose
Blank Mode	Select a particular blank mode from the nine choices provided.
Custom	Specify one or more blank wells and specify the other wells that these blank results are to be subtracted from. **NOTE:** To use this tab, you must select **Custom** at the bottom of the Blank Mode tab.

Figure 4-36 Blank Mode Dialog Box



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2. Select the Calculation Mode you want by clicking on the appropriate radio button. The Blank Modes to choose from are described below.

Blank Mode	Description
None	Default blank mode. Blank samples do not need to be specified on the template.
Previous	Use the last blank value from the previous plate.
Plate	Subtracts the value obtained for each well of a previously run plate from the corresponding well on the current plate. You will be prompted for the blank plate ID at run time.
Individual	Each time the Reader encounters a Blank sample, the blank value is updated. This value is then subtracted from subsequent RLU readings until another Blank sample is read. Wells are read in the order A1 to H1, A2 to H2, etc.
Average	(This is commonly used) Average the RLUs from all of the Blank samples you define on the template. The average blank value is then subtracted from all the other well RLUs on the plate.
Row	Each row is treated separately with a separate blank sample. If no blanks were assigned to a row, no blank is used.
Column	Each column is treated separately with a separate blank sample. If no blanks are assigned to a column, no blank is used.
Paired	Used for subtracting the results of one test from the other (delta method). One blank sample is associated with each Test, Standard, or Control sample. The total number of blank samples must be equal to the total number of all the well type samples. Blank samples are assigned to different well types in the following order: 1 Tests 2 Standards 3 Controls 4 Positive Control 5 Negative Control 6 Cut-Off Control 7 User-Defined Controls #1 - #8 For a plate with 3 Standard samples and 8 Test samples, 11 Blank samples are required. The Blanks would be assigned in the following way: Blanks 1-8 are assigned to Test samples 1-8 Blanks 9-11 are assigned to Standard samples 1-3.
Custom	Allows you to assign specific Blanks to specific samples. See the following procedure for further details.

3. If you want to use the blank value from a plate that was run earlier, click on **Select** to display the Plate Data Files dialog box. Select the data file and then click on **OK**.

4. Click on **OK** in the Blank Mode dialog box.

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5. If you selected **Custom** in the previous step, click on the Custom tab. The Custom dialog box will be displayed (Figure 4-37), allowing you to assign specific Blanks to specific samples. The assigned Blanks will be subtracted from the specified samples at runtime.

6. All of the blank samples defined on the template are listed in the **Blanks:** box. Select any blank from this list.

7. Specify the sample that the blank is to be subtracted from by clicking on it in the **Subtract From:** Then, click on **Add**. The sample you specified will be displayed in the **Check list:** box.

   NOTE: If you want to subtract the blank from all of the samples, click on **Add All**.

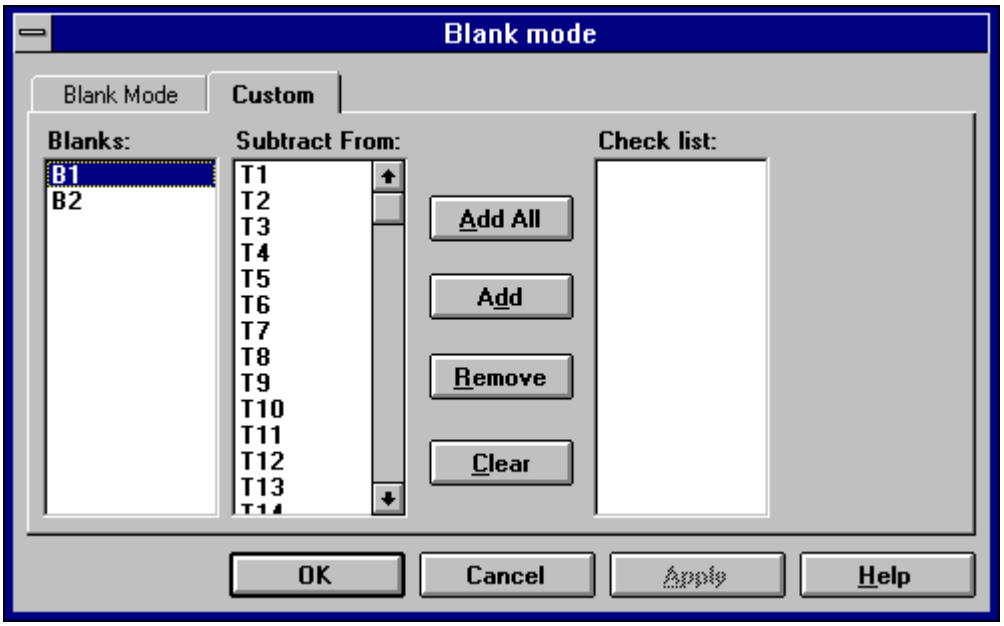
8. Repeat Step **5** to specify additional samples that the highlighted blank is to be subtracted from. As you add each sample, it will be added to the list in the **Check list:** box.

   NOTE: If you want to remove a sample from the **Check list:** box, highlight the sample in the box and then click on **Remove**.
   If you want to remove all of the samples from the **Check list:** box, click on **Clear**.

9. Repeat Steps **4** and **5** to specify a new blank and the samples that the highlighted blank is to be subtracted from. The list of samples in the **Check list:** box will be replaced with the new list you are creating.

10. Click on **OK**.

Figure 4-37 Custom Dialog Box



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QC: Raw Data

Use this feature to validate the RLU values that are obtained as the plate is being read. This extremely powerful function on the MLX allows you to automatically check RLU readings and verify whether RLU data for a particular sample or the entire plate are valid.

The Q.C.: Raw Data tests are carried out using **expressions** that you define. The expressions are similar to those used in computer programming and they must follow a prescribed format. Usually, more than one expression is used when defining the Q.C.: Raw Data test.

Creating an expression is not difficult, but it must be done correctly so that the MLX carries out the instruction that you intend.

NOTE: Q.C.: Raw Data tests are performed on the RLU readings obtained by the MLX. The tests are carried out before any secondary calculations such as Curve Fit or Threshold are performed.

Elements of an Expression

An expression is similar to a formula. An expression always consists of at least two of the following elements:

Element	Description
RLU Value:	A symbol that designates the RLU value that you are validating or the RLU value that you are comparing to.
RLU of a Particular Replicate	The well type designation, followed by the sample number, followed by the replicate number. The sample number and replicate number must be separated by a period. For example, the symbol “PC4.3” designates the RLU of replicate 3 of positive control sample number 4.
RLU of a Particular Sample	The well type designation, followed by the sample number. The RLU is the mean RLU across all of the replicates for that sample. For example, the symbol “PC4” designates the mean RLU obtained on positive control sample number 4.
RLU of Each Sample of a Well Type	The well type designation, followed by the letter “i”. The RLU is the mean RLU across all of the replicates for each sample. For example, the symbol “PCi” designates the mean RLU obtained on each positive control sample.
Mean RLU of a Well Type	The well type designation. The RLU is the mean RLU across all of the samples for that well type. For example, the symbol “PC” designates the mean RLU obtained on all of the positive control samples.
Operator:	A symbol that tells the expression what operation to perform on an identifier or other element. For example, “+” means that other values are added.
Arithmetic Operator	Position between two RLU values to designate ordinary mathematical calculations. For example, the ”+” operator means that the RLU values on either side of the symbol are added. The arithmetic operators that you can use are described below:

Symbol	Meaning
+	Add
-	Subtract
*	Multiply
/	Divide

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Element	Description
Operator: (Continued):	
Logarithmic Operator	Position before an RLU value to designate a logarithmic function of the RLU value. For example, the "log" symbol before an RLU value means the base 10 logarithm of the RLU value. The logarithmic operators that you can use are described below:

Symbol	Meaning
**	Raised to the power of (follow by a number)
log	Base 10 logarithm
alog	Inverse Base 10 logarithm
ln	Natural logarithm
exp	Inverse natural logarithm

Statistical Operator — Position before an RLU value to designate a particular statistic of the RLU values. For example, the expression "CV(PC)" means the coefficient of variation of the positive control RLUs. The statistical operators that you can use are described below:

Symbol	Meaning
SD	Standard Deviation
CV	Coefficient of Variation
Max	Maximum Value
Min	Minimum Value
Median	Median Value

NOTE: When you use the SD or a CV operator, the element that follows must be in parentheses. For example: CV(PC)<10.

Comparison Operator — Position after an RLU value or other element to designate comparison with another element. For example, the expression "CV(PC)<10" means that the coefficient of variation of the positive control RLUs must be less than 10. The comparison operators are described below:

Symbol	Meaning
==	Equals
=	Assign a value to (not the same as "equals")
<	Less than
<=	Less than or equal to
>	Greater than
>=	Greater than or equal to

Conditional Operator — Position before or after an RLU value or element to designate the conditions that you are applying to more than one RLU value. For example, the expression "If PC<10 then NC<2" means that if the mean RLU of the positive control is less than 10, then the mean RLU of the negative control must be less than 2.

Symbol	Meaning
If	Defines a base condition
then	Defines the state that is dependent
else	Defines the remaining outcome

NOTE: Insert a space after **if** and before and after **else** and **then**. This is the only place in an expression where a space is permitted.

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Element	Description
Other Elements:	Other elements, described below, allow you to enter specific numerical limits and to create an expression of greater complexity.
Numerals	Use to define a limit for an RLU, CV, SD, etc. In the expression "CV(PC)<10", the limit of 10 is entered as numerals (typed).
Parentheses	Parentheses are to specify the elements to precede or follow an operator, when the expression is ambiguous. For example, in the expression "PC-2*SD(PC)<=PCi<= PC+2*SD(PC)", the parentheses are necessary to specify that the RLU of each positive control must be within 2SD of the mean RLU of all positive controls. Up to six levels of parentheses can be used.
Commands:	Although commands are not required in an expression, it may be convenient to use one of the commands shown below.
Variable	Use to denote a variable included in Lot Specific Data that you define at run time. You can define up to 99 variables in this manner. For example, if you define three variables, they will be written as "Variable1", "Variable 2", and "Variable 3".
Valid	Use to define the minimum number of acceptable samples that must remain after other tests have been carried out to remove outliers, etc. For example, the expression "Valid(PC)>=2" means that there must be at least two positive controls with acceptable data. **NOTE:** When you use the Valid command, the element that follows must be in parentheses.
Verify	Use to check each sample or replicate against specified criteria, remove any values that do not pass from subsequent calculations, and check that the number of wells or replicates that pass meet or exceed the number specified. An expression containing "Verify" is of the form "Verify(Well ID,Value,Mode)", where Well ID: The wells to be tested. For example, if you want to check positive controls (PC), this can be either PC (for checking each sample), or PC1 (for checking each replicate). Value: A value to be tested against. This can be an absolute RLU (e.g. 123), a percentage value (e.g. 10%), a number of standard deviations (e.g. 1.5SD) or a measure of the coefficient of variation (e.g. 5.0CV). Mode: The calculation mode to be used. This can be either **0** (mean of the wells), **1** (exclusive mean, i.e. the mean of the other wells excluding the current well) or **2** (median of the wells). Verify will check each of the samples (or wells) against the defined Value. If the well being checked does not meet the condition it is removed from the calculations; otherwise it is deemed to be okay. When all of the wells have been checked, the Verify function will check that the number of wells or replicates that pass meet or exceed the number specified. When the value being tested against is either CV or SD, Verify works differently. Replicates or wells are removed one by one until the CV (or SD) of the remaining replicates or wells drops below the limit specified. The well that is removed is determined by calculating the difference between the data value and the mean of the group as specified by the "Mode" parameter. The replicate or well with the largest difference is removed first.
Commands:	Verify is used to streamline a set of expressions. For example, if you have

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Element	Description
Verify (Continued)	4 negative control wells (NC1.1 to NC1.4), and you want to use only those wells where the RLU is within 25% of the mean RLU of the other wells, and you want at least three wells to be valid, you can use the expressions: 0.75*(4*NC1-NC1i)/3<NC1i<1.25*(4*NC1-NC1i)/3 Valid(NC1)>=3 Or, using "Verify" the above expressions can be replaced with: Verify(NC1,25%,1)>=3

Constructing an Expression

Any expression you construct always defines the criteria for acceptability of the RLU value contained in the expression. For example, the expression “CV(PC)<10” means that whenever the CV of positive controls is less than 10, the RLUs of the positive controls will be considered acceptable.

Raw data quality control tests are usually defined for control samples.

NOTE: If Q.C.: Raw Data tests are applied to unknown samples, they typically are used to specify that RLU readings on replicate wells must be within a particular range.

Place the elements in an expression in the same order that you would state the criteria for acceptability. Therefore, if one of your criteria is “the CV of positive controls must be less than 10”, the expression should read “CV(PC)<10”. Or, if another one of your criteria is “the mean RLU of all positive controls must be between 500 and 600, the expression should read “500<=PC<=600”.

The expression may be relatively simple, such as those in the examples above, or they may be complex. For example, you can specify that the average RLU obtained on negative controls must not be greater than 0.2 except when the average RLU obtained on positive controls is greater than 0.7. In this case, you will accept an average RLU obtained on negative controls of up to 0.3:

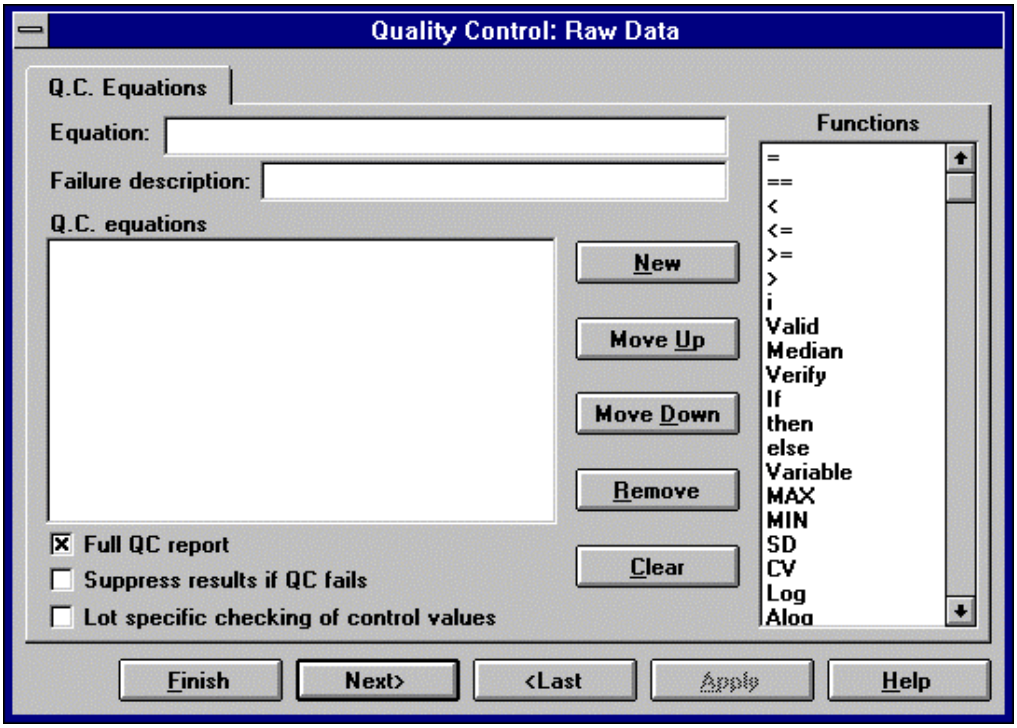
If PC>0.7 then NC<=0.3 else NC<=0.2

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To define Q.C.: Raw Data expressions:

1. Click on **Settings** and then click on **Q.C. Raw Data**. The Quality Control: Raw Data dialog box will be displayed (Figure 4-38).
2. Click on **New**.
3. Enter the expression in the **Equation:** text box. For convenience, you can select most elements that you need to build the expression from the **Functions** box. Type any numerical values or arithmetic operators.
4. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Equation:** box to the **Q.C. Equations** box. You can then construct the next expression by repeating the procedure from Step **3**.
5. If necessary, you can rearrange the expressions that are contained in the **Q.C. Equations** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.
6. You can remove an expression from the **Q.C. Equations** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Q.C. Equations** box by clicking on **Clear**.
7. Click in the **Failure Description:** text box. Then, type the message that you want displayed if the Q.C. Raw Data test is not passed.

Figure 4-38 Quality Control: Raw Data Dialog Box



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8. If you want all of the QC data (all equations, values, etc.) to be included in the results report, place an **X** in the **Full QC Report** check box. If you want to include only the QC equations that fail, leave the check box blank.

   NOTE: Use Report Options (page 174) to specify whether or not QC data are included in the results report.

9. If you do not want results to be printed if Raw Data QC Fails, place an **X** in the **Suppress Results if QC Fails** check box.

10. To check the recovered values on controls against lot-specific values, place an **X** in the **Lot specific checking of control values** check box.

    NOTE: If you select this option, a message box will prompt you to enter the expected values at run time if the values for the lot being used have not already been entered.

11. Click on **OK** when you have completed defining the Q.C. Raw Data expressions.

Multiple Expressions

It is likely that you will use more than one and possibly many expressions to define a complete test for Q.C. Raw Data. The expressions must be placed in the proper order. The order that you use will follow the particular logic that you establish for the test.

An unlimited number of Q.C.: Raw Data expressions can be defined for an assay. If the Q.C. Raw Data tests are not passed, assay results on the plate may not be valid. In this case, the results will either be flagged or suppressed, depending upon the selection you made in the Preferences dialog box (see Chapter 3) when setting up the MLX.

For example, you may want to subject the raw data from a plate to the following tests:

1. First, eliminate any positive control with a mean RLU that is not within 360 to 450.
2. Second, verify that at least two positive control samples remain.
3. Third, examine the RLU obtained for each test sample and verify that it is within the range of 20 to 600.

To carry out this test as outlined, construct the following expressions in the order shown below:

```
360<=PCi<=450
Valid(PC)>=2
20<=Ti<=600
```


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Threshold

The Threshold command is used for qualitative or semi-quantitative assays. The Threshold command allows you to specify RLU values that correspond to positive, negative, and intermediate cut-off points.

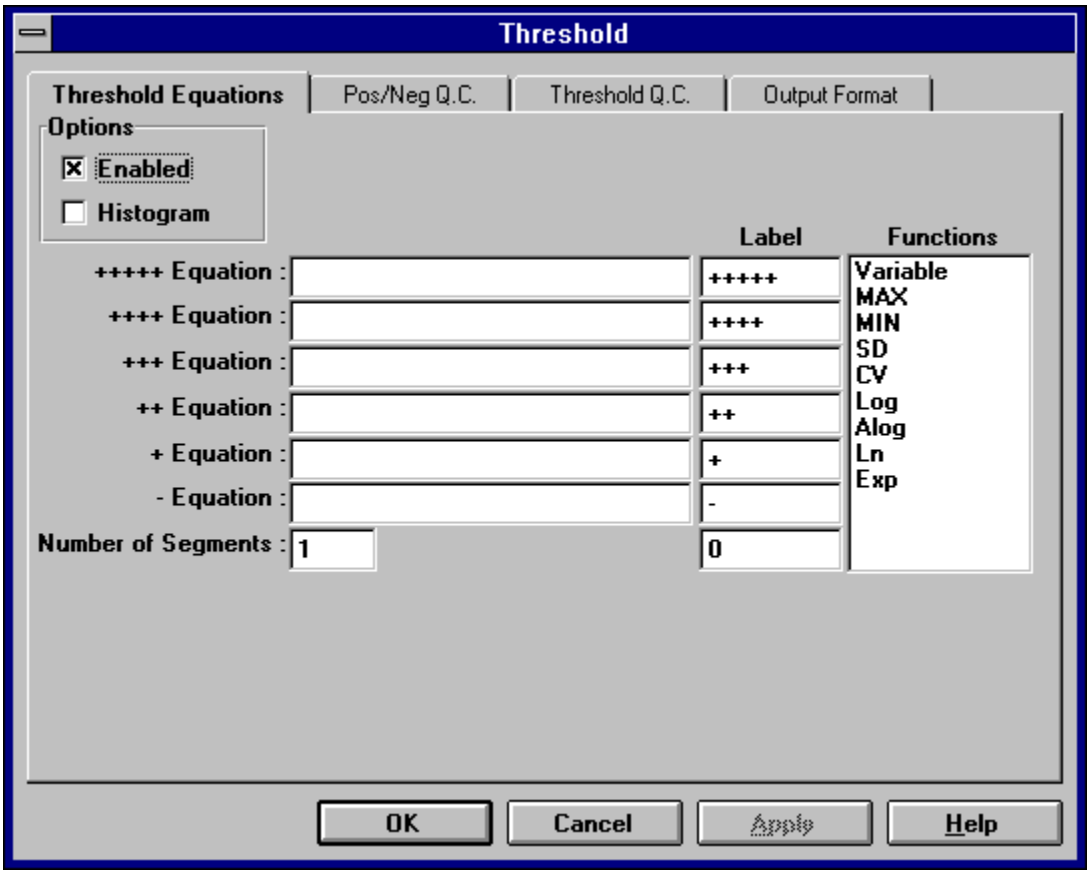
To specify Threshold settings:

1. Click on **Settings** and then click on **Threshold**. Four tabbed Threshold dialog boxes will be displayed (Figure 4-39). Select the appropriate tab as outlined below:

Tab	Purpose
Threshold Equations	Define positive and negative threshold cut-off points. For further information, see "Threshold Equations" on page 132.
Pos/Neg Q.C.	Assign a result (+++++, ++++, +++, ++, + or –) to each well type. For further information, see "Pos/Neg Q.C." on page 133.
Threshold Q.C.	Define an unlimited number of Threshold Quality Control (QC) equations which specify criteria for the results. If these criteria are not met you may choose to abort the test.
Output Format	Specify the output format of the processed results.

2. Instructions for using each dialog box are contained in the following sections.

Figure 4-39. Threshold Equations Dialog Box



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Threshold Equations

The Threshold Equations dialog box (Figure 4-39) allows you to enter RLU values that correspond to cut-off points for negative, positive, and intermediate qualitative or semi-quantitative results. Or, you can define these cut-off points relative to the RLU obtained on a specified well type such as a positive control.

A **negative limit** is set using the – equation, and a **positive limit** is set using the + equation. Positive and negative limits are always required. Four additional positive levels (++, +++, ++++ and +++++) are also available if you want to report semi-quantitative results.

Threshold limits can be entered directly as RLU values or as a simple expression. When RLU values are entered, a result is:

positive whenever its RLU is **greater than or equal to** the threshold
negative whenever its RLU is **less than** the threshold.

If the – value is lower than the + value, then RLU values below the – value are negative, RLU values equal to or greater than the + value are positive, and values in between are indeterminate (gray zone). If RLU values are inverse (a high RLU corresponds to a negative sample), then RLU values below the + value are positive and RLU values above the – value are negative.

If you use an expression for a cut-off value, use the designators for well RLU values and operators (described on page 125), numerals, and the functions that are shown in the **Functions** box. For example, you may decide that a negative sample will be any sample with an RLU that is 10 RLU units less than the mean RLU obtained on negative controls. This would be defined by the expression “NC-10”.

To define threshold equations:

1. ***Options:*** Place an **x** in the **Enabled** check box to enable using threshold equations.
   Place an **x** in the **Histogram** check box if you want to include a histogram of the results of the test samples in the results report. The histogram will graphically show the number of +++++, ++++, +++, ++, + and – results.
2. Create the expression that defines the negative (-) cut-off point. Type the expression in the **- Equation** text box.
3. Create the expression that defines the positive (+) cut-off point. Type the expression in the **+ Equation** text box.
4. If needed, create expressions that defines the intermediate (++, +++, ++++, and +++++) cut-off points. Type each expression in the appropriate text box.
5. ***Label:*** These boxes designate how each result will be labeled in the results report. To change a label, click on the corresponding box and delete the existing label. Type in the new label, of up to five letters or symbols.

   NOTE: Revelation also assigns labels to other types of data: ***** (Unread Well), * (Data Out of Range), and # (Difference Data). These labels can not be changed.

6. ***Number of Segments:*** If there is a large difference between the positive and negative cut-off points, many readings may fall between the two. This neutral area (the **grey zone**) can be divided into up to ten segments that are numbered 0 to 9. To specify the number of segments, type the number in the text box.
7. Click on **OK**.

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Pos/Neg Q.C.

The Pos/Neg Q.C. dialog box (Figure 4-40) allows you to assign an expected result (+++++, ++++, +++, ++, + or –) to each control well type. The RLU values corresponding to these qualitative results are those that are established in Threshold Equations (see page 132).

After averaging the replicate RLU values, the qualitative result that is obtained for each well (+++++, ++++, +++, ++, + or –) must agree with the expected result that you specify in Pos/Neg Q.C. or the well type will fail QC.

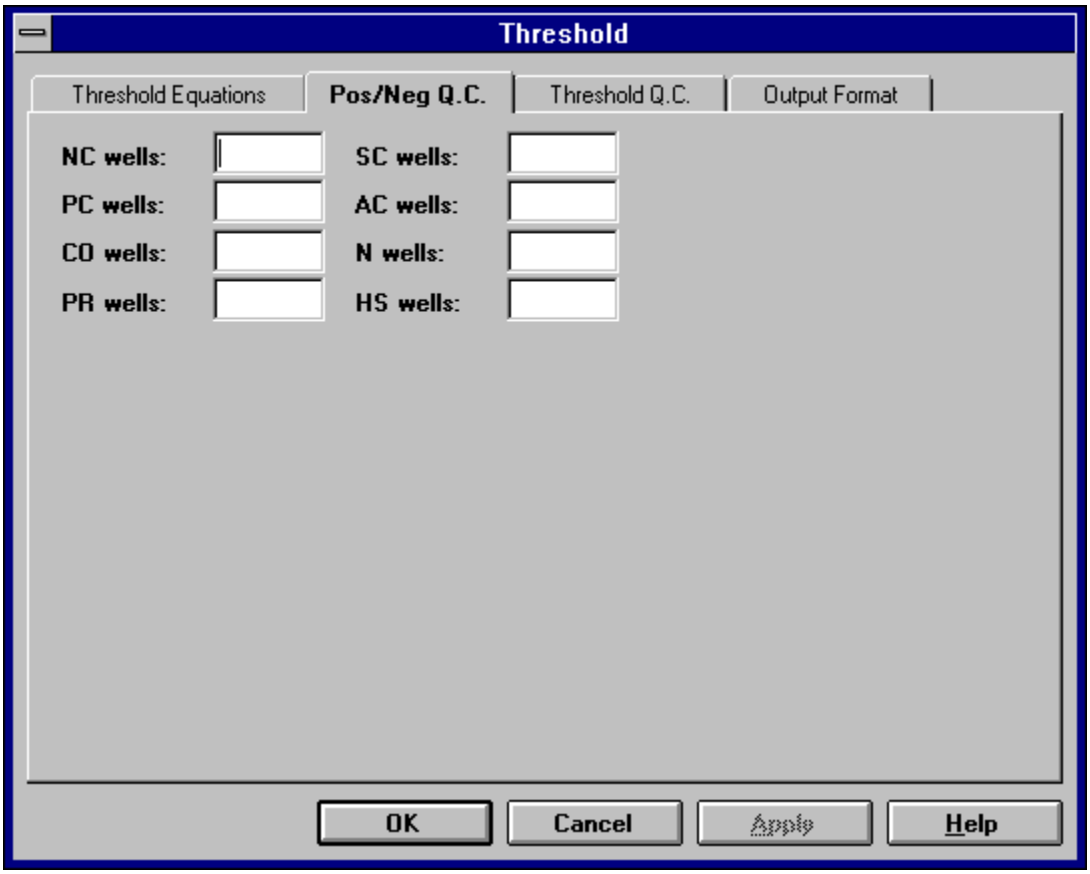
To define Pos/Neg Q.C. criteria:

1. For each well type, type the qualitative result that you expect.

   NOTE: Any of the boxes can be left blank. Or, you can type “RLU” in any box to cause those results to be displayed after the run so that you can manually verify the control results.

2. Click on **OK**.

Figure 4-40. Pos/Neg Q.C. Dialog Box



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Threshold Q.C.

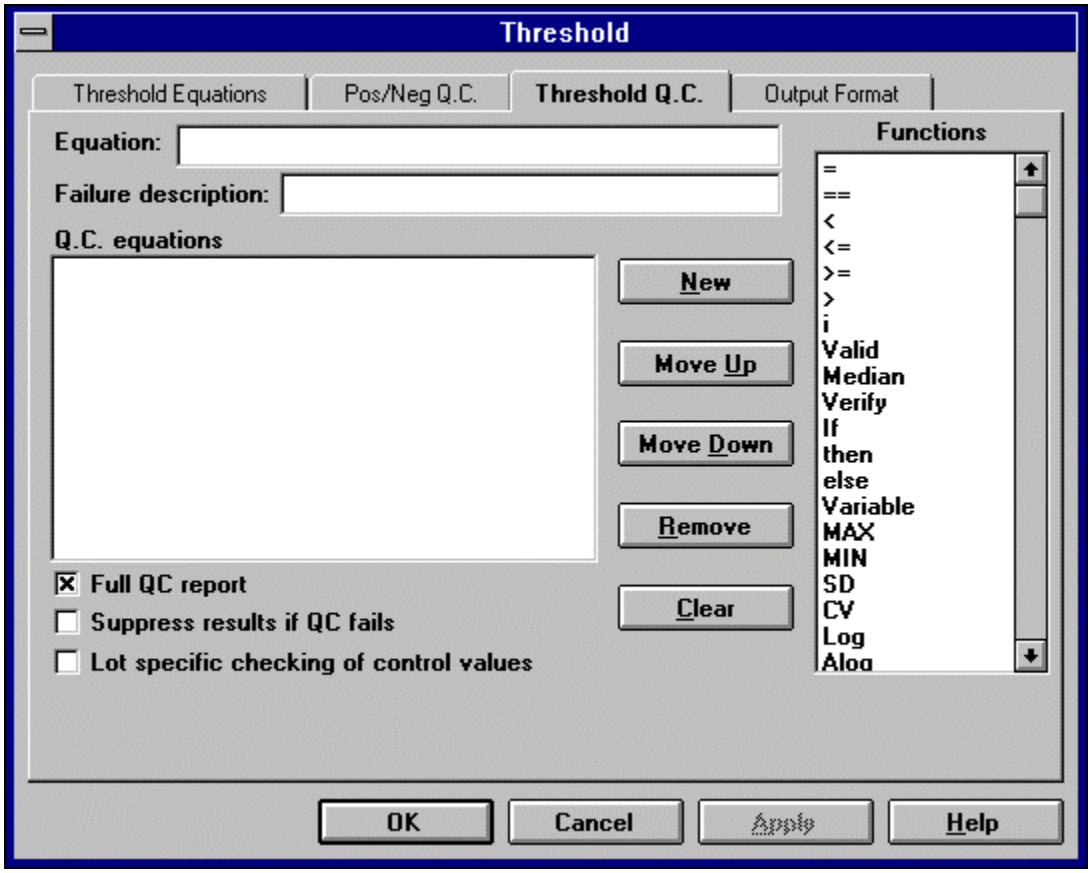
The Threshold Q.C. dialog box (Figure 4-41) allows you to define an unlimited number of Threshold Quality Control (QC) expressions, which specify criteria for the results. If these criteria are not met you may choose to abort the test.

The procedure for creating Threshold Q.C. Expressions is identical to the procedure for creating Q.C.Raw Data expressions. Refer to page 125 for detailed instructions on creating appropriate expressions.

To define Threshold Q.C. criteria:

1. Click on **New**.
2. Enter the expression in the **Equation:** text box. For convenience, you can select most elements that you need to build the expression from the **Functions** box. Type any numerical values or arithmetic operators.
3. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Equation:** box to the **Q.C. Equations** box. You can then construct the next expression by repeating the procedure from Step **2**.

Figure 4-41. Threshold Q.C. Dialog Box



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4. If necessary, you can rearrange the expressions that are contained in the **Q.C. Equations** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.
5. You can remove an expression from the **Q.C. Equations** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Q.C. Equations** box by clicking on **Clear**.
6. Click in the **Failure Description:** text box. Then, type the message that you want displayed if the Threshold Q.C. test is not passed.
7. If you want all of the QC data (all equations, values, etc.) to be included in the results report, place an **X** in the **Full QC Report** check box. If you want to include only the QC equations that fail, leave the check box blank.

   NOTE: Use Report Options (page 174) to specify whether or not QC data are included in the results report.

8. If you do not want results to be printed if Threshold QC Fails, place an **X** in the **Suppress Results if QC Fails** check box.
9. To check the recovered values on controls against lot-specific values, place an **X** in the **Lot specific checking of control values** check box.

   NOTE: If you select this option, a message box will prompt you to enter the expected values at run time if the values for the lot being used have not already been entered.

10. Click on **OK** when you have completed defining the Threshold Q.C. expressions.

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Threshold Output Format

The Output Format dialog box (Figure 4-42) allows you to specify the manner in which results are presented in the results report.

To specify the output format:

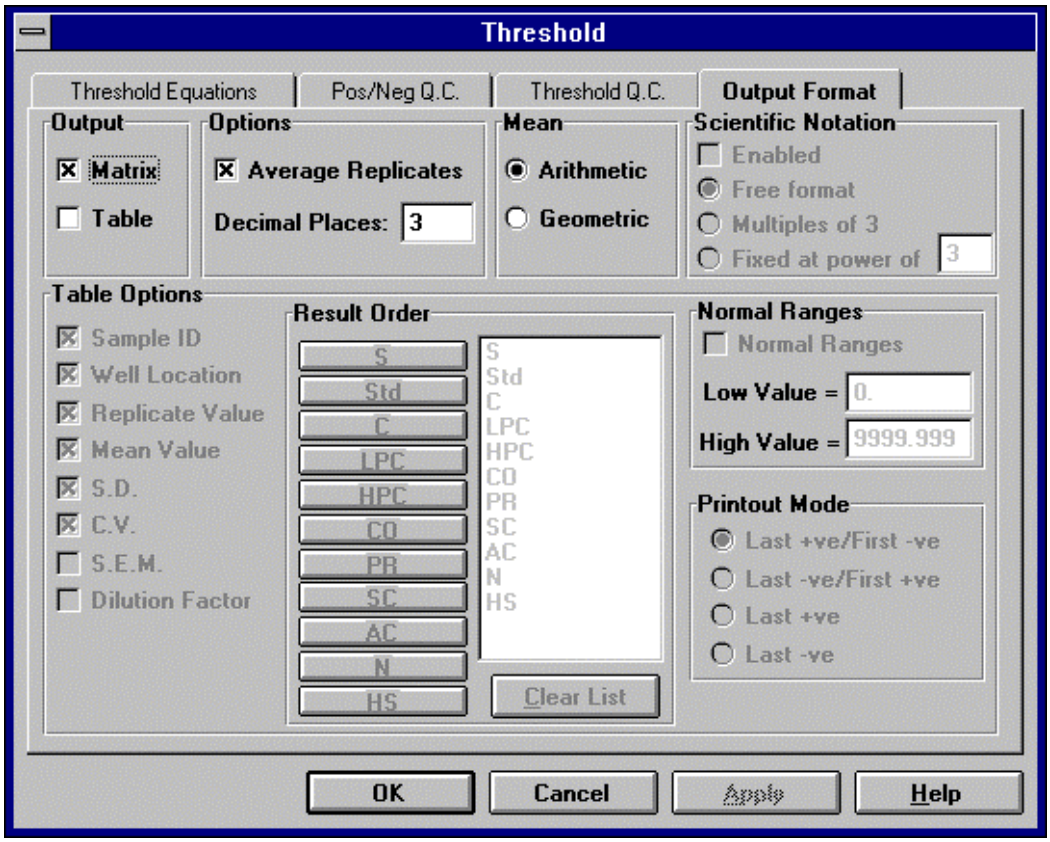
1. ***Output:*** Place an **x** in the **Matrix** check box to display the results from each well location on a diagram of the template. Place an **x** in the **Table** check box to display the results from each well location in a table organized by well type.
2. ***Options:*** Place an **x** in the **Average Replicates** check box if you want to average the calculated RLU values. Then, type the number of decimal places to be used for data calculations.

   NOTE: The default number of decimal places is **3**, and this is suitable for most applications. The maximum number of decimal places you can specify is **6**. The minimum number is **0**.

3. ***Mean:*** Click on the **Arithmetic** radio button to report the arithmetic mean of the replicates. Click on the **Geometric** radio button to report the geometric mean of the replicates.

   NOTE: Reporting the geometric mean (the antilog of the mean of the logarithms) is useful when the imprecision is directly proportional to the level.

Figure 4-42. Output Format Dialog Box



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4. ***Scientific Notation:*** Place an **x** in the **Enabled** check box to set the display mode for the results. Then, select whether you want to specify a **Free Format**, **Multiples of 3**, or **Fixed at power of** where you enter the exponent.

   NOTE: The default setting is for all results to be limited to eight significant figures. If you check the box you have three further options. **Free Format** allows the software to use scientific notation when appropriate. **Multiples of 3** limits the exponent to multiples of 3. **Fixed at power of** allows you to enter the exponent.

5. ***Table Options:*** (If **Table** is checked in Step **1**.) Place an **x** in the appropriate check boxes to specify the additional data to be displayed in the results table.

   NOTE: The raw value for the well type sample is always shown. Some table options are not available for certain data processing options, while some are obligatory. Options which are unavailable will be grayed. Options which are obligatory will also be grayed, and the corresponding check box will be checked.

6. ***Result Order:*** (If **Table** is checked in Step **1**.) Click on each well type in the order that you want the results to be presented. The order of results presentation will be shown in the adjacent box.

   NOTE: To delete the current order and create a new one, click on **Clear List** and repeat Step **5**. To place a well type at the top of the list, double-click on it. You can only click on **OK** when you have placed all the well types on the list.

7. ***Normal Ranges:*** Place an **x** in the **Normal Ranges** check box to flag with an asterisk (*) any results which fall outside the normal range you specify. Then, type the high and low values for the normal range.

   Note: If you have included a number of different processing options and used a number of tables in the output, Revelation may overwrite sections of data in the output. If this happens, try using matrices instead of tables, or remove one of the processing options.

8. ***Printout Mode:*** This specifies how results are printed when there are different sample types within a particular sample and the results are independently calculated. For example, if serial dilutions are defined (page 120), you may want to print the last positive (**Last +ve**) and/or the first negative (**First -ve**) results.

   Click on the **Last +ve/First -ve** button if you want the last positive and first negative samples to be reported in that order.

   Click on the **Last -ve/First +ve** button if you want the last negative and first positive samples to be reported in that order.

   Click on the **Last +ve** button if you want the last positive sample to be reported.

   Click on the **Last -ve** button if you want the last negative sample to be reported

9. After you have completed defining Output Format, click on **OK**.

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Curve Fit

The Curve Fit command allows you to specify all of the parameters that are required in order to report a quantitative result on the MLX. Ten dialog boxes are accessed by this command.

To specify Curve Fit settings:

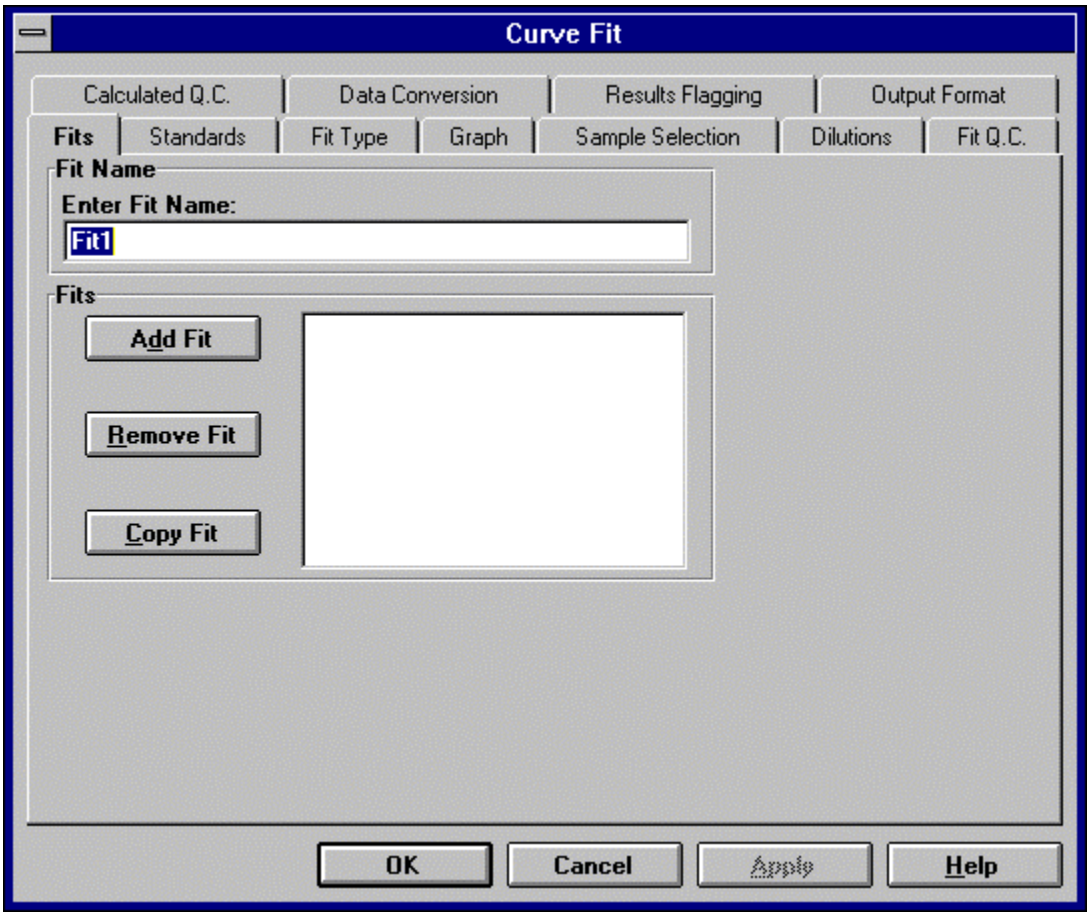
1. Click on **Settings** and then click on **Curve Fit**. Ten tabbed Curve Fit dialog boxes will be displayed (Figure 4-43). Select the appropriate tab as outlined below:

Tab	Purpose
Fits	Enter a name for the fit being defined, and add, remove, or copy fits from the displayed list. See "Fits" on page 139.
Standards	Select the standards for the curve fit calculations. See "Standards" on page 140.
Fit Type	Select the type of curve fit formula to be used and specify axes scales and results format. See "Fit Type" on page 142.
Graph	Use this dialog box if you want a graph to be included in your results report. Specify a graph name, axis labels and scaling, if a graph is requested. See "Graph" on page 145.
Sample Selection	Select the samples which are to be included in the curve fit. See "Sample Selection" on page 147.
Dilutions	Enter any dilutions made to the Test and Control samples. This option is only available if Concentration results format is selected. See “Dilutions” on page 148.
Curve Fit Quality Control	Apply QC expressions to the curve fit. See “Fit Q. C.” on page 150.
Calculated Q.C.	Create curve fit Quality Control equations. See "Calculated Q.C." on page 152.
Data Conversion	Convert data into different units. See "Data Conversion" on page 155.
Results Flagging	Define criteria for flagging of results or replacing out-of-range results with a phrase. See "Results Flagging" on page 157.
Output Format	Specify the output format of the processed results. See “Output Format" on page 159.

2. Instructions for using each dialog box are contained in the following sections.

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Figure 4-43. Curve Fit Dialog Box



Fits

The Fits dialog box (Figure 4-43) allows you to enter a name for the fit being defined and to add, remove, or copy fits from the displayed list. It is useful to copy an existing fit, give it a new name, and then edit it if you wish to reanalyze a plate with a fit that is different.

To add, remove, or copy a Fit:

1. ***Fit Name:*** Type the name that you will assign to the fit. Each fit must be given a name so that it can be distinguished from other fits used in the test.

   NOTE: You can also use **Fit Name** to change the name of a fit.

2. Click on **Add Fit** to add the fit name to the list displayed in the **Fits** box.
3. To remove a fit from the list, click on the fit and then click on **Remove Fit**.
4. To copy a fit from the list, click on the fit and then click on **Copy Fit**. The copied fit will be added to the list under the name you enter in Step **1**.
5. Click on **OK**.

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Standards

The Standards dialog box (Figure 4-44) allows you to select the standards that will be used to construct the standard curve.

To select the standards:

1. ***Select:*** This box displays all of the Standard samples that have been defined on the template. Click on the first Standard that you want to use for the standard curve. Then, click on **Add**. The standard that you selected will be displayed in the **Check list:** box.
2. Repeat Step **1** for each standard you want to use in the standard curve.

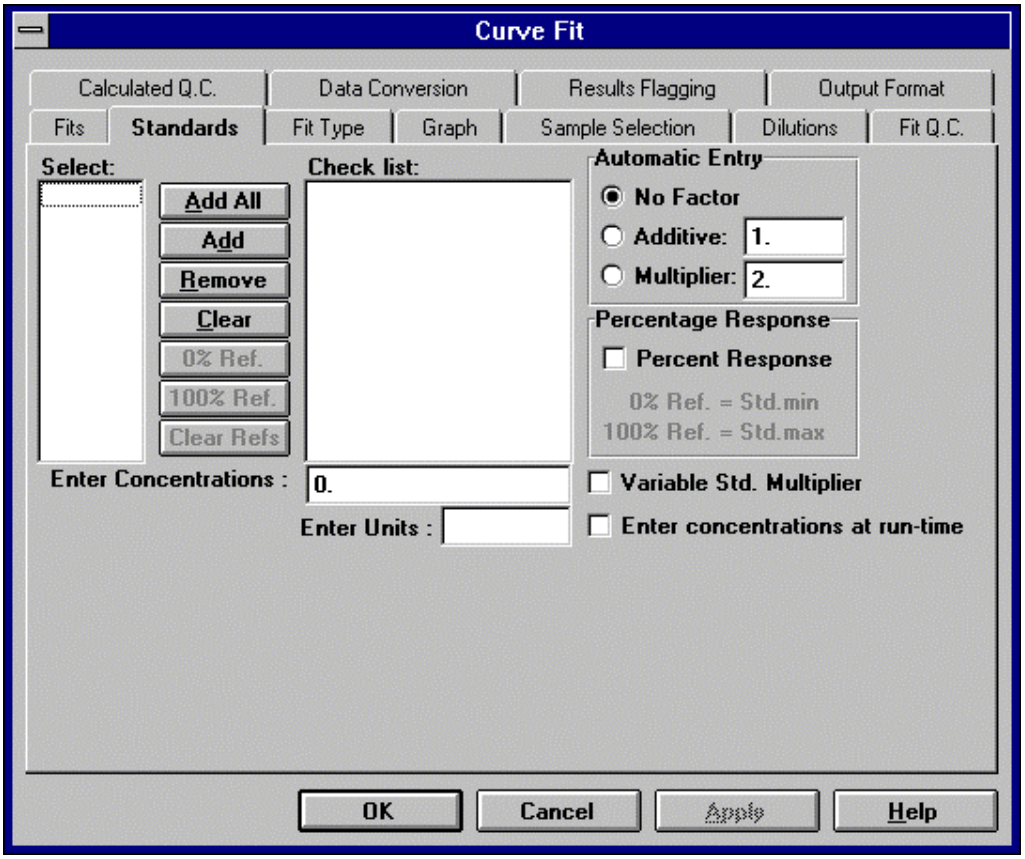
   NOTE: To select all of the standards, click on **Add All**.

3. Examine the standards that are listed in the **Check list:** box. Verify that only the standards you want to be used for the standard curve are displayed.

   NOTE: If necessary, you can remove a standard from the **Check List** box by clicking on it and then clicking on **Remove**. Or, you can remove all of the standards by clicking on **Clear**.

4. Click on one of the standards listed in the **Check list:** box. Then, type the concentration for that standard. Then, press **Enter** on the keyboard.

Figure 4-44 Standards Dialog Box



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5. Repeat Step **4** for each standard that is listed in the **Check list:** box.
6. Click in the **Enter Units:** box and type the units for the concentration. The concentration and units will be displayed next to the standards in the **Check list:** box.
7. ***Automatic Entry:*** If you prefer, concentration values for the standards can be calculated automatically. This can be done either by adding a specified value to the concentration of the lowest standard or by multiplying the concentration of the lowest standard by a specified value.

   NOTE: The default condition is for **No Factor** to be activated. Under those conditions, specify the concentration as outlined in the previous steps.

   To enter standard values by adding a specified value to the concentration of the lowest standard, click on **Additive:** and type the concentration value that is to be added. Then, click on the lowest concentration standard in the **Check list:** box, click in the **Enter Concentrations:** box, and type the concentration for that standard. Then, press **Enter** on the keyboard.

   To enter standard values by multiplying the concentration of the lowest standard by a specified value, click on **Multiplier:** and type the multiplication factor. Then, click on the lowest concentration standard in the **Check list:** box, click in the **Enter Concentrations:** box, and type the concentration for that standard. Then, press **Enter** on the keyboard.
8. ***Percentage Response:*** If you prefer, results can be expressed as a percentage of the RLU observed for the highest and/or lowest standard or as a percentage of the 10%, 50%, and/or 90% lethal concentration values.

   NOTE: By default, Revelation uses the lowest Standard sample RLU (**Smin**) as a reference point for the 0% and the highest Standard sample RLU (**Smax**) as a reference point for the 100% standard values.

   To express results as a percentage of the RLU observed for the highest and/or lowest standard, place a check mark in the **Percent Response** box. Then click in the **Check list:** box on the standard you specify as the 0% value and then click on **0% Ref**. Then, click in the **Check list:** box on the standard you specify as the 100% value and then click on **100% Ref**.

   To express results as the 10%, 50%, and/or 90% lethal concentration values, place a check mark in the **LC10**, **LC50**, and or **LC90** box.

   NOTE: Click on **Clear Refs** to reset to the default values.
9. Place an **x** in the **Variable Std. Multiplier:** box to multiply the concentration (or other units as appropriate) value of all the standards by a specified factor when the plate is run. If you select this option, a message box will be displayed when the plate is run, prompting you to enter the factor.
10. Finally, place an **x** in the **Enter concentrations at run-time** box if you want a message box to be displayed when the plate is run, prompting you to enter the labelled concentration values for each standard.
11. Click on **OK**.

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Fit Type

The Fit Type dialog box (Figure 4-45) allows you to select the curve fit formula that will be used, specify the scale for the standard curve axes, and specify the results format.

To define the fit type:

1. ***Fit Type:*** The curve fit formulas that you can choose from are described below. Select the curve fit formula by clicking on the appropriate radio button.

Fit Type	Description	Minimum Number of Standards
Linear	linear polynomial regression	1
Quadratic	quadratic polynomial regression	3
Cubic	cubic polynomial regression	4
Quartic	quartic polynomial regression	5
Polygon	point to point straight line fit	3
Cubic Spline	smooth curve cubic spline fit	3
Michaelis-Menten	hyperbolic fit	3
Sigmoid	S-shaped curve fit - four definable parameters.	4
Log/Logit	S-shaped curve fit - with fixed parameters	4
Akima	smooth curve which passes through all data points	3
Parallel	parallel line bioassay	4
Stored Curve...	See "Stored Curve" for complete details.	0

NOTE: A detailed description of each curve type is contained in the *Appendix*.

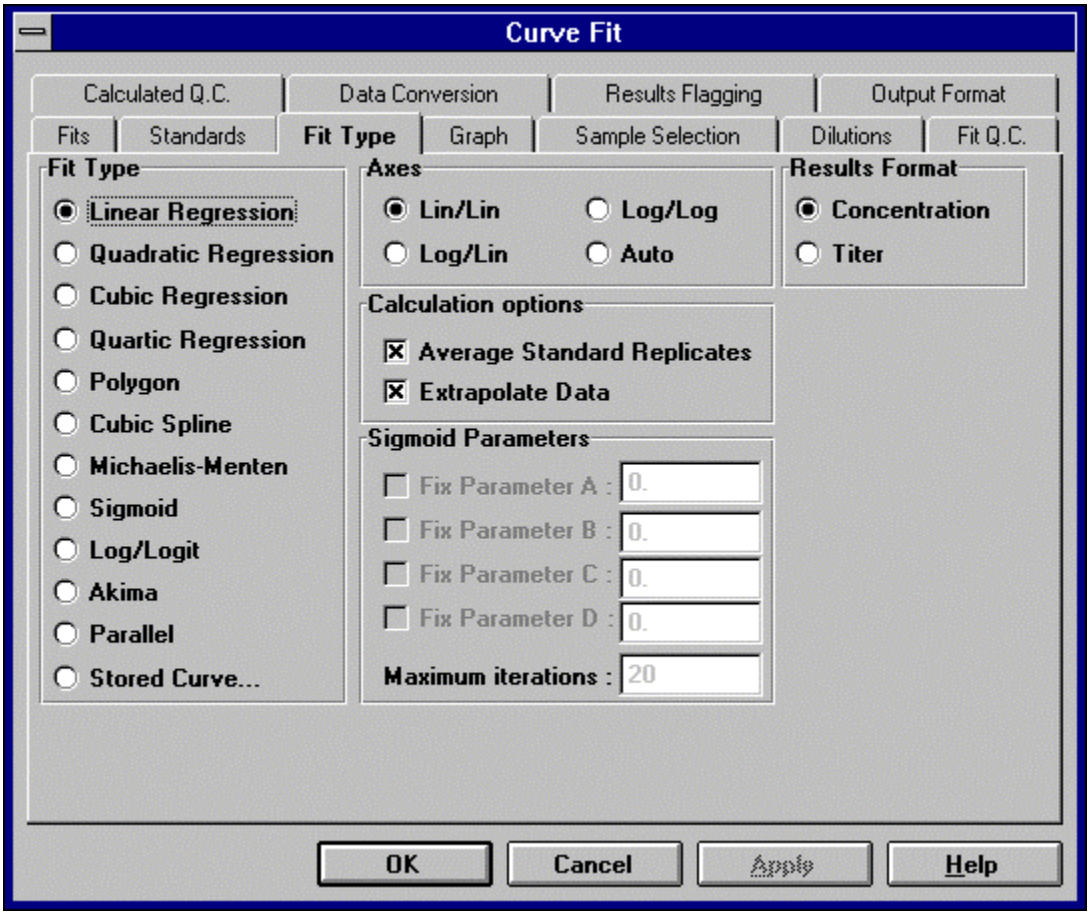
2. ***Axes:*** Scaling options for the axes are described below. Select a scaling option by clicking on the appropriate radio button.

NOTE: When you choose a fit type, scaling options which are not available for that type are grayed. If only one scaling option is available, all of the other options will be grayed and the required option will be automatically selected.

Scaling Option	Description
Lin/Lin	A linear scale is used for both axes.
Log/Log	A logarithmic scale is used for both axes.
Log/Lin	A logarithmic scale is used for the x axis and a linear scale is used for the y axis.
Auto	The best axis fit is automatically selected.

Figure 4-45 Fit Type Dialog Box

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3. ***Calculation Options:*** Place an **x** in the **Extrapolate Data** check box to extrapolate the concentration graph beyond the range of the Standard values.

   Place an **x** in the **Average Standard Replicates** check box if you want the individual standard replicates to be averaged before they are used for curve fitting.

   NOTE: The R-squared value will be increased when standard replicates are averaged. As a result, the automatic removal of outliers will be less effective. Refer to Fit Q.C. (page 150) for additional information.

4. ***Sigmoid Parameters:*** Place an **x** in the check box of any parameter you want to define. Then, type a value in the box.

   If required, type a value for the maximum iterations (the maximum number of times that the software will attempt to construct a satisfactory plot). The default value is **20**, and this is acceptable for most applications. If the plot can not be created within the maximum number of retries it is aborted.

   NOTE: The minimum number of iterations is **1**. The maximum number is **29,999**.

5. ***Results Format:*** If the test is a titer test, click on the **Titer** radio button. If the test measures concentration, click on the **Concentration** radio button.

6. Click on **OK**.

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Stored Curve

This option allows you to use a standard curve that was previously obtained to calculate the values obtained in the current assay.

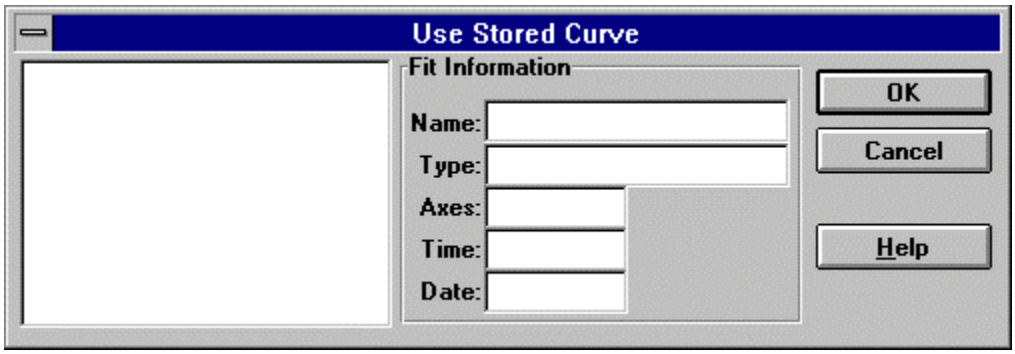
To specify a stored curve:

1. Select **Stored Curve** as the Fit Type (see previous procedure). The Use Stored Curve dialog box (Figure 4-46) is displayed.

2. Click on the curve that you wish to use. The Name, Type, and Axes of the curve you selected and the time and date that the curve was obtained will be displayed in the **Fit Information** box.

3. Click on **OK**.

Figure 4-46. Use Stored Curve Dialog Box



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Graph

The Graph dialog box (Figure 4-47) allows you to specify whether a graph of the standard curve is included in the results report. It also allows you to define the layout of the concentration graph by specifying a graph name, and axis labels and scaling.

To define the graph :

1. Place an **x** in the **Produce a Graph** check box if you want a graph of the standard curve to be included in the results report.

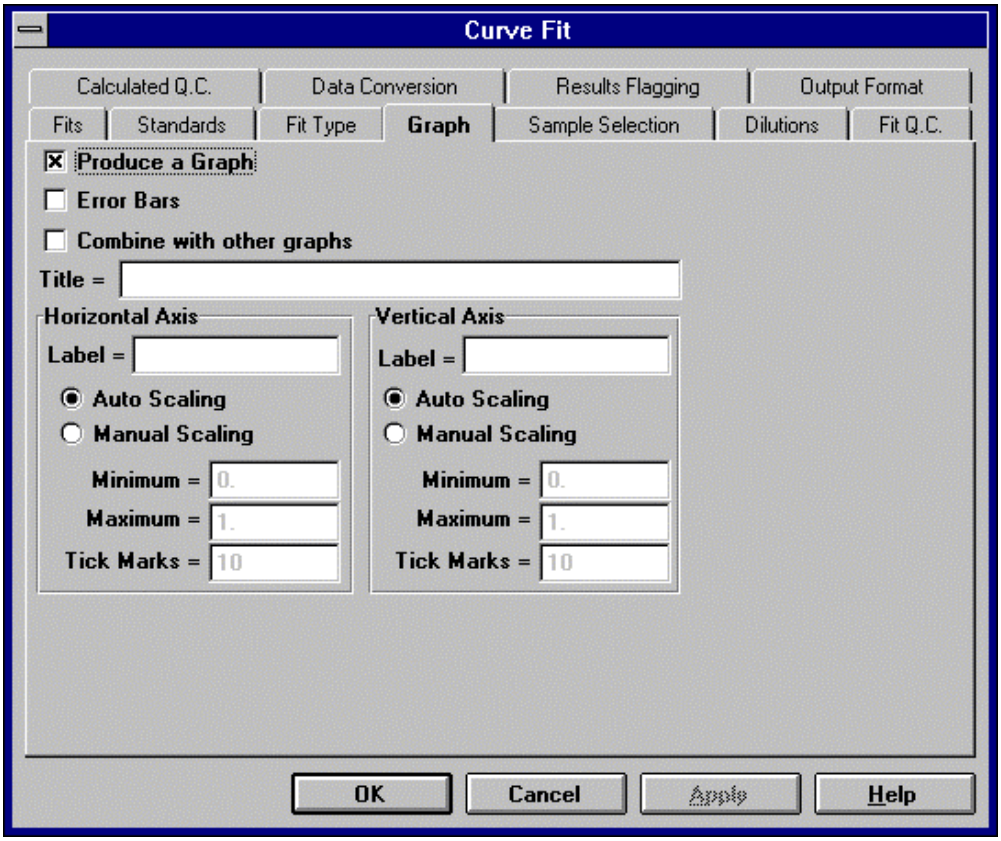
   NOTE: This box must be checked if you want a graph of the results to be displayed. Use Report Options (page 174) to specify whether or not standard curve data are included in the results report.

2. Place an **x** in the **Error Bars** check box if you want error bars included in the report. The error bars indicate the range of values for replicates.
3. Place an **x** in the **Combine with other graphs** check box if you want more than one standard curve to be shown on the graph.

   NOTE: The **Combine with other graphs** check boxes for the other standard curves must also be checked.

4. ***Title:*** Type the title that you want to be displayed above the graph.

Figure 4-47 Graph Dialog Box



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5. ***Horizontal Axis:*** Type the label that you want displayed for the horizontal axis. Then, select **Auto Scaling** to allow the system to automatically define the axis scale or select **Manual Scaling** to specify the scale.

   If you select **Manual Scaling**, click on **Minimum** and type the value for the axis minimum. Then, click on **Maximum** and type the value for the axis maximum. Finally, click on **Tick Marks** and type the number of divisions for the axis.

   NOTE: The first division is the start of the axis and the last division is the end of the axis.
   The minimum number of divisions is **2**, and the maximum number is **10**.

6. ***Vertical Axis:*** Specify the label and scaling for the vertical axis in the same manner you did for the horizontal axis.

7. Click on **OK**.

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Sample Selection

The Sample Selection dialog box (Figure 4-48) allows you to specify the samples which will be analyzed using the standard curve and will appear in the results report. Samples that are not selected will not be displayed in the report.

To select samples:

1. ***Samples:*** This box usually displays all of the test samples and controls that have been defined on the template. Click on the first samples for which results will be calculated. Then, click on **Add**. The sample that you selected will be displayed in the **Check list:** box.
2. Repeat Step **1** for each sample for which results will be calculated.

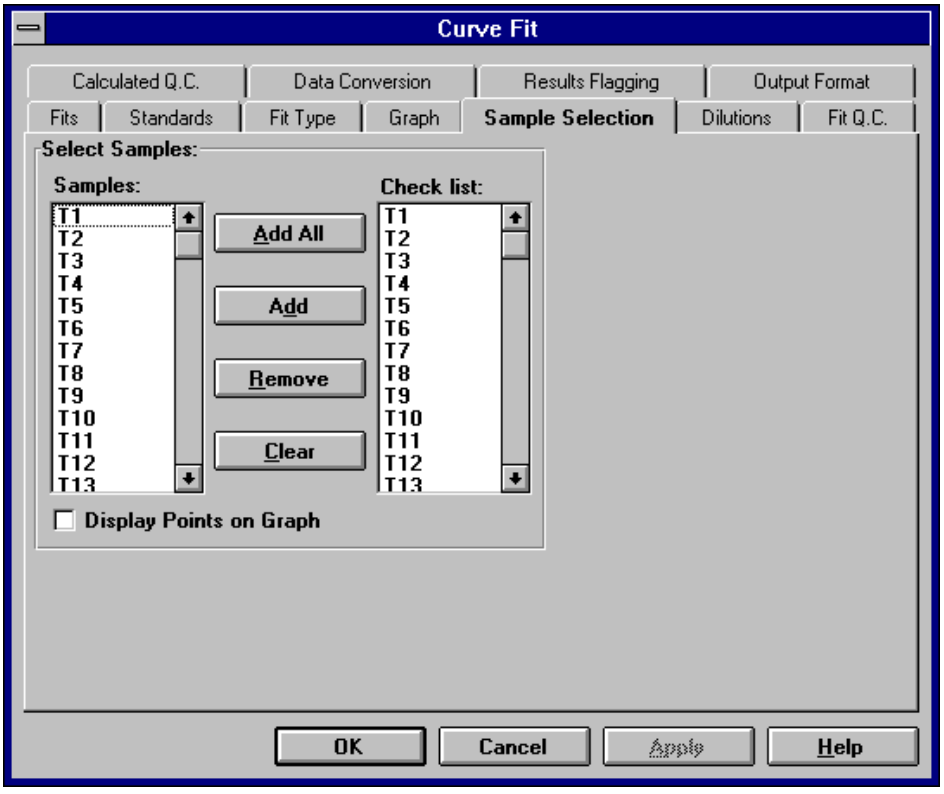
   NOTE: To select all of the samples, click on **Add All**.

3. Examine the samples that are listed in the **Check list:** box. Verify that only the samples for which results are to be calculated are displayed.

   NOTE: If necessary, you can remove a sample from the **Check List:** box by clicking on it and then clicking on **Remove**. Or, you can remove all of the samples by clicking on **Clear**.

4. Place an **x** in the **Display Points on Graph** check box if you want a point corresponding to the value obtained for each sample to be displayed on the standard curve.
5. Click on **OK**.

Figure 4-48 Sample Selection Dialog Box



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Dilutions

The Dilutions dialog box (Figure 4-49) allows you to enter any dilutions you will make to Test and Control samples.

NOTE: This option is only available if the **Concentration** results format has been selected.

To specify dilutions:

1. ***Enter Dilution:*** Click on one of the samples listed in the **Samples** box. Then, type the dilution factor for that sample. Then, press **Enter** on the keyboard.

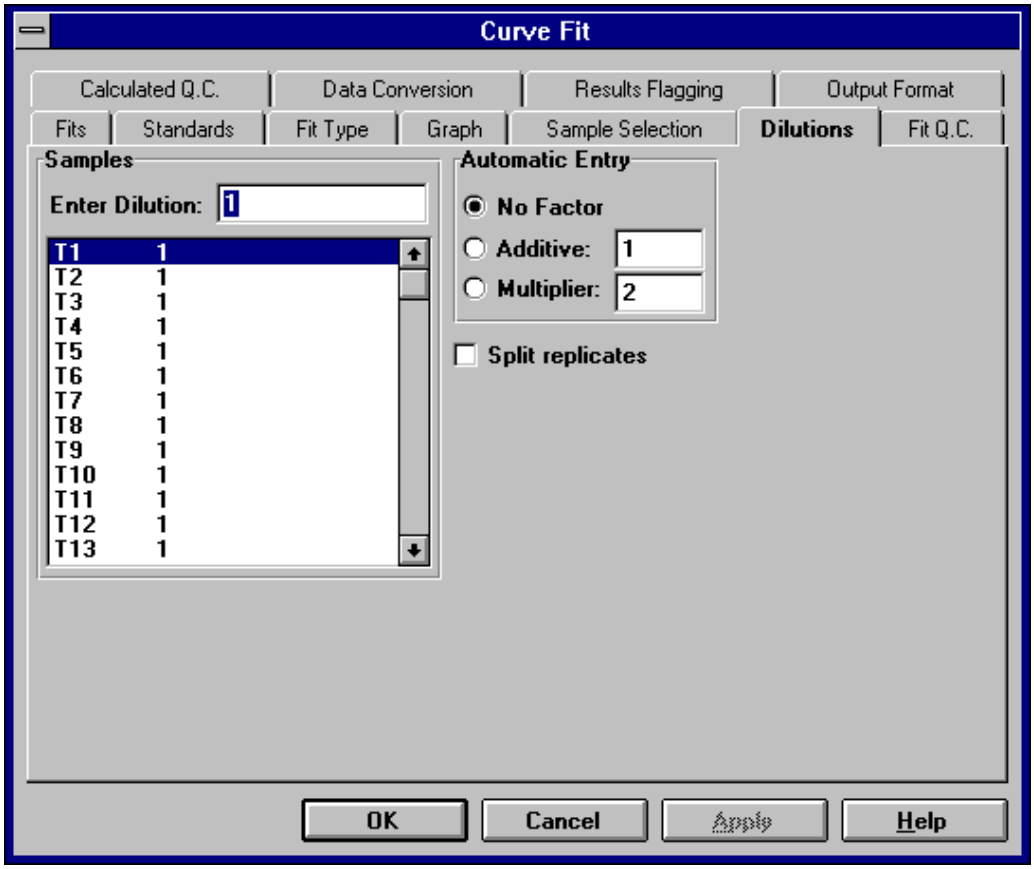
   NOTE: The dilution factor you enter is the inverse of the fractional reduction in concentration due to dilution. For example, if you dilute **1** mL of sample with **3** mL of diluent, the dilution factor is **4**.

2. Repeat Step **1** for each sample that is listed in the **Samples** box.

3. ***Automatic Entry:*** If you prefer, dilution factors can be entered automatically. This can be done either by incrementing successive dilutions by a specified value or by multiplying successive dilutions by a specified value.

   NOTE: The default condition is for **No Factor** to be activated. Under those conditions, specify the dilutions as outlined in the previous steps.

Figure 4-49 Dilutions Dialog Box



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To enter dilution factors by incrementing successive dilutions by a specified value, click on **Additive:** and type the incremental value that is to be added. Then, click on the lowest concentration sample in the **Samples** box and type the dilution factor for that sample. Then, press **Enter** on the keyboard. The incremental dilution will be applied to successive dilutions of the sample.

To enter standard values by multiplying successive dilutions by a specified value, click on **Multiplier:** and type the multiplication factor. Then, click on the lowest concentration sample in the **Samples** box and type the dilution factor for that sample. Then, press **Enter** on the keyboard. The incremental dilution will be applied to successive dilutions of the sample.

4. Place an **x** in the **Split Replicates** check box if you want the results on each replicate to be multiplied by the dilution factor. Leave the check box blank if you want the results to be multiplied after the replicates have been averaged.

5. Click on **OK**.

CAUTION: If a template is changed after dilution factors are entered, the dilution factors are NOT reset to **1**. Each dilution factor will remain associated with a well position (A1, D7, etc.) regardless of the well type (T, C, S, etc.) assigned in the template. Therefore, all dilutions should be reset to **1** before changing a template.

To reset dilutions to **1** for wells that will be redefined as Standards, click on **Settings**, click on **Curve Fit**, and then click on **Dilutions**. Change the dilution factor and save the assay. Then, click on **Settings**, click on **Template**, and make the appropriate well type changes.

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Fit Q. C.

The Fit Q.C. dialog box (Figure 4-50) allows you to apply statistical checks to the standard curve to verify that the relationship between the values obtained on the standards meet the criteria that you specify. These checks can help verify whether the standard curve you are using is acceptable. For example, if the slope obtained for the standard curve is less than the specified minimum, you may conclude that the run does not exhibit the required sensitivity. In addition, you can automatically remove outlier values when calculating the standard curve.

The statistical checks that are performed are described below.

Statistical Check	Description
R-Squared	The proportion of the variance of RLU values that can be attributed to the linear regression of the standard values. R-squared can range from 0 to 1.0. If R-squared is 1.0, there is no scatter in RLU values.
Residual	An estimate of the error of each observation. The residuals are expressed as a percentage of the value.
Slope	The change in RLU (the y-axis) relative to the change in the standard value (the x-axis).
Y-Intercept	The RLU value (the y-axis value) that corresponds to a standard value (x-axis value) of zero on the standard curve.

To specify curve fit quality control:

1. ***Quality Control:*** Place an **x** in the **Curve Fit Quality Control** check box if you want to enable curve fit quality control.
2. Type a value for **Minimum R-Squared**.

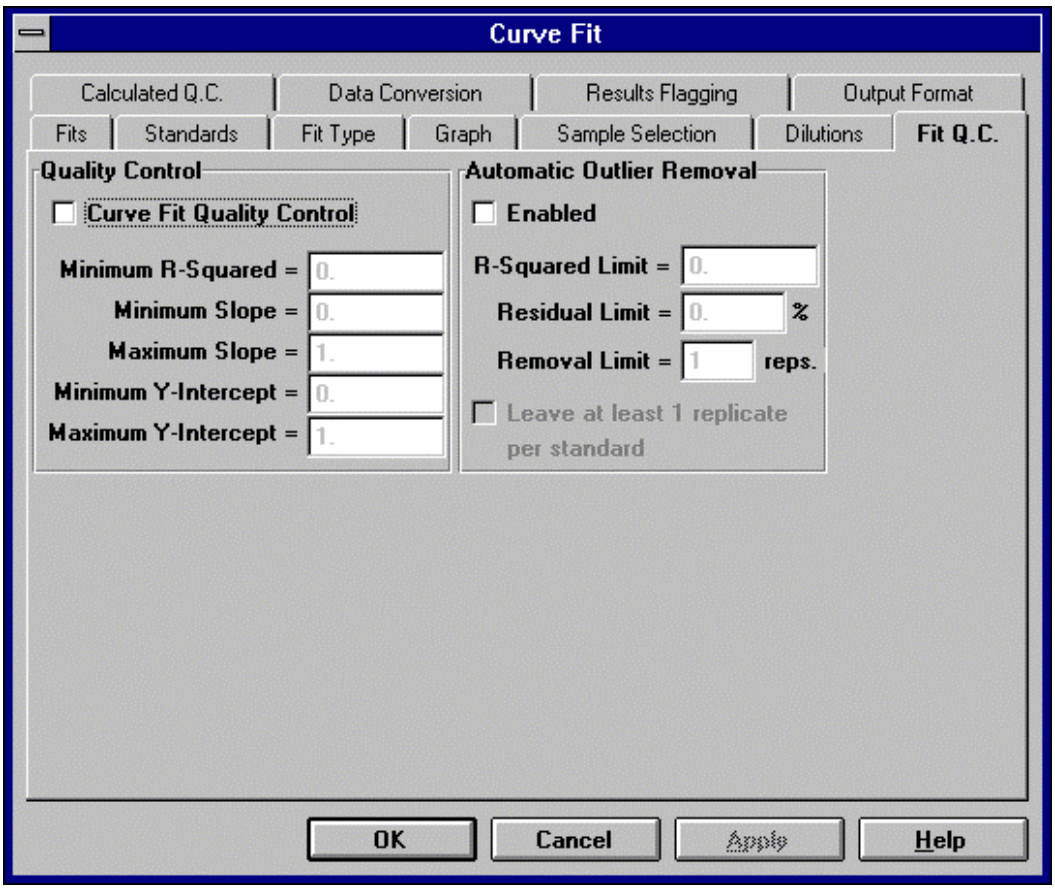
   NOTE: The value for **Minimum R-Squared** must be between **0.0** and **1.0**. The closer the value you enter is to **1.0**, the tighter the requirements are for scatter of values about the line.

3. Type values for **Minimum Slope** and **Maximum Slope**.
4. Type values for **Minimum Y-Intercept** and **Maximum Y-Intercept**.
5. ***Automatic Outlier Removal:*** Place an **x** in the **Enabled** check box if you want to enable automatic outlier removal.
6. Type a value for **R-Squared Limit**.

   NOTE: The value for **R-Squared Limit** must be between **0.0** and **1.0**.

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Figure 4-50. Fit Q.C. Dialog Box



7. Type a value for **Residual Limit**.

8. Type a value for **Removal Limit**.

9. Place an **x** in the **Leave at Least 1 Replicate** check box if you want to leave at least one replicate for each standard level.

10. Click on **OK**.

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Calculated Q.C.

The Calculated Q.C. checks allow you to verify that the calculated numerical results obtained on certain samples fall within the criteria that you establish. Typically, the Calculated Q.C. function is used to verify that the results obtained on control samples fall within the allowable range.

NOTE: Calculated Q.C. tests are performed on the values obtained by the MLX **after** secondary calculations such as Curve Fit or Threshold are performed. Use the Q.C. Raw Data function (see page 125) to check RLU values **before** calculations are performed.

The Calculated Q.C. tests are constructed using expressions in the same manner as you constructed tests for Q.C. Raw Data (see page 125). The only difference is that the elements you are testing refer to the calculated result rather than an RLU value. These elements are described below.

Element	Description
Calculated Value:	A symbol that designates the calculated value that you are validating or the calculated value that you are comparing to.
Value of a Particular Replicate	The well type designation, followed by the sample number, followed by the replicate number. The sample number and replicate number must be separated by a period. For example, the symbol “PC4.3” designates the calculated value of replicate 3 of positive control sample number 4. **Note:** Usually, you will have averaged RLU values of replicates before calculation of values, and you will not use this designation.
Value of a Particular Sample	The well type designation, followed by the sample number. The value is the mean value across all of the replicates for that sample. For example, the symbol “PC4” designates the mean value obtained on positive control sample number 4.
Value of Each Sample of a Well Type	The well type designation, followed by the letter “i”. The value is the mean value across all of the replicates for each sample. For example, the symbol “PCi” designates the mean value obtained on each positive control sample.
Mean Value of a Well Type	The well type designation. The value is the mean value across all of the samples for that well type. For example, the symbol “PC” designates the mean value obtained on all of the positive control samples.

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The expressions you construct define the criteria for acceptability of the calculated values. For example, you can check the calculated concentration value obtained for each positive control sample to verify that it is within an acceptable range of 72 µg/dL to 88 µg/dL. Then, assuming you are running two positive control samples on the plate, you can specify that both must pass this test for the assay results on the plate to be valid.

In this example, two expressions must be defined:

1. The first expression will test the concentration obtained on each positive control sample to verify that it is within an acceptable range of 72 µg/dL to 88 µg/dL:

   72<PC<88

2. The second expression specifies that both positive control samples must satisfy the criteria for the results to be valid:

   valid(PC)>=2

The expressions that you define must be stated in the same order that they are to be carried out. In the above example, testing of the concentration obtained on each positive control sample must be carried out before the number of samples that satisfy the criteria can be determined.

An unlimited number of Calculated Q.C expressions can be defined for an assay. If the tests are not passed, assay results on the plate may not be valid. In this case, the results will either be flagged or suppressed, depending upon the selection you made in the Preferences dialog box (see Chapter 3) when configuring the MLX.

To define Calculated Q.C. expressions:

1. Enter the expression in the **Equation:** text box.

   NOTE: Refer to "QC: Raw Data" on page 125 for the convention you must use to refer to the concentration of a sample and the components of the expression.

2. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Equation:** box to the **Q.C. Equations** box. You can then construct the next expression by repeating the procedure from Step **1**.

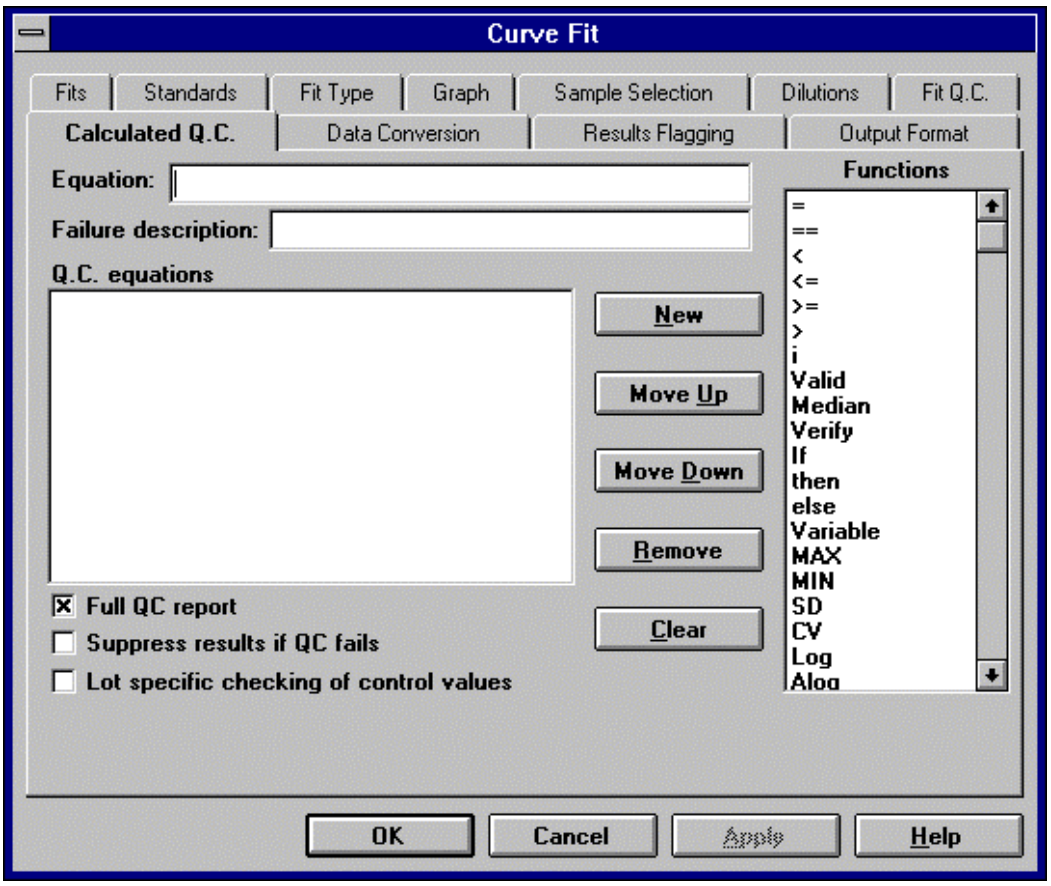
3. If necessary, you can rearrange the expressions that are contained in the **Q.C. Equations** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.

4. You can remove an expression from the **Q.C. Equations** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Q.C. Equations** box by clicking on **Clear**.

5. If you want a message describing the Q.C. failure to be displayed or printed on the results report when a failure is experienced, type the message in the **Failure Description:** box.

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Figure 4-51. Calculated Q.C. Dialog Box



6. If you want all of the QC data (all equations, values, etc.) to be included in the results report, place an **X** in the **Full QC Report** check box. If you only want to include only the QC equations that fail, leave the check box blank.

   NOTE: Use Report Options (page 174) to specify whether or not QC data are included in the results report.

7. If you do not want results to be printed if Calculated QC Fails, place an **X** in the **Suppress Results if QC Fails** check box.

8. To check the recovered values on controls against lot-specific values, place an **X** in the **Lot specific checking of control values** check box.

   NOTE: If you select this option, a message box will prompt you to enter the expected values at run time if the values for the lot being used have not already been entered.

9. Click on **OK**.

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Data Conversion

The Data Conversion dialog box (Figure 4-52) allows you to convert the data into different units. An expression of up to 50 characters can be defined to convert the results from one set of units to another.

NOTE: This feature is typically used to convert units to or from Scientific International Units (SIU).

The expression to convert the data is constructed using the same rules and many of the same elements that are used for constructing the Q.C.: Raw Data expression (page 125). The only difference is that a new element ("Sample") is used designate the current calculated value (the value before conversion) for each well. The use of "Sample" is outlined below:

Element	Description
Sample	If you insert "Sample" into an expression, the data conversion will be performed on each well to create new individual results.
Sample*n*	If you insert "Sample" followed by a numeral into an expression, the data conversion will be performed on the designated replicate of each well.

For example, you may wish to convert RLU values into a percentage value, where 0% is the negative control RLU and 100% is the positive control RLU. To do this, create the expression

100*(Sample-NC)/(PC-NC).

Or, you may wish to convert concentration values of mg/dL into SIU values of mmol/L. If 1 mg/dL of the analyte is equivalent to a concentration of 3 mmol/L in S.I. Units, then the expression

Sample*3

will convert the results to the S.I. Units of mmol/L.

To define data conversion:

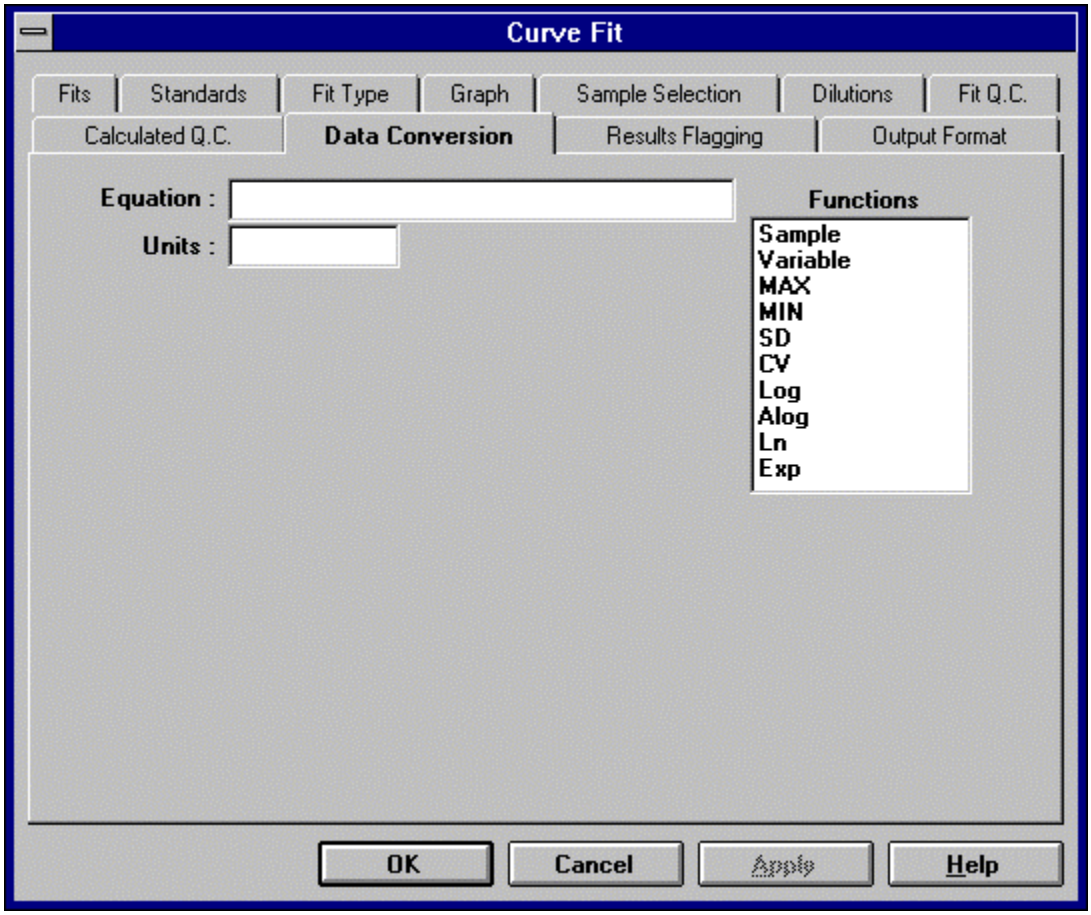
1. Enter the expression for converting the data in the **Equation:** text box. For convenience, you can select each function that you need to build the expression from the **Functions** box. Type any numerical values or arithmetic operators.

   NOTE: Refer to "QC: Raw Data" on page 125 for the convention you must use to refer to the concentration of a sample and the components of the expression.

2. After you have completed the expression, type the new units in the **Units** box.
3. Click on **OK**.

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Figure 4-52 Data Conversion Dialog Box



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Results Flagging

The Results Flagging dialog box (Figure 4-53) allows you to specify a phrase that either replaces a numerical result or is displayed next to the result. The phrase or flag will be initiated whenever the numerical result meets criteria that you establish. For example, you may want to report the phrase "<20 mg/dL" and suppress the actual numerical result whenever the results are lower than 20 mg/dL. Or, you may want to display a flag (for example "Abnormal High") next to the numerical result whenever the result exceeds the linear range of an assay. Results Flagging does not fail an assay but allows you to alter the presentation of the data.

NOTE: Results Flagging is performed on raw RLU values or calculated sample values obtained by the MLX **after** other calculations such as Threshold and/or Ratio are performed. If a phrase or flag is initiated, it will replace the result generated by a previous operation.

The Results Flagging criteria are constructed using expressions in the same manner as you construct tests for Q.C. Raw Data (see page 125). Note that, unlike Q.C. equations, Results Flagging conditions must evaluate as true for the result or flag to take effect.

Several unique elements are used for constructing a Results Flagging expression. These are described below.

Element	Description
Type( )	Use the "Type" keyword to designate the well type (Standards, Controls, Tests, etc.) to which a Results Flagging equation is applied. For example, the expression "Type (C)==0" means that this equation applies only to the control wells.
Sample (for RLU Values)	Use the "Sample" keyword to designate the RLU value of a particular sample, replicate or well type in the same manner as you used them for Q.C. Raw Data.
Result(Sample) (for Calculated Values)	Use the "Result(Sample)" construct to designate the calculated concentration of a sample instead of the RLU values that were obtained. For example, you can create the expression Result(Sample)<123 to set a lower limit of 123 mg/dL for the calculated value of any test sample. Then, whenever the value is less than 123 mg/dL, the phrase you enter for that expression or the Flag you designate will be triggered.
and, **or** Operators	Use the "and" or "or" operator in a Results Flagging phrase to test multiple conditions.

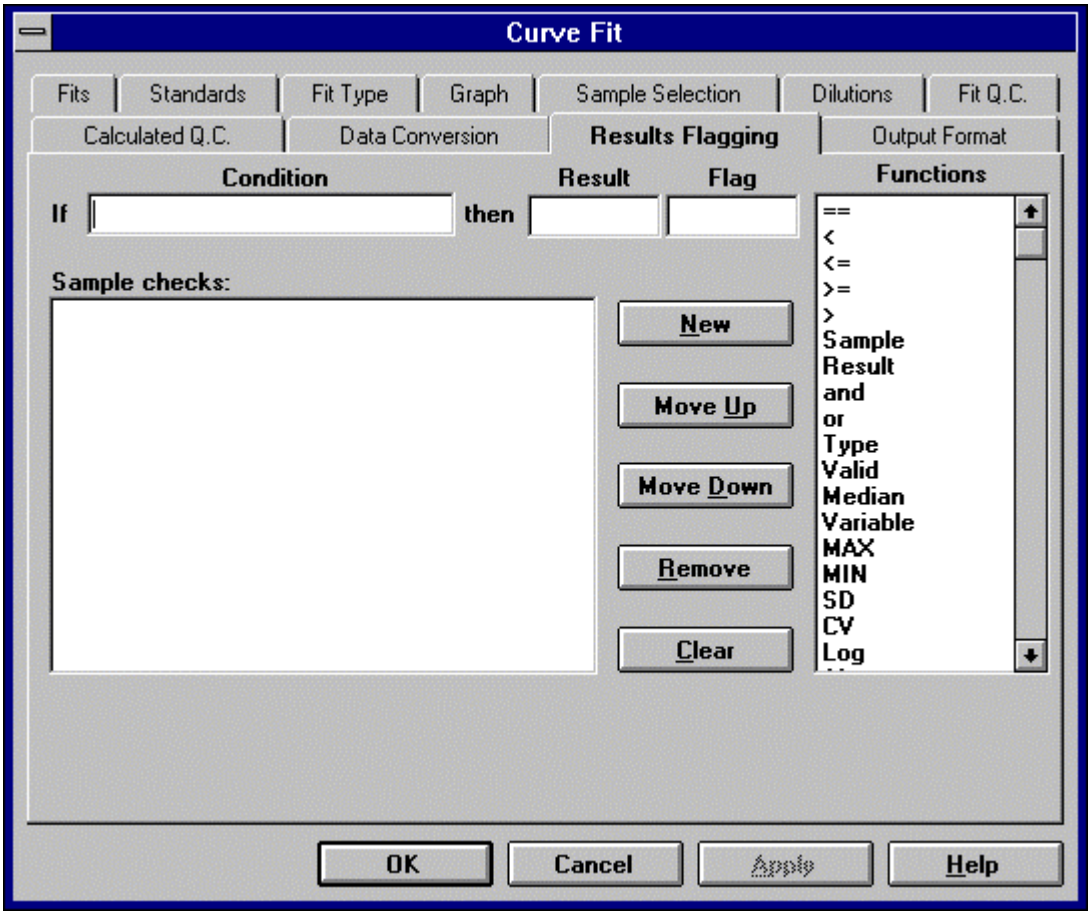
An unlimited number of Results Flagging expressions can be defined for an assay.

To define Results Flagging expressions:

1. Enter the expression in the **Condition** text box.
2. If you want the numerical result to be replaced by a phrase when the condition is exceeded, enter the phrase for that expression in the **Result** text box. If you want the numerical result to be accompanied by a flag when the condition is exceeded, enter the phrase for that flag in the **Flag** text box.

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Figure 4-53 Results Flagging Dialog Box



3. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Condition** box to the **Sample checks:** box. You can then construct the next expression by repeating the procedure from Step **1**.
4. If necessary, you can rearrange the expressions that are contained in the **Sample checks:** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.
5. You can remove an expression from the **Sample checks:** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Sample checks:** box by clicking on **Clear**.
6. Click on **OK**.

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Output Format

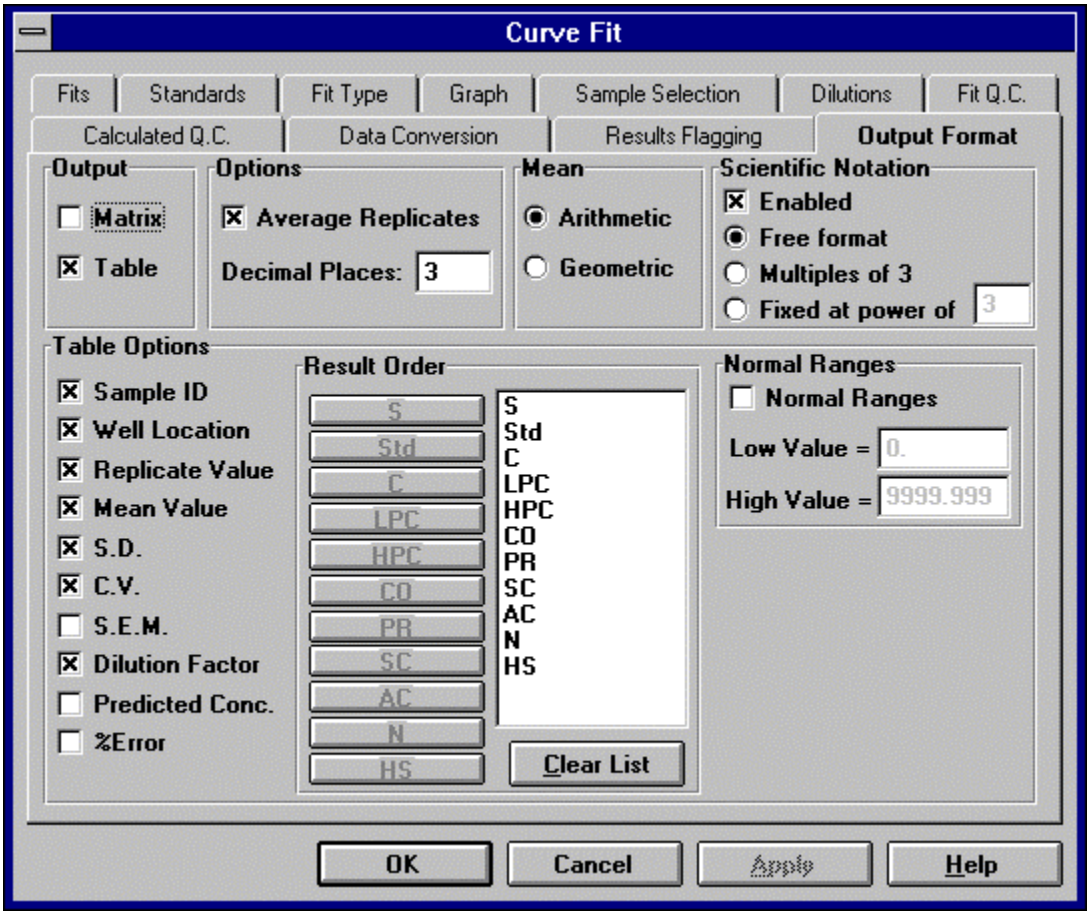
The Output Format dialog box (Figure 4-54) allows you to specify the manner in which results are presented in the results report.

To specify the output format:

1. ***Output:*** Place an **x** in the **Matrix** check box to display the results from each well location on a diagram of the template. Place an **x** in the **Table** check box to display the results from each well location in a table organized by well type.
2. ***Options:*** (If **Matrix** is checked in the previous step.) Place an **x** in the **Average Replicates** check box if you want to average the calculated RLU values. Then, type the number of decimal places to be used for data calculations.

> **NOTE:** The default number of decimal places is **3**, and this is suitable for most applications. The maximum number of decimal places you can specify is **6**. The minimum number is **0**.

Figure 4-54 Output Format Dialog Box



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3. ***Mean:*** Click on the **Arithmetic** radio button to report the arithmetic mean of the replicates. Click on the **Geometric** radio button to report the geometric mean of the replicates.

   NOTE: Reporting the geometric mean (the antilog of the mean of the logarithms) is useful when the imprecision is directly proportional to the level.

4. ***Scientific Notation:*** Place an **x** in the **Enabled** check box to set the display mode for the results. Then, select whether you want to specify a **Free Format**, **Multiples of 3**, or **Fixed at power of** where you enter the exponent.

   NOTE: The default setting is for all results to be limited to eight significant figures. If you check the box you have three further options. **Free Format** allows the software to use scientific notation when appropriate. **Multiples of 3** limits the exponent to multiples of 3. **Fixed at power of** allows you to enter the exponent.

5. ***Table Options:*** (If **Table** is checked in Step **1**.) Place an **x** in the appropriate check boxes to specify the additional data to be displayed in the results table.

   IMPORTANT: If many Table Options are selected, the data may not fit in the results table. In this case, not all data will be displayed.

   NOTE: The raw value for the well type sample is always shown. Some table options are not available for certain data processing options, while some are obligatory. Options which are unavailable will be grayed. Options which are obligatory will also be grayed, and the corresponding check box will be checked.

   The **Predicted Conc.** and **%Error** options allow you to display the predicted concentration and percent error of the actual value versus the labeled value for the standards.

6. ***Result Order:*** (If **Table** is checked in Step **1**.) Click on each well type in the order that you want the results to be presented. The order of results presentation will be shown in the adjacent box.

   NOTE: To delete the current order and create a new one, click on **Clear List** and repeat Step **5**. To place a well type at the top of the list, double-click on it. You can only click on **OK** when you have placed all the well types on the list.

7. ***Normal Ranges:*** Place an **x** in the **Normal Ranges** check box to flag with an asterisk (*) any results which fall outside the normal range you specify. Then, type the high and low values for the normal range.

   NOTE: If you have included a number of different processing options and used a number of tables in the output, Revelation may overwrite sections of data in the output. If this happens, try using matrices instead of tables, or remove one of the processing options.

8. After you have completed defining Output Format, click on **OK**.

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Ratio

The Ratio command is used for expressing results as a percentage or ratio. A typical use of Ratio is to express the results of unknowns in terms of the percentage of RLU values obtained on a control.

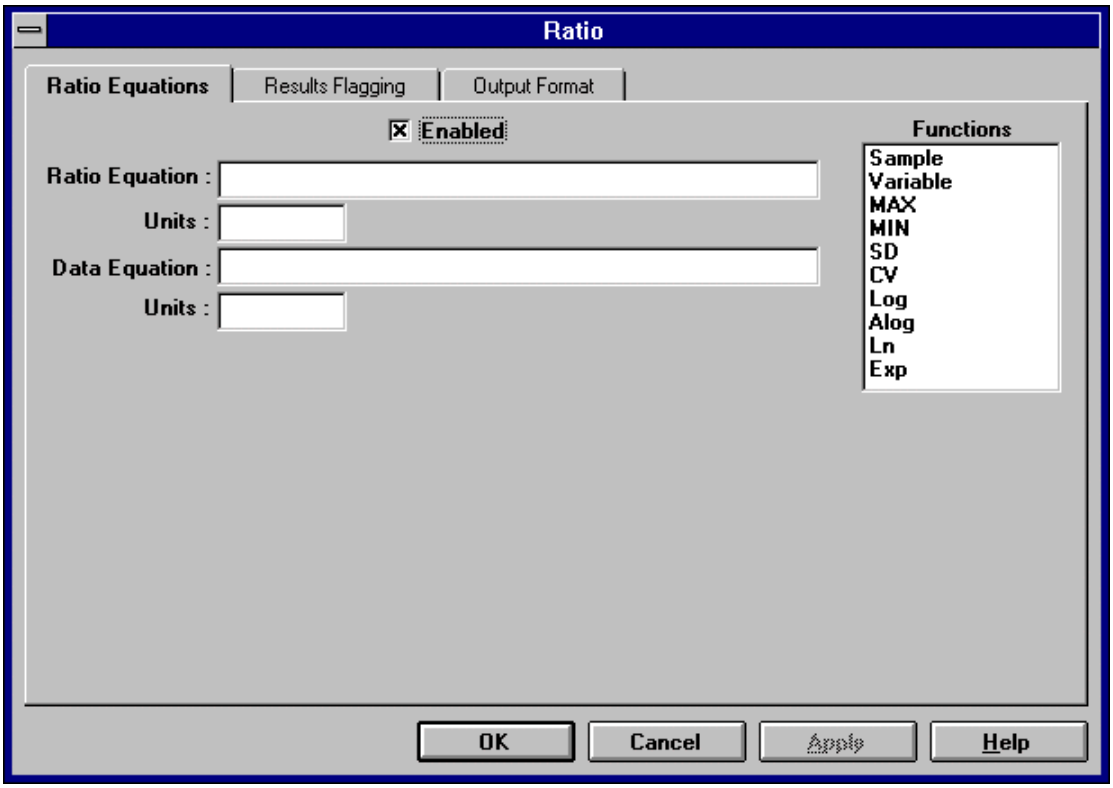
To specify Ratio settings:

1. Click on **Settings** and then click on **Ratio**. Two tabbed Ratio dialog boxes will be displayed (Figure 4-55). Select the appropriate tab as outlined below:

Tab	Purpose
Ratio Equations	Create and enter an expression that will express the results as a percentage or ratio. See page 162.
Output Format	Specify the output format of the processed results. See page 163.

2. Instructions for using each dialog box are contained in the following sections.

Figure 4-55. Ratio Equations Dialog Box



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Ratio Equations

The Ratio Equations dialog box (Figure 4-55) allows you to convert data using a ratio expression. The expression can consist of up to 50 characters. A single expression is used. The dialog box also allows you to prepare a second expression to report the resulting data in different units.

The expressions to convert the data and report the data in different units are constructed using the same rules and many of the same elements that are used for constructing the Q.C.: Raw Data expression (page 125). The only difference is that a new element ("Sample") is used designate the current calculated value (the value before conversion) for each well. The use of "Sample" is outlined below:

Element	Description
Sample	If you insert "Sample" into an expression, the ratio calculation will be performed on each well to reach new individual ratio results.
Sample*n*	If you insert "Sample" followed by a numeral into an expression, the ratio calculation will be performed on the designated replicate of each well.

For example, you may wish to convert RLU readings into a percentage of the maximum RLU on the plate. To do this, create the expression:

100∗Sample/Max.

Then, you may wish to convert concentration values of mg/dL into SIU values of mmol/L. If 1 mg/dL of the analyte is equivalent to a concentration of 3 mmol/L in S.I. Units, create the expression:

Sample∗3.

The first expression is entered in the **Ratio Equation** box, and the second expression is entered in the **Data Equation** box.

To define Ratio equations:

1. Place an **x** in the **Enabled** check box to enable the use of Ratio equations.
2. Create the expression that defines the ratio. Type the expression in the **Ratio Equation** text box.
3. Determine the units that you will use for the resulting ratio. Type the units in the first **Units** text box.
4. If you want to convert the units of the reported result, create the expression that defines the conversion. Type the expression in the **Data Equation** text box.
5. Determine the units that you will use for the resulting ratio. Type the units in the second **Units** text box.
6. Click on **OK**.

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Results Flagging

The Results Flagging dialog box (Figure 4-56) allows you to specify a phrase that either replaces a numerical result or is displayed next to the result. The phrase or flag will be initiated whenever the numerical result meets criteria that you establish. For example, you may want to report the phrase "<20 mg/dL" and suppress the actual numerical result whenever the results are lower than 20 mg/dL. Or, you may want to display a flag (for example "Abnormal High") next to the numerical result whenever the result exceeds the linear range of an assay. Results Flagging does not fail an assay but allows you to alter the presentation of the data.

NOTE: Results Flagging is performed on raw RLU values or calculated sample values obtained by the MLX **after** other calculations such as Threshold and/or Ratio are performed. If a phrase or flag is initiated, it will replace the result generated by a previous operation.

The Results Flagging criteria are constructed using expressions in the same manner as you construct tests for Q.C. Raw Data (see page 125). Note that, unlike Q.C. equations, Results Flagging conditions must evaluate as true for the result or flag to take effect.

Several unique elements are used for constructing a Results Flagging expression. These are described below.

Element	Description
Type( )	Use the "Type" keyword to designate the well type (Standards, Controls, Tests, etc.) that a Results Flagging equation is applied to. For example, the expression "Type (C)==0" means that this equation applies only to the control wells.
Sample (for RLU Values)	Use the "Sample" keyword to designate the RLU value of a particular sample, replicate or well type in the same manner as you used them for Q.C. Raw Data.
Result(Sample) (for Calculated Values)	Use the "Result(Sample)" construct to designate the calculated concentration of a sample instead of the RLU values that were obtained. For example, you can create the expression Result(Sample)<123 to set a lower limit of 123 mg/dL for the calculated value of any test sample. Then, whenever the value is less than 123 mg/dL, the phrase you enter for that expression or the Flag you designate will be triggered.
and, **or** Operators	Use the "and" or "or" operator in a Results Flagging phrase to test multiple conditions.

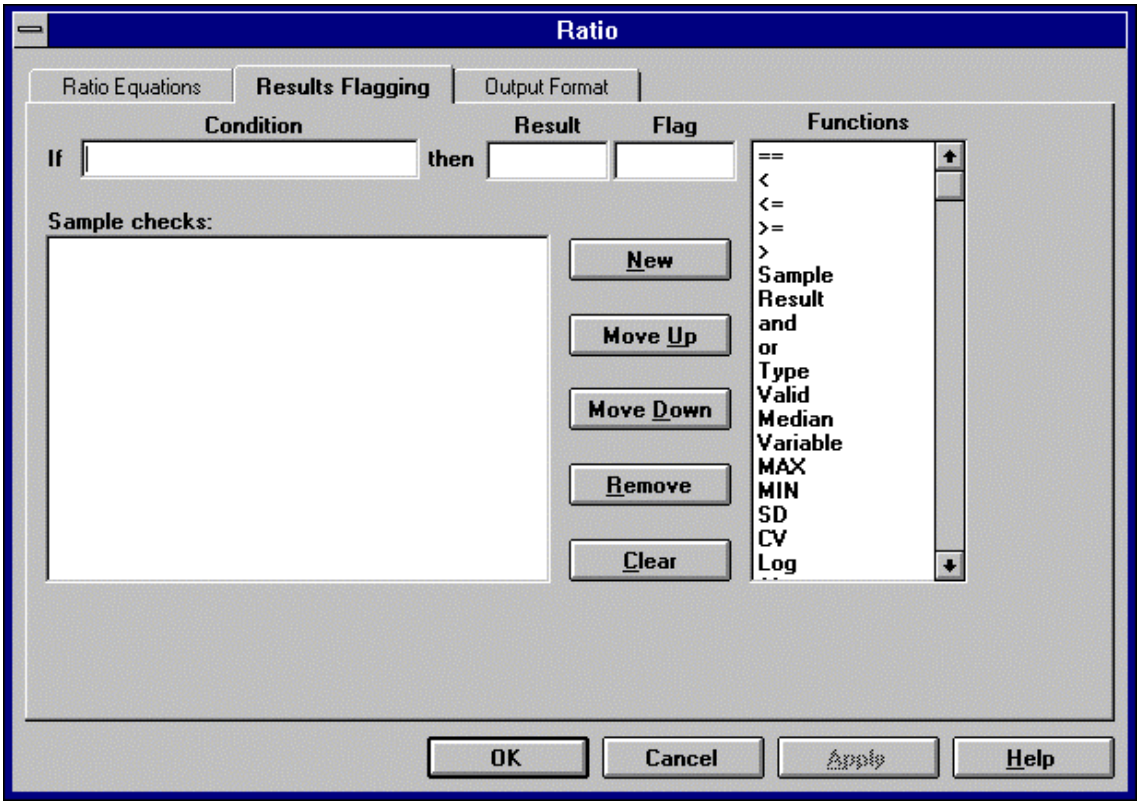
An unlimited number of Results Flagging expressions can be defined for an assay.

To define Results Flagging expressions:

1. Enter the expression in the **Condition** text box.
2. If you want the numerical result to be replaced by a phrase when the condition is exceeded, enter the phrase for that expression in the **Result** text box. If you want the numerical result to be accompanied by a flag when the condition is exceeded, enter the phrase for that flag in the **Flag** text box.

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Figure 4-56 Results Flagging



3. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Condition** box to the **Sample checks:** box. You can then construct the next expression by repeating the procedure from Step **1**.
4. If necessary, you can rearrange the expressions that are contained in the **Sample checks:** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.
5. You can remove an expression from the **Sample checks:** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Sample checks:** box by clicking on **Clear**.
6. Click on **OK**.

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Output Format

The Output Format dialog box (Figure 4-57) allows you to specify the manner in which results are presented in the results report.

To specify the output format:

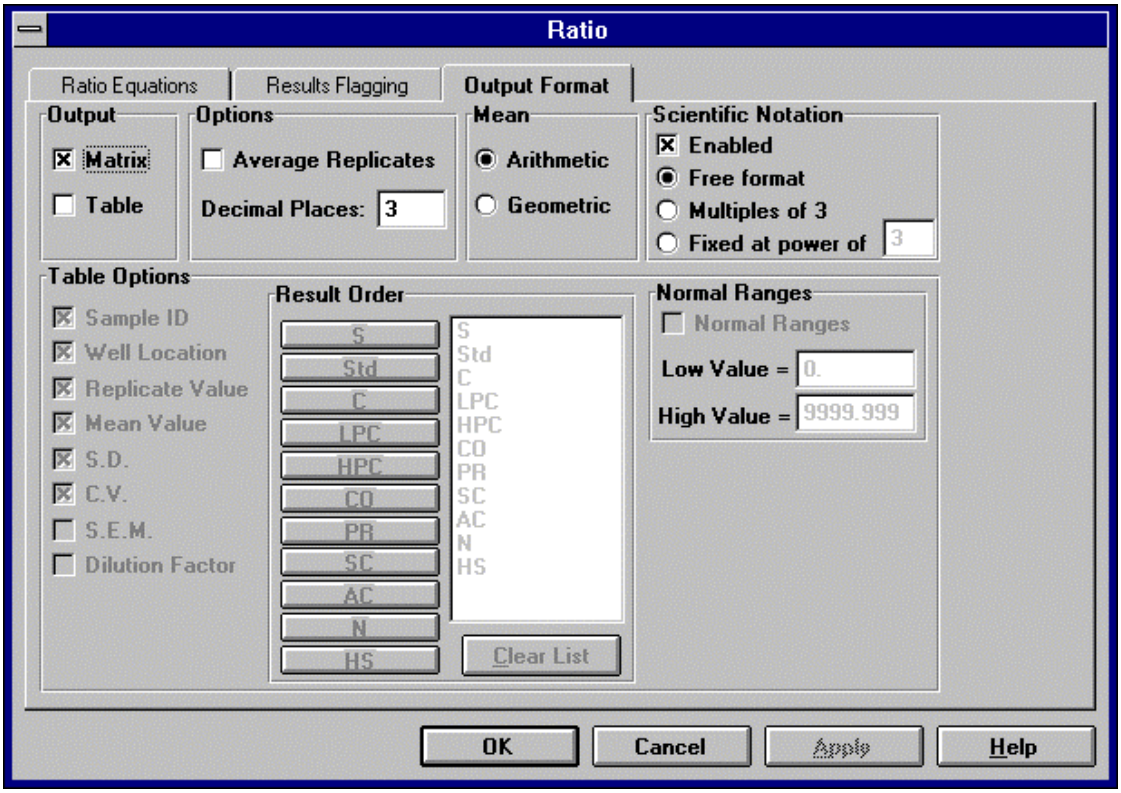
1. ***Output:*** Place an **x** in the **Matrix** check box to display the results from each well location on a diagram of the template. Place an **x** in the **Table** check box to display the results from each well location in a table organized by well type.
2. ***Options:*** (If **Matrix** is checked in the previous step.) Place an **x** in the **Average Replicates** check box if you want to average the calculated RLU values. Then, type the number of decimal places to be used for data calculations.

   NOTE: The default number of decimal places is **3**, and this is suitable for most applications. The maximum number of decimal places you can specify is **6**. The minimum number is **0**.

3. ***Mean:*** Click on the **Arithmetic** radio button to report the arithmetic mean of the replicates. Click on the **Geometric** radio button to report the geometric mean of the replicates.

   NOTE: Reporting the geometric mean (the antilog of the mean of the logarithms) is useful when the imprecision is directly proportional to the level.

Figure 4-57. Output Format Dialog Box



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4. ***Scientific Notation:*** Place an **x** in the **Enabled** check box to set the display mode for the results. Then, select whether you want to specify a **Free Format**, **Multiples of 3**, or **Fixed at power of** where you enter the exponent.

   NOTE: The default setting is for all results to be limited to eight significant figures. If you check the box you have three further options. **Free Format** allows the software to use scientific notation when appropriate. **Multiples of 3** limits the exponent to multiples of 3. **Fixed at power of** allows you to enter the exponent.

5. ***Table Options:*** (If **Table** is checked in Step **1**.) Place an **x** in the appropriate check boxes to specify the additional data to be displayed in the results table.

   NOTE: The raw value for the well type sample is always shown. Some table options are not available for certain data processing options, while some are obligatory. Options which are unavailable will be grayed. Options which are obligatory will also be grayed, and the corresponding check box will be checked.

6. ***Result Order:*** (If **Table** is checked in Step **1**.) Click on each well type in the order that you want the results to be presented. The order of results presentation will be shown in the adjacent box.

   NOTE: To delete the current order and create a new one, click on **Clear List** and repeat Step **5**. To place a well type at the top of the list, double-click on it. You can only click on **OK** when you have placed all the well types on the list.

7. ***Normal Ranges:*** Place an **x** in the **Normal Ranges** check box to flag with an asterisk (*) any results which fall outside the normal range you specify. Then, type the high and low values for the normal range.

   NOTE: If you have included a number of different processing options and used a number of tables in the output, Revelation may overwrite sections of data in the output. If this happens, try using matrices instead of tables, or remove one of the processing options.

8. After you have completed defining Output Format, click on **OK**.

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Spreadsheet

The Spreadsheet command is used for calculating results using the RLU values from particular wells.

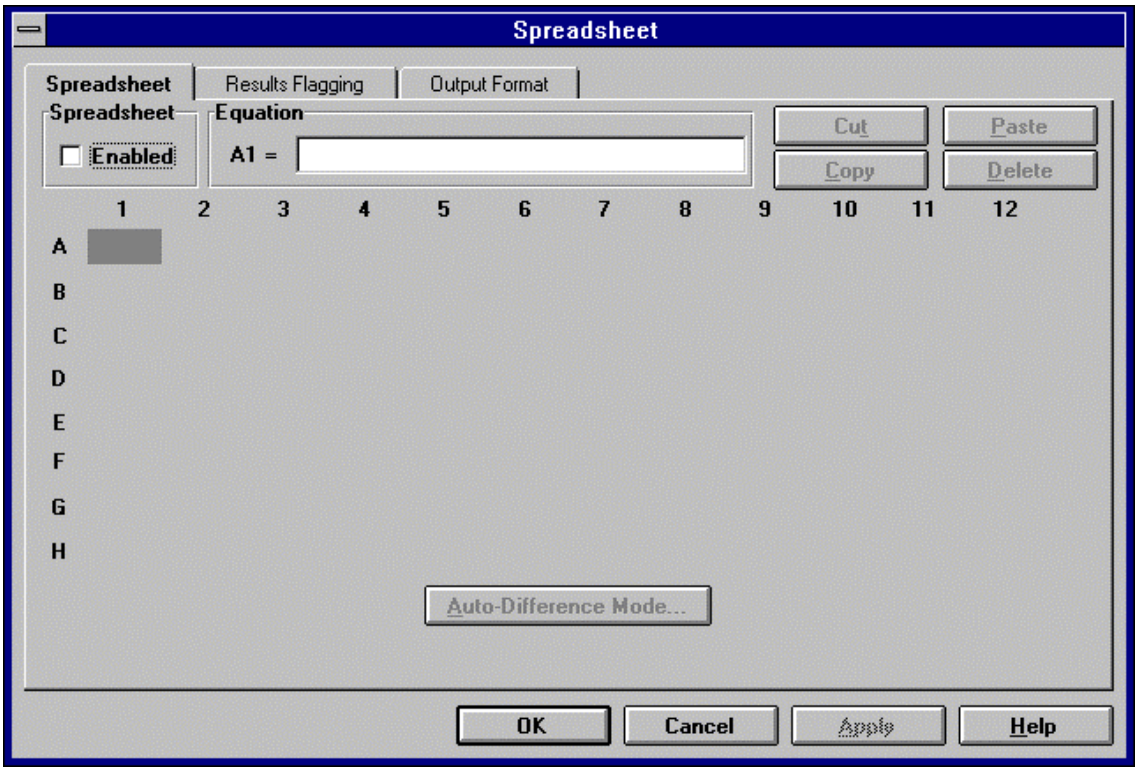
To specify Spreadsheet settings:

1. Click on **Settings** and then click on **Spreadsheet**. Two tabbed Spreadsheet dialog boxes will be displayed (Figure 4-58). Select the appropriate tab as outlined below:

Tab	Purpose
Spreadsheet	Create and enter expressions that will express the results for designated wells. See page 168.
Output Format	Specify the output format of the processed results. See page 170.

2. Instructions for using each dialog box are contained in the following sections.

Figure 4-58. Spreadsheet Dialog Box



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Spreadsheet

The Spreadsheet dialog box (Figure 4-58) allows you to express the results from a particular well in combination with the results from one or more additional wells. For example, you can use Spreadsheet to automatically subtract the results of a separate sample blank well for each unknown. Different calculation equations can be used for different wells.

The expressions to convert the data and report it in different units are constructed using rules that are similar to those used for constructing the Q.C.: Raw Data expression (page 125). However, fewer elements are used. The elements that you can use in constructing Spreadsheet equations are described below.

Element	Description
Well Locations	A particular well location is designated by its column number followed by its row number. For example, the first well in the upper left corner of the template is designated as A1.
Arithmetic Operators	Designate ordinary mathematical calculations. The arithmetic operators that you can use are described below: Symbol — Meaning + — Add - — Subtract * — Multiply / — Divide
Logarithmic Operators	Designate a logarithmic function. The logarithmic operators that you can use are described below: Symbol — Meaning ** — Raised to the power of (follow by a number) log — Base 10 logarithm alog — Inverse Base 10 logarithm ln — Natural logarithm exp — Inverse natural logarithm
Numerals 0 - 9	Specify a quantity for an element, usually in combination with an arithmetic operator.
Parentheses	Specify the elements to precede or follow, when the expression is ambiguous. Up to six levels of parentheses can be used.

For example, you may wish to subtract the RLU reading of well A3 from the RLU reading of well A2 when calculating the results. To do this, enter the expression:

A2-A3.

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To define Spreadsheet equations:

1. Place an **x** in the **Enabled** check box to enable the use of Spreadsheet equations.
2. Click on the first well that you wish to create an equation for. The well location will be highlighted in the dialog box and the location of the well will be displayed in the **Equation** field:

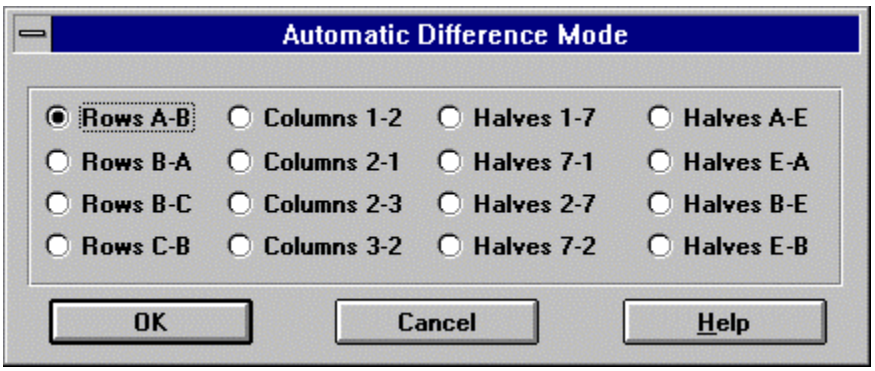
   A2 =.
3. Create the equation and type it in the **Equation** text box. When you have completed typing the equation, click on the next well that you wish to create an equation for.
4. Repeat Step **3** for any other wells that you wish to create an equation for.
5. You can copy an equation that is displayed in the **Equation** text box by clicking on **Copy**. Then, you can paste that equation into a new well by clicking on the new well and clicking on **Paste**.

   NOTE: Well locations in a pasted equation are changed to make them consistent with the current well. For example, if the equation "A1-E1" is pasted into well A12, the equation becomes "A12-E12". This can result in equations containing well locations which do not exist.

   To avoid this, insert the **$** character before the row and column designations in the equation before copying it. In the example above, if you modify the equation to "A1-E1", the equation will remain the same wherever you paste it.
6. You can remove an equation that is displayed in the **Equation** text box by clicking on **Cut** (if you want to paste the removed equation into another location) or by clicking on **Delete**.
7. If you prefer, you can utilize the Auto Difference mode to automatically calculate the difference using a pre-programmed subtraction calculation. To use this mode, click on **Auto-Difference Mode**. The Auto Difference Mode dialog box will be displayed (Figure 4-59). Select the subtraction calculation by clicking on the appropriate radio button and then click on **OK**.

   NOTE: The auto-difference calculations are described in the dialog box. For example, the calculation "Rows A-B" designates that RLU values in Row B are subtracted from RLU values in Row A.
8. After you have completed defining Spreadsheet equations, click on **OK**.

Figure 4-59. Auto Difference Mode Dialog Box



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Results Flagging

The Results Flagging dialog box (Figure 4-60) allows you to specify a phrase that either replaces a numerical result or is displayed next to the result. The phrase or flag will be initiated whenever the numerical result meets criteria that you establish. For example, you may want to report the phrase "<20 mg/dL" and suppress the actual numerical result whenever the results are lower than 20 mg/dL. Or, you may want to display a flag (for example "Abnormal High") next to the numerical result whenever the result exceeds the linear range of an assay. Results Flagging does not fail an assay but allows you to alter the presentation of the data.

NOTE: Results Flagging is performed on raw RLU values or calculated sample values obtained by the MLX **after** other calculations such as Threshold and/or Ratio are performed. If a phrase or flag is initiated, it will replace the result generated by a previous operation.

The Results Flagging criteria are constructed using expressions in the same manner as you construct tests for Q.C. Raw Data (see page 125). Note that, unlike Q.C. equations, Results Flagging conditions must evaluate as true for the result or flag to take effect.

Several unique elements are used for constructing a Results Flagging expression. These are described below.

Element	Description
Type( )	Use the "Type" keyword to designate the well type (Standards, Controls, Tests, etc.) that a Results Flagging equation is applied to. For example, the expression "Type (C)==0" means that this equation applies only to the control wells.
Sample (for RLU Values)	Use the "Sample" keyword to designate the RLU value of a particular sample, replicate or well type in the same manner as you used them for Q.C. Raw Data.
Result(Sample) (for Calculated Values)	Use the "Result(Sample)" construct to designate the calculated concentration of a sample instead of the RLU values that were obtained. For example, you can create the expression Result(Sample)<123 to set a lower limit of 123 mg/dL for the calculated value of any test sample. Then, whenever the value is less than 123 mg/dL, the phrase you enter for that expression or the Flag you designate will be triggered.
and, **or** Operators	Use the "and" or "or" operator in a Results Flagging phrase to test multiple conditions.

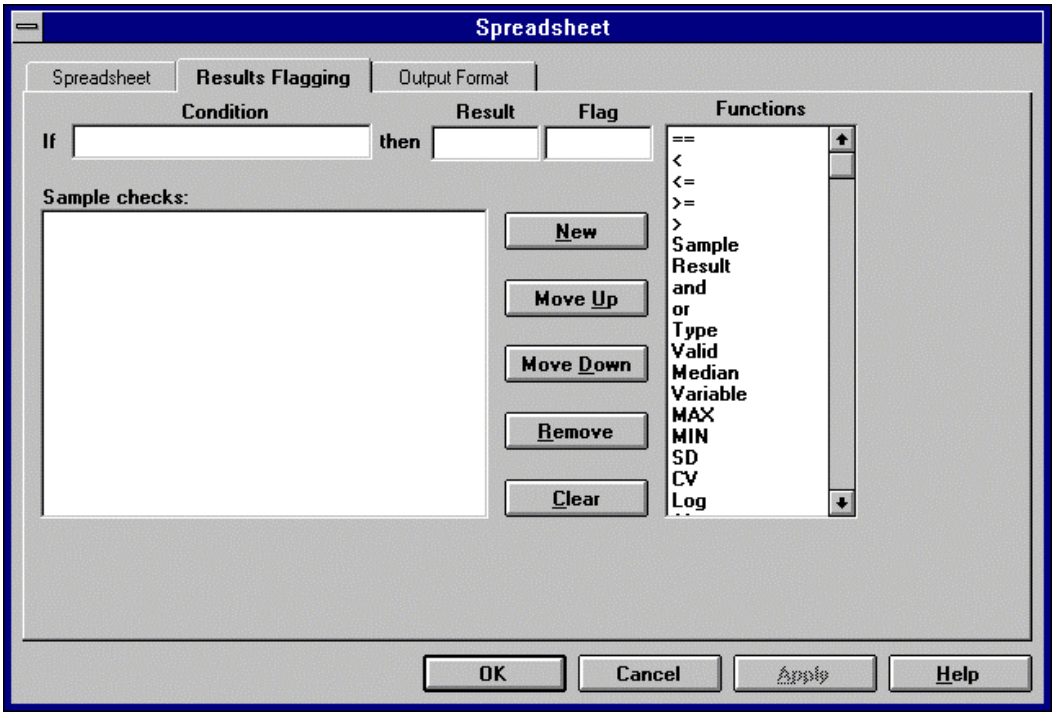
An unlimited number of Results Flagging expressions can be defined for an assay.

To define Results Flagging expressions:

1. Enter the expression in the **Condition** text box.
2. If you want the numerical result to be replaced by a phrase when the condition is exceeded, enter the phrase for that expression in the **Result** text box. If you want the numerical result to be accompanied by a flag when the condition is exceeded, enter the phrase for that flag in the **Flag** text box.

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Figure 4-60 Results Flagging Dialog Box



3. After you have completed the first expression, click on **New** to begin a new expression. The first expression will be transferred from the **Condition** box to the **Sample checks:** box. You can then construct the next expression by repeating the procedure from Step **1**.

4. If necessary, you can rearrange the expressions that are contained in the **Sample checks:** box. To move an expression up one position, click on the expression and then click on **Move Up**. To move an expression down one position, click on the expression and then click on **Move Down**.

5. You can remove an expression from the **Sample checks:** box by clicking on the expression and then clicking on **Remove**. Or, you can remove all of the expressions from the **Sample checks:** box by clicking on **Clear**.

6. Click on **OK**.

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Output Format

The Output Format dialog box (Figure 4-61) allows you to specify the manner in which results are presented in the results report.

To specify the output format:

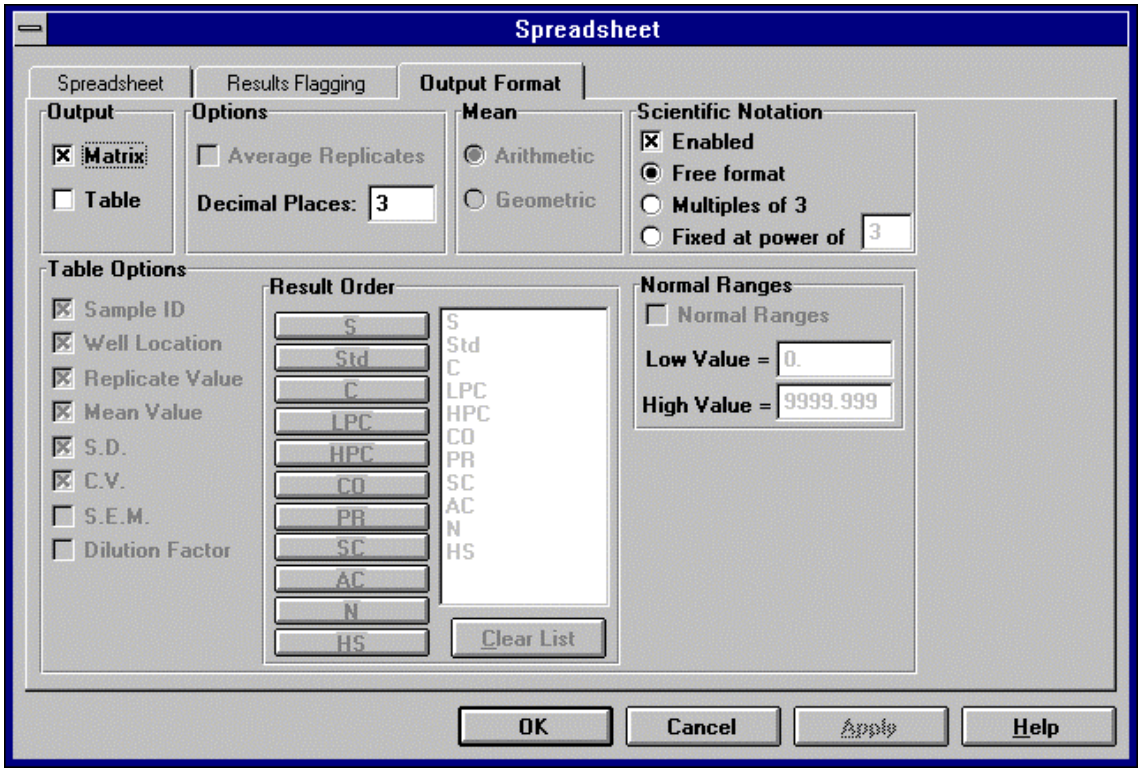
1. ***Output:*** Place an **x** in the **Matrix** check box to display the results from each well location on a diagram of the template. Place an **x** in the **Table** check box to display the results from each well location in a table organized by well type.
2. ***Options:*** (If **Matrix** is checked in the previous step.) Place an **x** in the **Average Replicates** check box if you want to average the calculated RLU values. Then, type the number of decimal places to be used for data calculations.

   NOTE: The default number of decimal places is **3**, and this is suitable for most applications. The maximum number of decimal places you can specify is **6**. The minimum number is **0**.

3. ***Mean:*** Click on the **Arithmetic** radio button to report the arithmetic mean of the replicates. Click on the **Geometric** radio button to report the geometric mean of the replicates.

   NOTE: Reporting the geometric mean (the antilog of the mean of the logarithms) is useful when the imprecision is directly proportional to the level.

Figure 4-61. Output Format Dialog Box



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4. ***Scientific Notation:*** Place an **x** in the **Enabled** check box to set the display mode for the results. Then, select whether you want to specify a **Free Format**, **Multiples of 3**, or **Fixed at power of** where you enter the exponent.

   NOTE: The default setting is for all results to be limited to eight significant figures. If you check the box you have three further options. **Free Format** allows the software to use scientific notation when appropriate. **Multiples of 3** limits the exponent to multiples of 3. **Fixed at power of** allows you to enter the exponent.

5. ***Table Options:*** (If **Table** is checked in Step **1**.) Place an **x** in the appropriate check boxes to specify the additional data to be displayed in the results table.

   NOTE: The raw value for the well type sample is always shown. Some table options are not available for certain data processing options, while some are obligatory. Options which are unavailable will be grayed. Options which are obligatory will also be grayed, and the corresponding check box will be checked.

6. ***Result Order:*** (If **Table** is checked in Step **1**.) Click on each well type in the order that you want the results to be presented. The order of results presentation will be shown in the adjacent box.

   NOTE: To delete the current order and create a new one, click on **Clear List** and repeat Step **5**. To place a well type at the top of the list, double-click on it. You can only click on **OK** when you have placed all the well types on the list.

7. ***Normal Ranges:*** Place an **x** in the **Normal Ranges** check box to flag with an asterisk (*) any results which fall outside the normal range you specify. Then, type the high and low values for the normal range.

   NOTE: If you have included a number of different processing options and used a number of tables in the output, Revelation may overwrite sections of data in the output. If this happens, try using matrices instead of tables, or remove one of the processing options.

8. After you have completed defining Output Format, click on **OK**.

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Report Options

The Report Options command is used for specifying the data that are contained in the results report, the order in which the data is presented, and the format of the report.

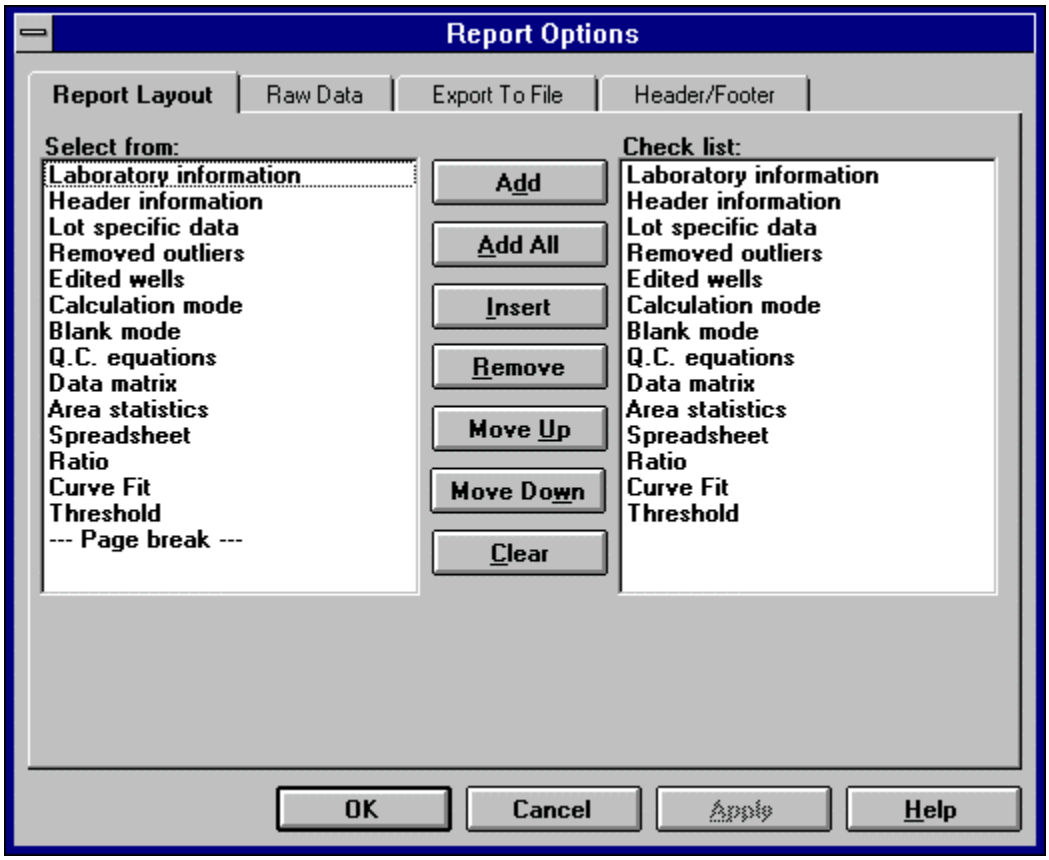
To specify report options:

1. Click on **Settings** and then click on **Report Options**. Four tabbed Report Options dialog boxes will be displayed (Figure 4-62). Select the appropriate tab as outlined below:

Tab	Purpose
Report Layout	Construct a report layout by selecting the data to be included and the order in which these items are presented.
Raw Data	Specify the manner in which results are presented.
Export to File	Export data to an ASCII file and to specify the data to be included in the exported file.
Header/Footer	Define the content of the report header and footer.

2. Instructions for using each dialog box are contained in the following sections.

Figure 4-62. Report Layout Dialog Box



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Report Layout

The Report Layout dialog box (Figure 4-62) allows you to specify the data that are included in the results report and the order in which the data are presented.

To specify the report layout:

1. ***Select From:*** Click on the first item in this list that you want to appear in the report. Then, click on **Add** to place the item in the **Check List** box.

   Note: To add all of the items to the **Check List** box, click on **Add All**.

2. Repeat Step **1** for each item in the **Select From** box that you want to place in the report.

3. After you have selected all of the items to appear in the report, examine the list of items in the **Check List** box. The items are listed in the order they will be displayed in the report.

   To change the order of an item, click on the item in the **Check List** box. Then, click on **Move Up** to move the item up one position or click on **Move Down** to move the item down one position.

   To add another item to the list at a specific position, click on the item in the **Select From** box. Then, click on the item in the **Check List** box that is immediately after where you want the new item to appear. Then, click on **Insert**.

   To remove an item from the **Check List** box, click on the item in the **Check List** box. Then, click on **Remove**. To remove all of the items from the **Check List** box, click on **Clear**.

   Note: Calculation Mode must be included in the **Check List** box if you want to display flash graphs.

4. After you have completed defining the report layout, click on **OK**.

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Raw Data

The Raw Data dialog box (Figure 4-63) allows you to specify the manner in which results are presented.

To specify the raw data matrix:

1. ***Output:*** Place an **x** in the **Matrix** check box to display the results from each well location on a diagram of the template. Place an **x** in the **Table** check box to display the results from each well location in a table organized by well type.
2. ***Matrix Options:*** (If **Matrix** is checked in the previous step.) Place an **x** in the **Calculated Data** check box if you want to display the calculated values.

   Place an **x** in the **Sample IDs** check box if you want to display the Sample ID for each sample.
3. ***Averaging Options:*** Click on the **Average Replicates** radio button to report the average of the replicates. Click on the **Arithmetic Mean** radio button to report the arithmetic mean of the replicates. Click on the **Geometric Mean** radio button to report the geometric mean of the replicates.

   NOTE: Reporting the geometric mean (the antilog of the mean of the logarithms) is useful when the imprecision is directly proportional to the level.
4. ***Decimal Places:*** Place an **x** in the **Free Format** or **Fixed at power of** to define where the exponent is entered.

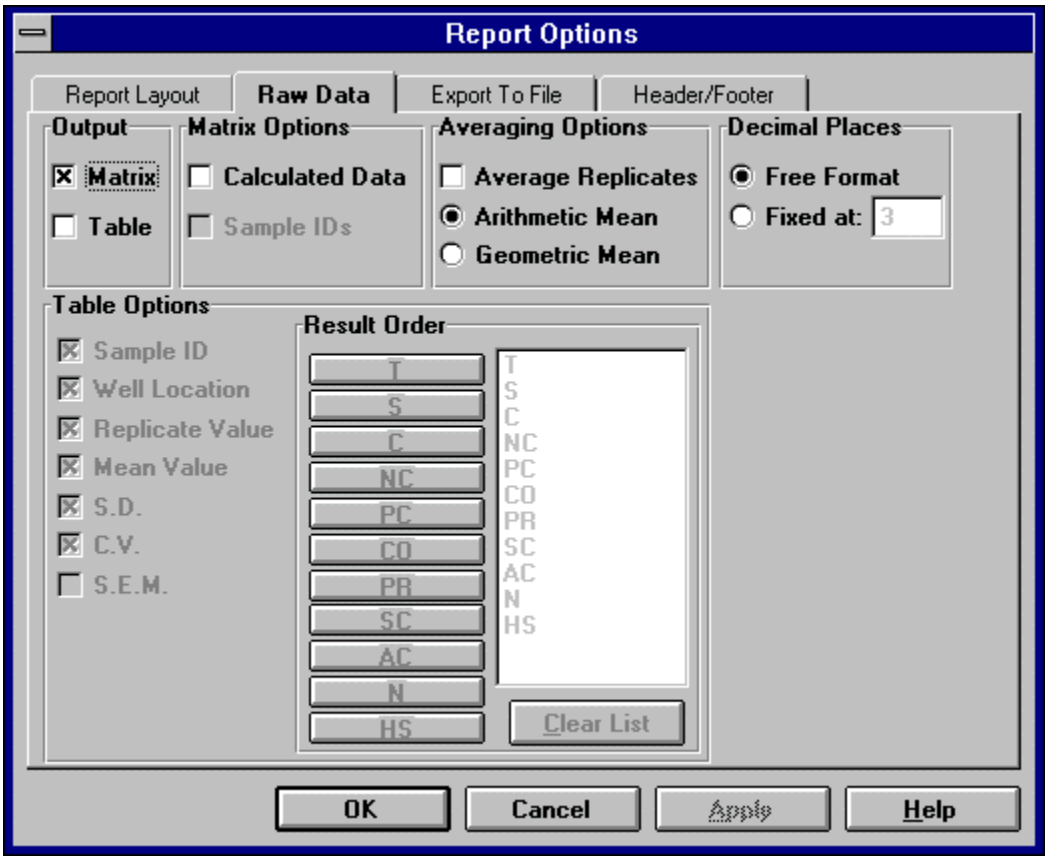
   NOTE: The default setting is for all results to be limited to eight significant figures. If you check the box you have three further options. **Free Format** allows the software to use scientific notation when appropriate. **Fixed at power of** allows you to enter the exponent.
5. ***Table Options:*** (If **Table** is checked in Step **1**.) Place an **x** in the appropriate check boxes to specify the additional data to be displayed in the results table.

   NOTE: The raw value for the well type sample is always shown. Some table options are not available for certain data processing options, while some are obligatory. Options which are unavailable will be grayed. Options which are obligatory will also be grayed, and the corresponding check box will be checked.
6. ***Result Order:*** (If **Table** is checked in Step **1**.) Click on each well type in the order that you want the results to be presented. The order of results presentation will be shown in the adjacent box.

   NOTE: To delete the current order and create a new one, click on **Clear List** and repeat Step **5**. To place a well type at the top of the list, double-click on it. You can only click on **OK** when you have placed all the well types on the list.
7. After you have completed defining Raw Data Options, click on **OK**.

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Figure 4-63. Raw Data Dialog Box



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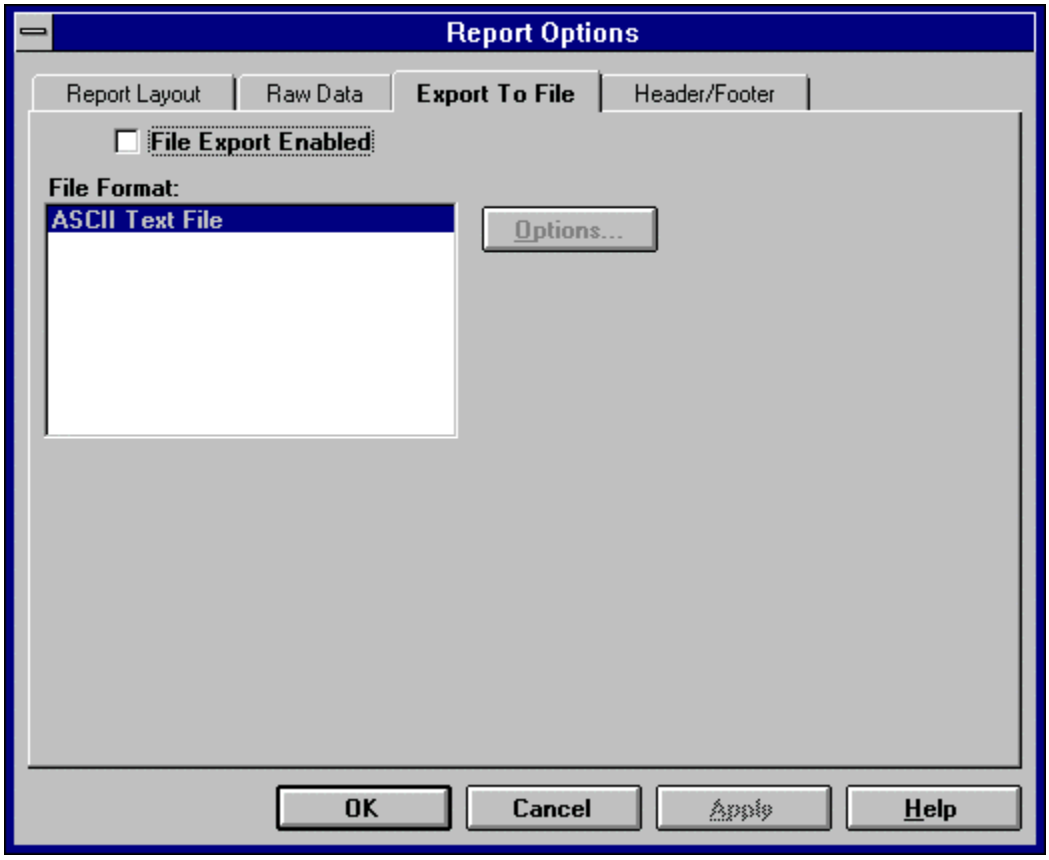
Export to File

The Export to File dialog box (Figure 4-64) allows you to export data to an ASCII file and to specify the data to be included in the exported file. If you plan to ever export data to an ASCII file, you must enter a plate ID.

To specify export parameters:

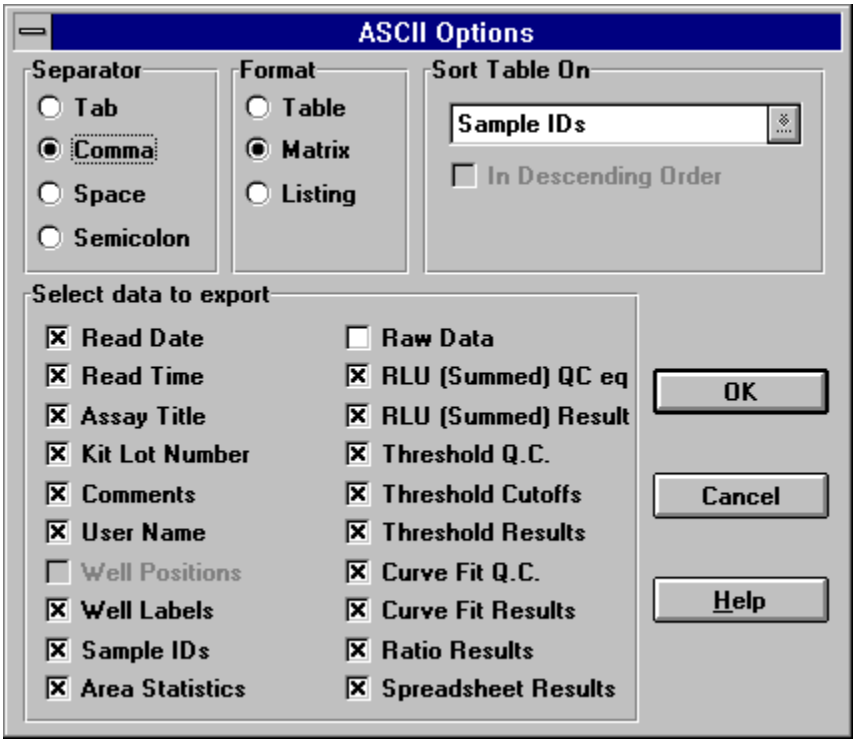
1. ***Output:*** Place an **x** in the **File Export Enabled** check box to enable the file exporting option.
2. Select the file format that you will export.
3. Click on **Options** to display the ASCII Options dialog box (Figure 4-64).
4. ***Separator:*** Click on the appropriate radio button to select whether you want file information divided into well locations using tabs, commas, spaces or semicolons.
5. ***Format:*** Click on the **Matrix** button to display the results from each well location on a diagram of the template. Click on the **Table** button to display the results from each well location in a table organized by well type. Click on the **Listing** button to display the results from each well location in a listing.

Figure 4-64. Export to File Dialog Box



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Figure 4-65. ASCII Options Dialog Box



6. ***Sort Table On:*** Click on the drop-down arrow to display the variables on which you can sort the data for presentation order. Then, click on the parameter you want to sort on. Place an **x** in the **Descending** check box if you want to sort in descending order.

7. ***Select Data to Export:*** Place an **x** in the appropriate check boxes to select the data that you want to export.

   NOTE: For **Curve Fit Results**: If more than one fit is applied to the same wells, only the last fit that was defined is exported.

8. Click on **OK** in the ASCII Options dialog box. Then, click on **OK** in the Export to File dialog box.

   NOTE: The Export filename will be *txt directory\Plate ID.txt*. If the Plate ID is not specified, the Export filename will consist of *txt directory\Untitled.txt*.

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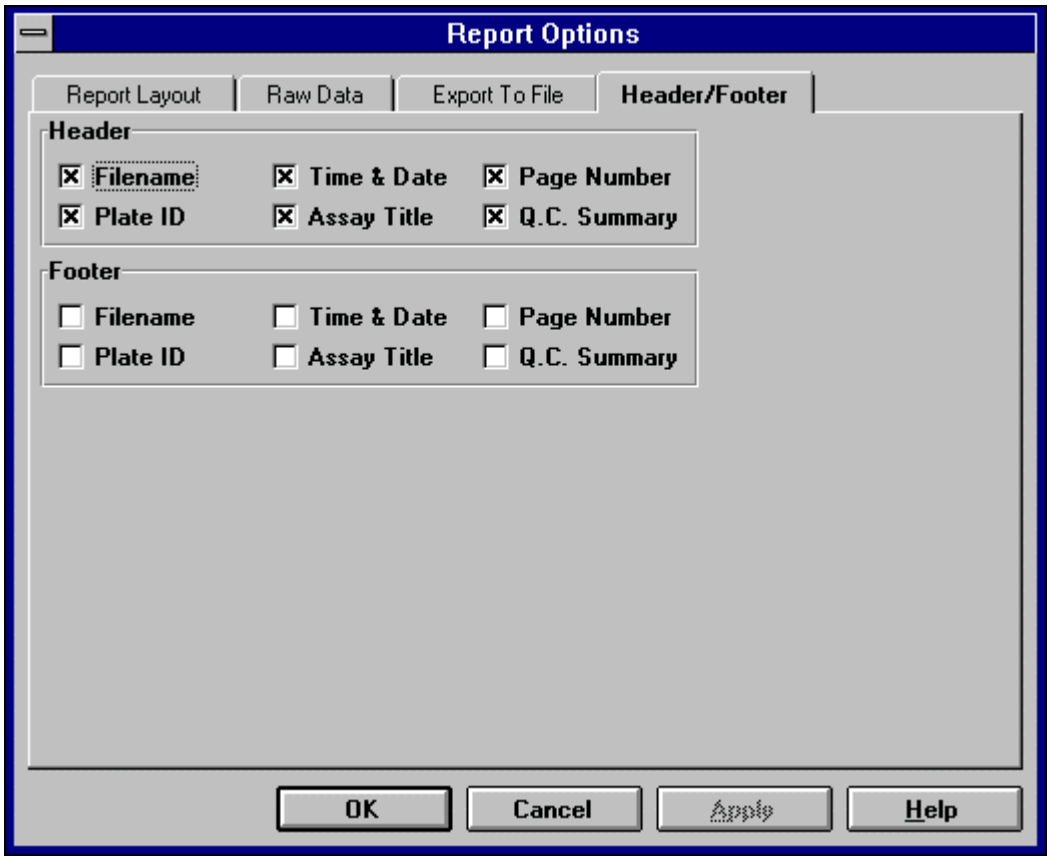
Header/Footer

The Header/Footer dialog box (Figure 4-66) allows you to define the information that will be displayed on the header and footer of the results report.

To specify the header and footer:

1. ***Header:*** Place an **x** in the appropriate check boxes to select the information that you want to display in the report header.
2. ***Footer:*** Place an **x** in the appropriate check boxes to select the information that you want to display in the report footer.
3. Click on **OK.**

Figure 4-66. Header/Footer Dialog Box



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Assay Options

The Assay Options command is used for specifying the data that are contained in the results report, the order in which the data is presented, and the format of the report.

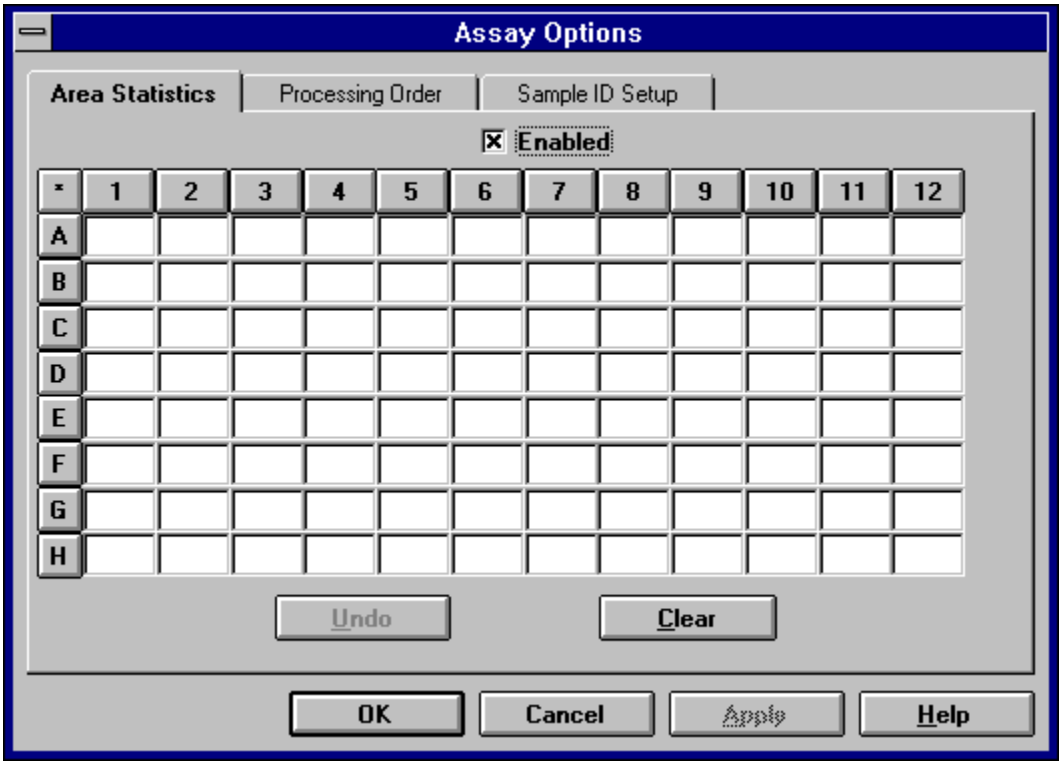
To specify assay options:

1. Click on **Settings** and then click on **Assay Options**. Three tabbed Assay Options dialog boxes will be displayed (Figure 4-67). Select the appropriate tab as outlined below:

Tab	Purpose
Area Statistics	Designate wells for which Revelation will calculate statistical information.
Processing Order	Specify the processing order if more than one data processing option is selected.
Sample ID Setup	Designate whether the results from each well should be calculated separately or as grouped values for each sample.

2. Instructions for using each dialog box are contained in the following sections.

Figure 4-67. Assay Options Dialog Box



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Area Statistics

The Area Statistics dialog box (Figure 4-67) allows you to designate wells within a plate to calculate statistics on each area you designate. For example, you may run two lots of controls within a single well type and want to determine the mean value and standard deviation of each lot. The statistics that you can obtain on each area include the mean value, the standard deviation (SD), the coefficient of variation (CV), and the standard error of the mean (SEM).

To specify area statistics:

1. Place an **x** in the **Enabled** check box to enable the area statistics option.
2. Define the first statistical area by typing the numeral **0** in each well within the area.

   NOTE: The wells that are contained in a particular statistical area can be located anywhere on the plate. In the example shown in Figure 4-67, the wells in Area **0** are at **A1**, **A2**, **H11**, and **H12**.

3. Define the next statistical area by typing the numeral **1** in each well within the area.

   NOTE: Up to 96 separate statistical areas can be defined.

4. To place an entire row in a statistical area, type a numeral into the first well in the row and then click on the letter next to the row. To place an entire column in a statistical area, type a numeral into the top well in the column and then click on the number above the column.

   To remove a single well from a statistical area, highlight the number and then delete it using the **Delete** key. To delete an entire row from a statistical area, delete the numeral from the first well in the row and then click on the letter next to the row. To delete an entire column from a statistical area, delete the numeral from the top well in the column and then click on the number above the column.

   To clear all of the statistical area assignments, click on **Clear**.

5. After you have completed defining the area statistics, click on **OK**.

The Area Statistics dialog box allows you to designate areas within a single plate for calculating statistics. You can also use Plate-Plate Statistics (page 191) to calculate the grand mean and the total SD, the total CV, and the total SEM across multiple plates.

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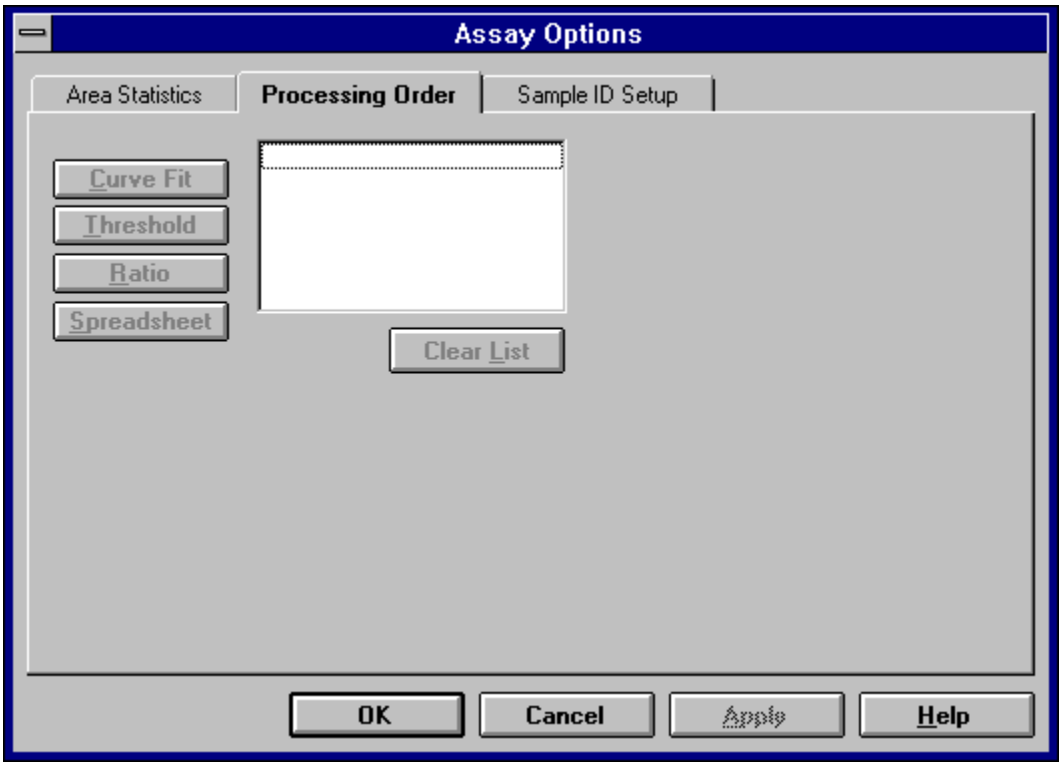
Processing Order

The Processing Order dialog box (Figure 4-68) allows you to define the order in which calculations are performed if you specify more than one calculation.

To specify processing order:

1. Click on **Clear List**.
2. Click on the appropriate button (**Curve Fit**, **Threshold**, **Ratio**, or **Spreadsheet**) to define the calculation that should be performed first. The calculation type will be displayed in the box.
3. Repeat Step **2** for each calculation that you are performing. Each calculation type will be displayed in the box in the order you specify.
4. After you have specified the calculation order, examine the list of calculations in the box. To remove all of the items from the box and start over, click on **Clear List**.
5. After you have defined the processing order, click on **OK**.

Figure 4-68. Processing Order Dialog Box



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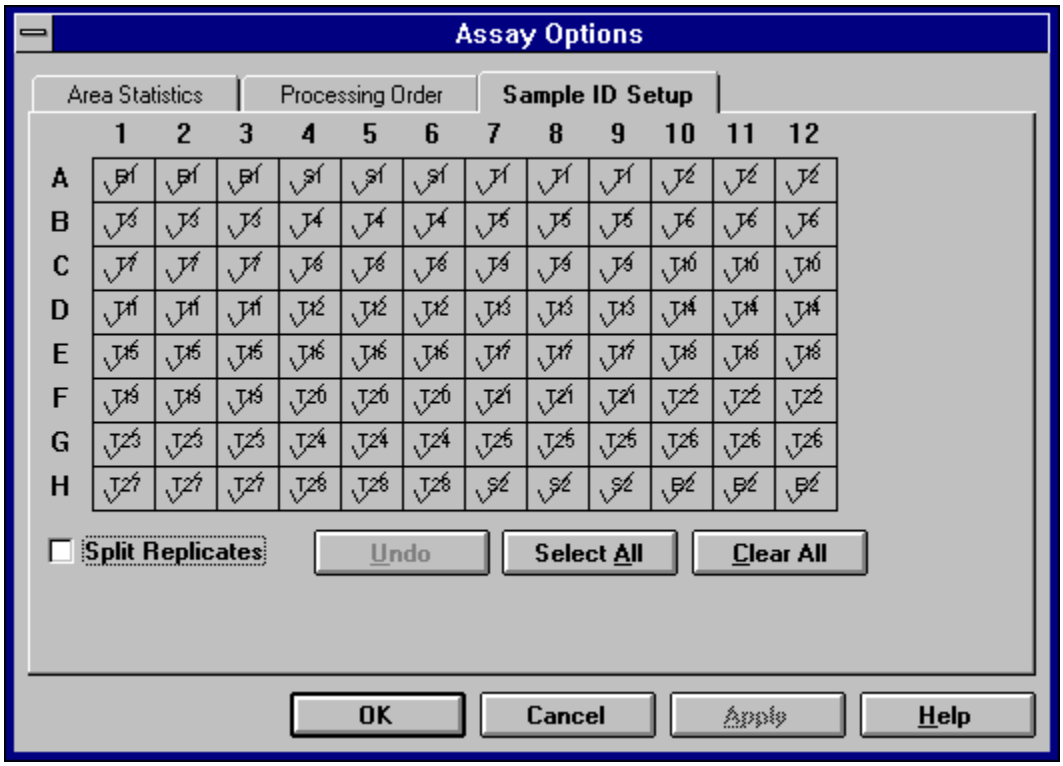
Sample ID Setup

The Sample ID Setup dialog box (Figure 4-69) allows you to specify wells for which a sample ID will be prompted at run time.

To set up sample IDs:

1. Place an **x** in the **Split Replicates** check box if you want the sample ID of each replicate to be entered separately.
2. Click on **Clear All** if you want to clear the template.
3. Click on each well for which you want a sample ID to be entered. To select all of the wells, click on **Select All**.
4. Click on **Undo** if you want to clear the last well you clicked in.
5. After you have set up the sample IDs, click on **OK**.

Figure 4-69. Sample ID Setup Dialog Box



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Using the Wizard

If you wish, you can use the Wizard to guide you through the Assay definition process on the MLX Luminometer. When the Wizard is used, each of the dialog boxes that is required for defining an assay is automatically displayed.

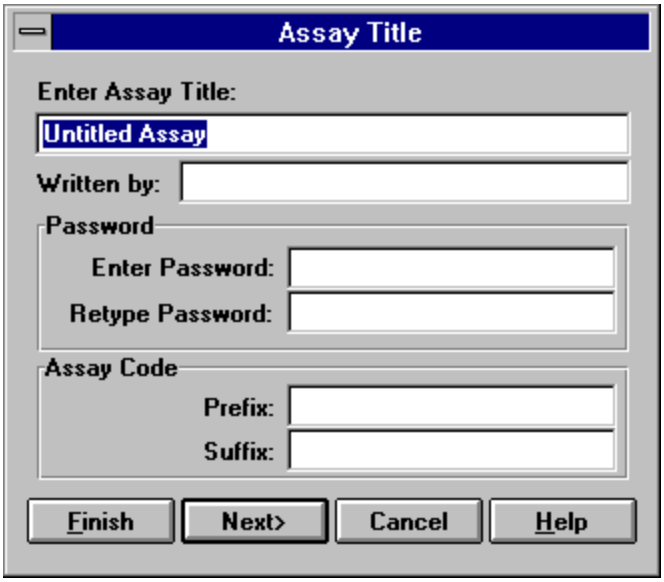
To use the Wizard:

1. Select **New Assay** or **Open** from the **File** Menu. The Luminometer Assay Type dialog box will be displayed.
2. Click on **Glow - Endpoint**, **Glow - Kinetic**, or **Flash** to select the type of assay you wish to create.
3. Click on the **Wizard** icon on the toolbar. The Assay Title dialog box (Figure 4-70) will be displayed.
4. Enter the information concerning the title, author, password, and assay code for the assay you want to define. Then, click on **Next**. The Luminometer Control dialog boxes (Figure 4-71) will be displayed.
5. Enter the information concerning the luminometer control for the assay. Click on each tab to display each luminometer control dialog box for the type of assay you are defining.

   When you are finished defining the luminometer control parameters, click on **Next**. The Template dialog box (Figure 4-72) will be displayed.

   Note: You can stop at any time by clicking on **Finish**. Or, you can return to a previous dialog box by clicking on **Last**.

Figure 4-70 Wizard "Assay Title" Dialog Box



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Figure 4-71 Wizard "Luminometer Control" Dialog Boxes

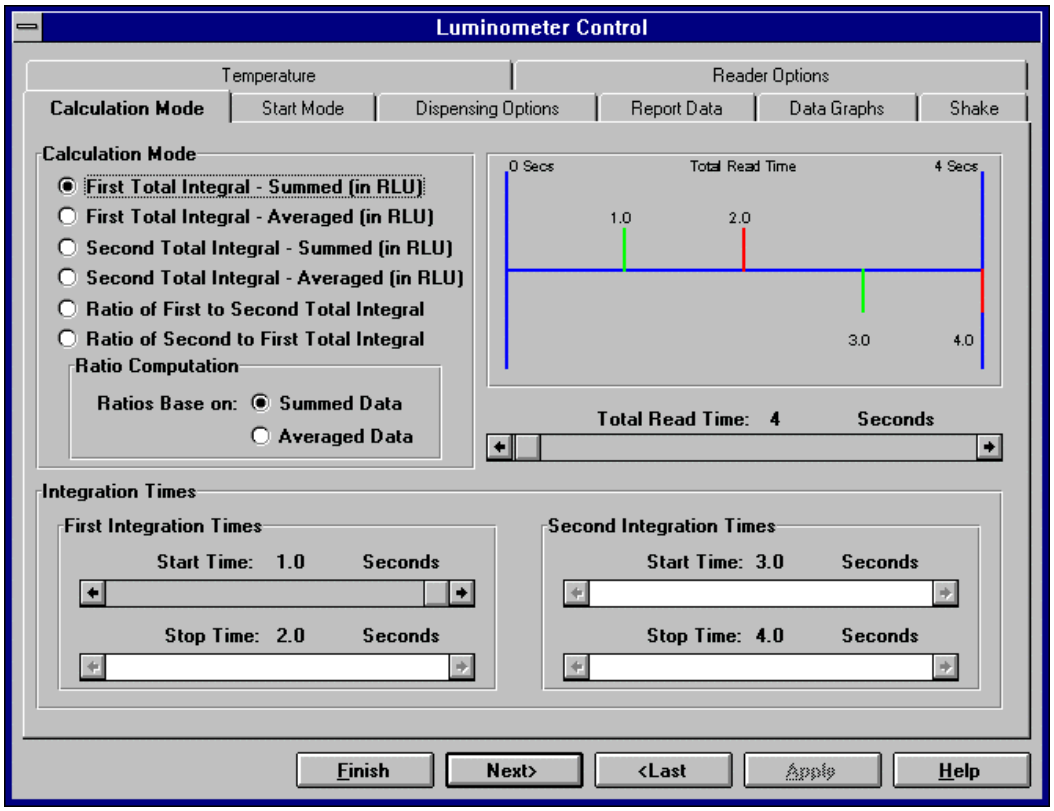
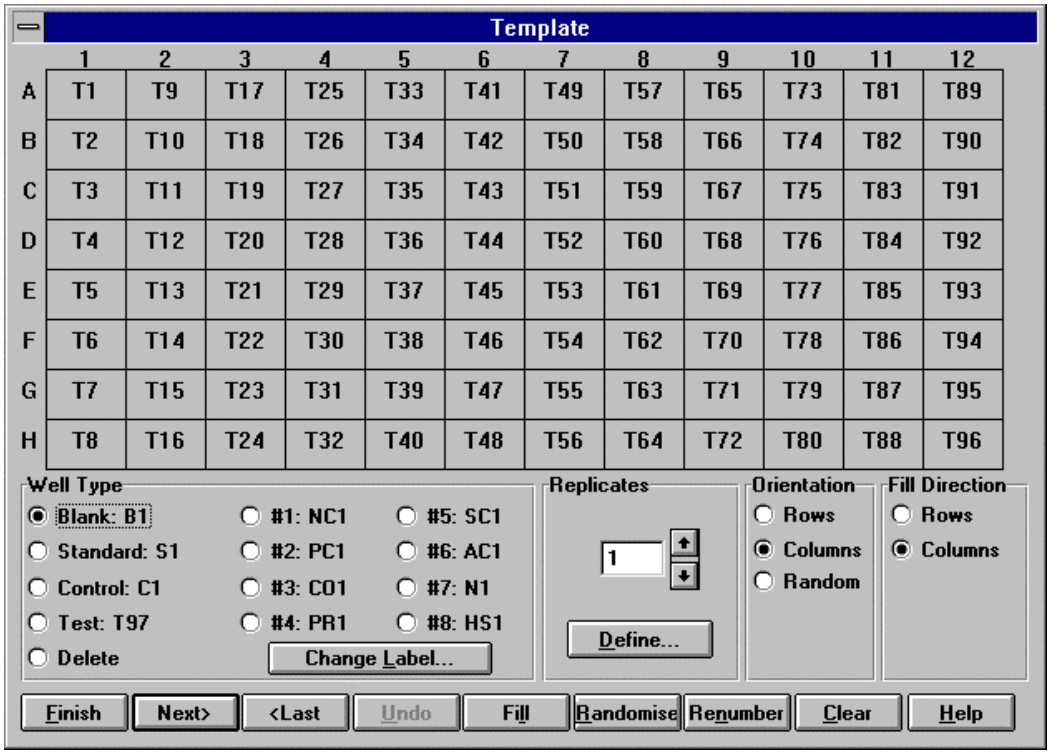


Figure 4-72 Wizard "Template" Dialog Box



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6. Define the template and enter the information concerning well type, replicates, orientation, and fill direction. Then, click on **Next**. The Blank Mode dialog boxes (Figure 4-73) will be displayed.

7. Enter the information concerning the blank mode for the assay. Click on each tab to display each of the blank mode dialog boxes. When you are finished, click on **Next**. The Quality Control - Raw Data dialog box (Figure 4-74) will be displayed.

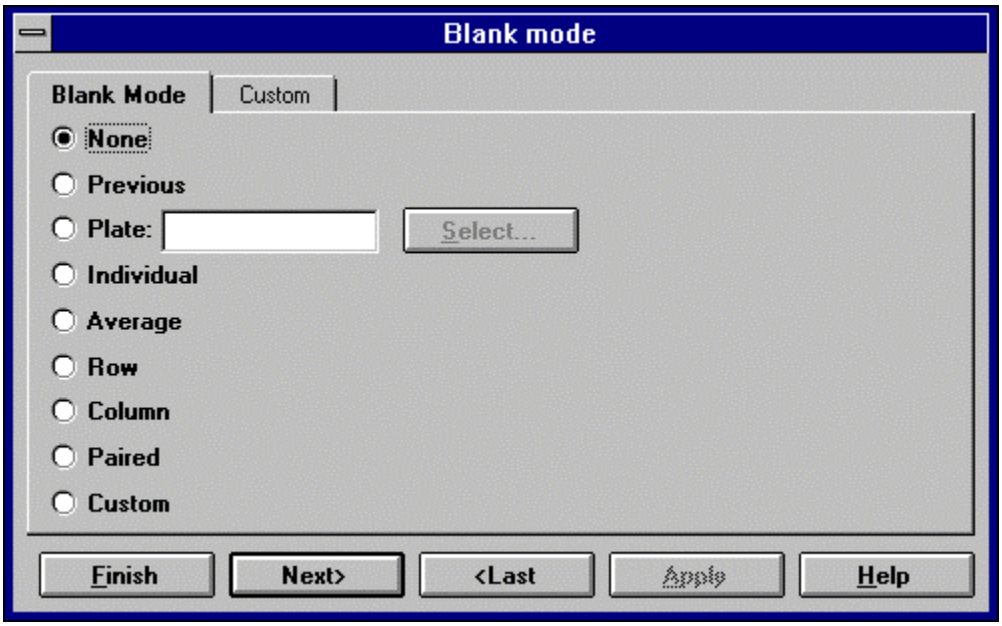
8. Enter the information concerning the quality control of the assay. When you are finished, click on **Next**. The Data Processing Options dialog box (Figure 4-75) will be displayed.

9. Click on the appropriate button to enter information for the threshold, curve fitting, ratio, and spreadsheet parameters for the assay. When you are finished, click on **Next**. The Report Options dialog box (Figure 4-76) will be displayed.

10. Select the appropriate report options for the assay. When you are finished, click on **Finish**. The Assay Summary window will be displayed.

Definition of the assay is now completed.

Figure 4-73 Wizard "Blank Mode" Dialog Boxes



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Figure 4-74 Wizard "Quality Control - Raw Data" Dialog Box

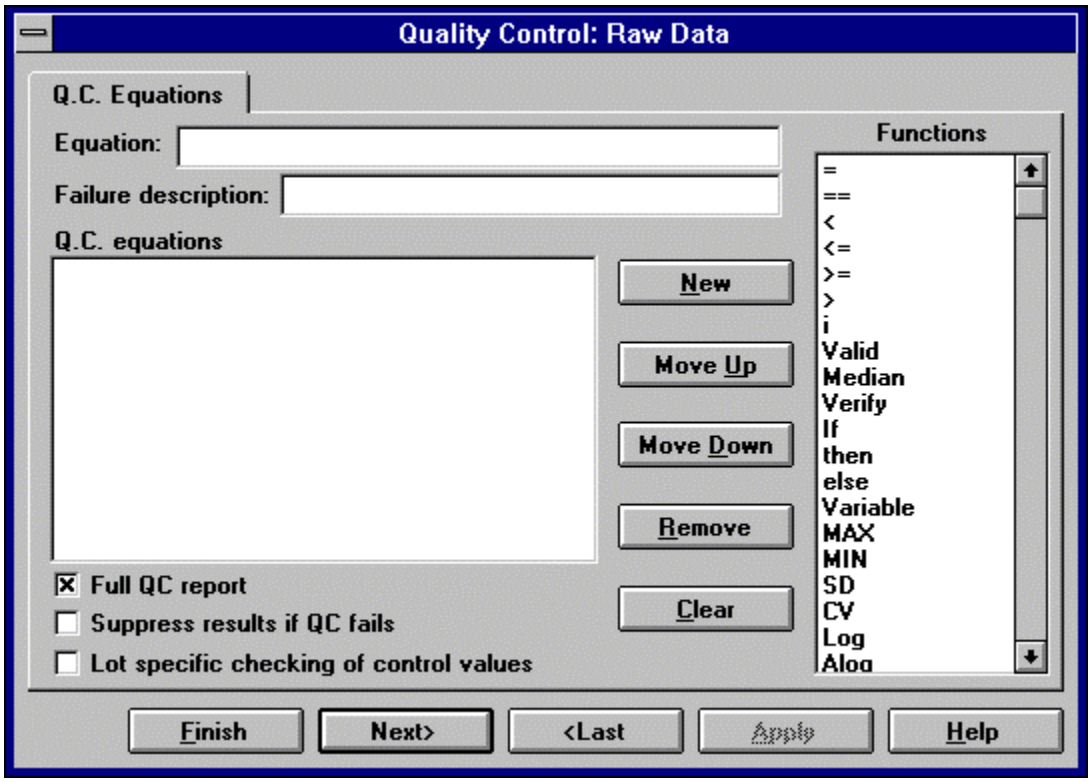
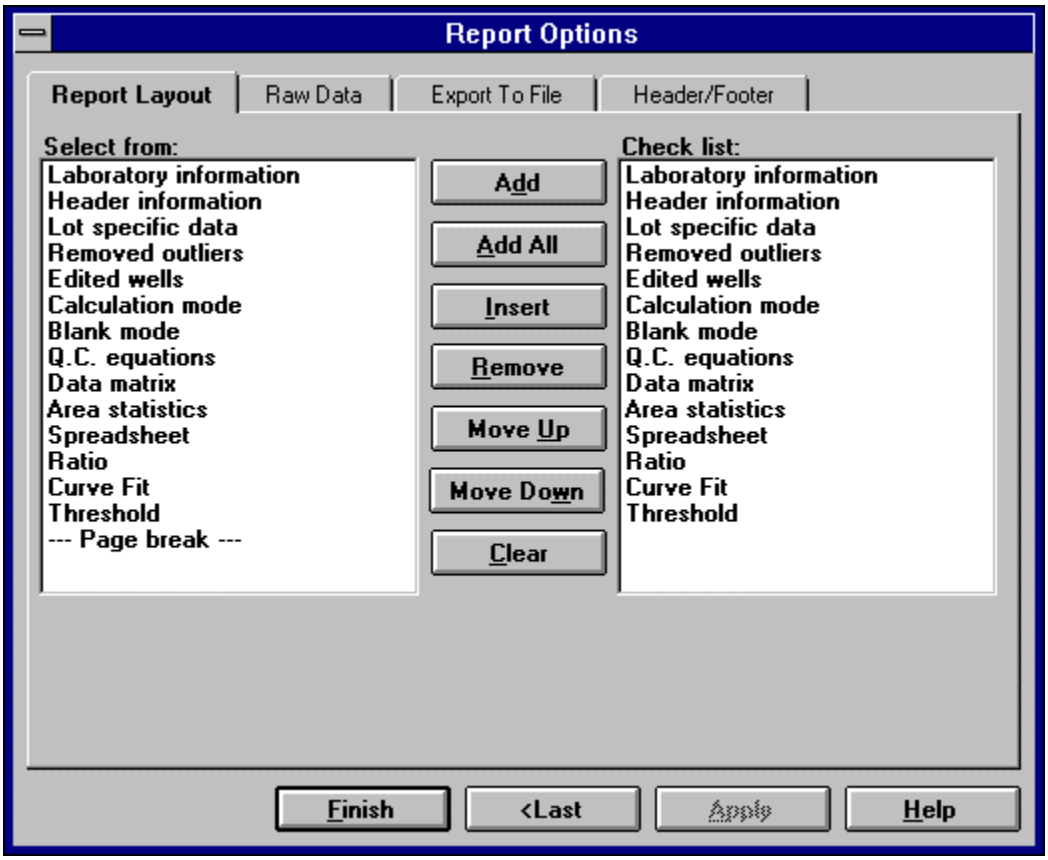


Figure 4-75 Wizard "Data Processing Options" Dialog Box



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Figure 4-76 Wizard "Report Options" Dialog Box



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5 Analyzing a Plate

Starting an Assay

An assay can be started in two ways:

- Click on the **Quick Operation** button corresponding to the assay to be run. This is the easiest way to run an assay.

  Note: When the MLX is delivered to your laboratory, the **Quick Glow** button is configured with an assay for 96 test wells (T1 through T96) using a 0.25 second read time per well with AutoGain enabled. The **Quick Flash** button is configured with an assay for 96 test wells (T1 through T96) using a single substrate injection of 150 µL from Dispenser A, using a two second integration time with AutoGain enabled, and graphing of the first column of data (wells A1 through H1) in the report.

  If you want to run an assay with a glowing substrate such as dioxetane or luminol, choose **Quick Glow**. If you want to run a flash assay such as aequorin, choose **Quick Flash**.

- Activate the **Run Plate** command from the File menu or click on the **Run Plate** icon. Then, select one or more assays that you want to run. This is the way to run an assay that is not assigned to a Quick Operation button.

  Note: If you are running more than one assay using the multiple assays feature, the selected assays must be of the same type.

Using the Quick Operation Buttons

The Quick Operation buttons (Figure 5-1) allow you to initiate an assay by simply clicking a button. This is useful for assays that are commonly used, and it saves the additional step of selecting the type of assay to be run when the Run Plate dialog box is displayed.

To run an assay assigned to a Quick Operation button:

1. If a Flash type assay is being run and the dispensers are being used, be sure that the proper reagents have been loaded and that the dispensers have been initialized and primed before running the assay. For further information concerning preparation of the dispensers, refer to Chapter 2.

2. Click on the **Quick Operation** button for the assay you want to run. The Run Plate dialog box (Figure 5-2) will be displayed and the plate carrier will move out automatically. The assay corresponding to the Quick Operation button will be displayed in the **Selected Assay(s)** box.

   Note: Be sure that the area in front of the MLX Luminometer is clear to prevent damage to the plate carrier when it moves out. Six inches of space is required in front of the system.

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Figure 5-1 The Quick Operation Buttons

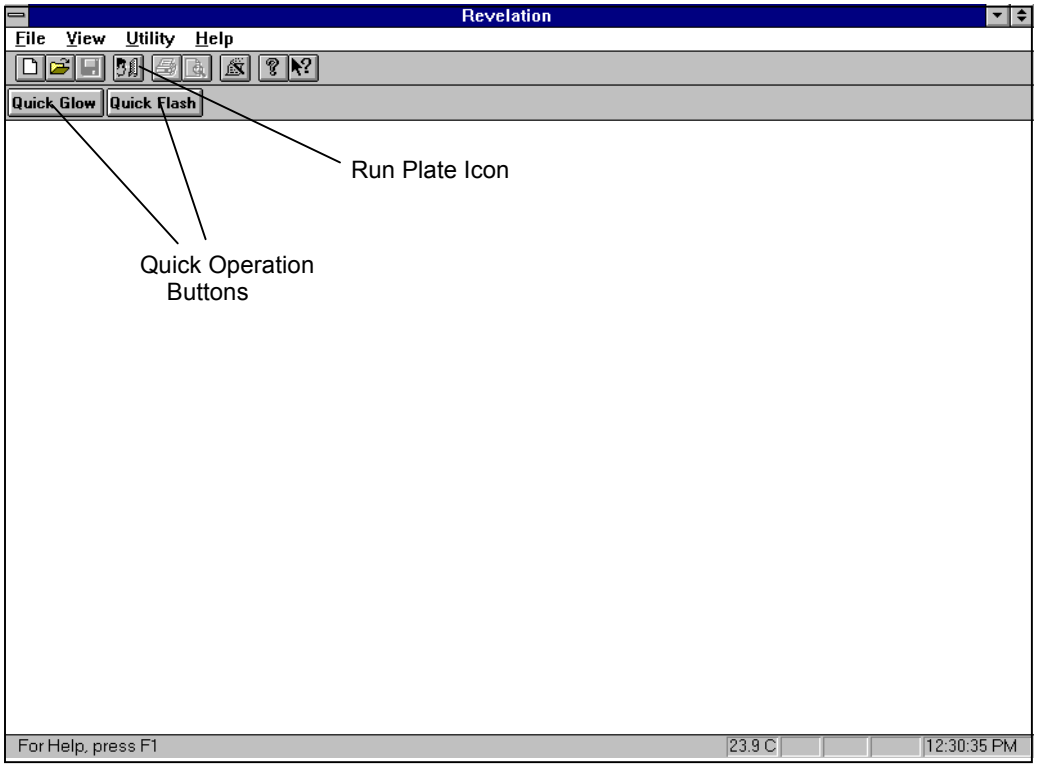
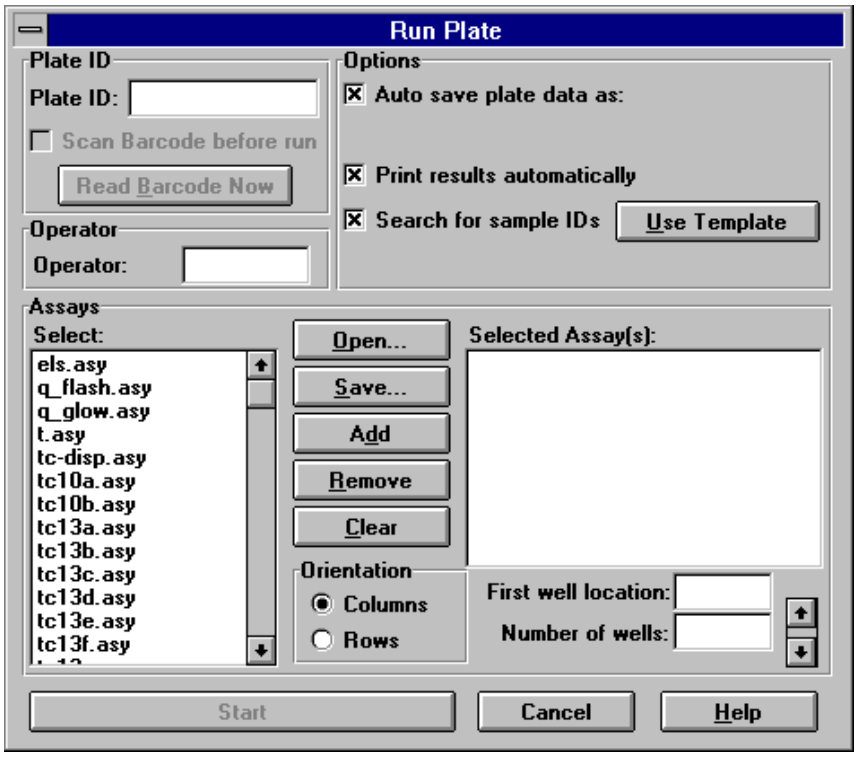


Figure 5-2 Run Plate Dialog Box



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3. Press the release button on the plate carrier (Figure 5-3). Lift up the plate cover, and place the Microtiter® plate in the plate carrier, observing proper orientation of the plate (Figure 5-4).
4. Gently close the plate cover until it locks in the down position.

Figure 5-3 Opening the Plate Carrier

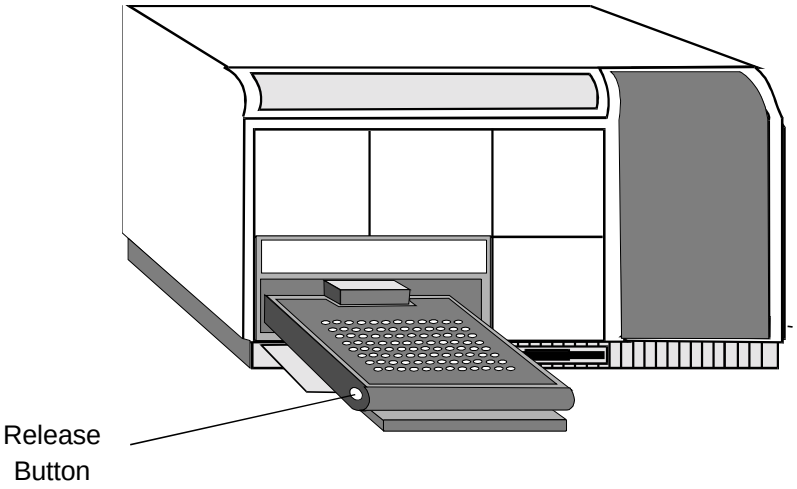
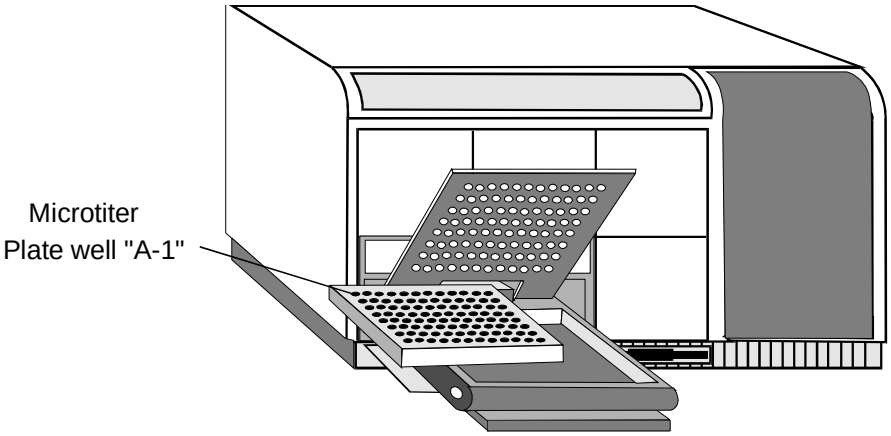


Figure 5-4 Inserting the Microtiter® Plate



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5. ***Plate ID*:** Type the Plate ID (up to eight characters) if you want it to appear in the report.

   Note: If you enabled **Default Plate ID** when defining the MLX Preferences, the next ascending Plate ID will be displayed in the **Plate ID** box. If you wish, you can change that Plate ID by highlighting it and retyping.

6. ***Scan Barcode Before Run*:** Place an **x** in the **Scan Barcode Before Run** check box to have the MLX system read the plate barcode before running the plate.

   Click on **Read Barcode Now** to have the MLX system read the plate barcode immediately.

   Note: If the barcode reader option is not installed on the MLX system, the barcode commands will be grayed (inactive) .

7. ***Operator*:** Type your name or operator code (up to eight characters) as you want it to appear in the report.

8. ***Options:*** Select one or more options by placing a check mark in the appropriate box(es):

   If you want the analyzed data to be automatically saved, select **Auto save plate data as:**. The file name in which the data will be saved is shown below the check box.

   If you want the analyzed data to be automatically printed, select **Print results automatically**.

   If you want to link Sample IDs contained in a separate file to the plate being analyzed, select **Search for Sample IDs**.

   Note: In order to link Sample IDs, **Search for Sample IDs enabled** must be selected when you define the MLX Preferences. Refer to page 59 for details.

9. ***Number of Wells:*** Type the total number of wells to be read. Or, click on the up or down arrow to increment up or down the number displayed in the box.

   Note: This step defines which wells on the plate will be read. If you do not make an entry, all of the wells specified in the template will be read.

10. Click on **Start**.

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Using the Run Plate Command

Use the Run Plate command to run an assay that is not assigned to a Quick Operation button. When using the Run Plate command, the assay to be run must be selected before proceeding. Otherwise, the procedure is identical to running an assay using a Quick Operation button.

To run an assay not assigned to a Quick Operation button:

1. If a Flash type assay is being run and the dispensers are being used, be sure that the proper reagents have been loaded and that the dispensers have been initialized and primed before running the assay. For further information concerning preparation of the dispensers, refer to Chapter 2.

2. Click on **File** and then click on **Run Plate**. The Run Plate dialog box (Figure 5-2) will be displayed and the plate carrier will move out automatically.

   Note: Be sure that the area in front of the MLX Luminometer is clear to prevent damage to the plate carrier when it moves out. Six inches of space is required in front of the system.

3. Press the release button on the plate carrier (Figure 5-3). Lift up the plate cover, and place the Microtiter® plate in the plate carrier, observing proper orientation of the plate (Figure 5-4).

4. Gently close the plate cover until it locks in the down position.

5. Specify the ***Plate ID***, ***Operator***, ***Barcode***, and ***Options*** selections as outlined in the previous section:

6. The assays that can be run are displayed in the **Select** box (Figure 5-2). Click on the assay that you want to run. And then click on Add to place the selected assay in the **Selected Assay(s)** box.

   Note: You can run more than one assay. The plate will be run using every assay that is placed in the **Selected Assay(s)** box.

   If you want to remove an assay from the **Selected Assay(s)** box, click on the assay and then click on **Remove**. If you want to remove all of the assays from the **Selected Assay(s)** box, click on **Clear**.

   If you want to open one of the assay files, click on the assay and then click on **Open**. Click on **Save** to save the assay (if you have modified it).

7. Select the ***Number of Wells*** as outlined in the previous section.

8. Click on **Start**.

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Lot-Specific Data

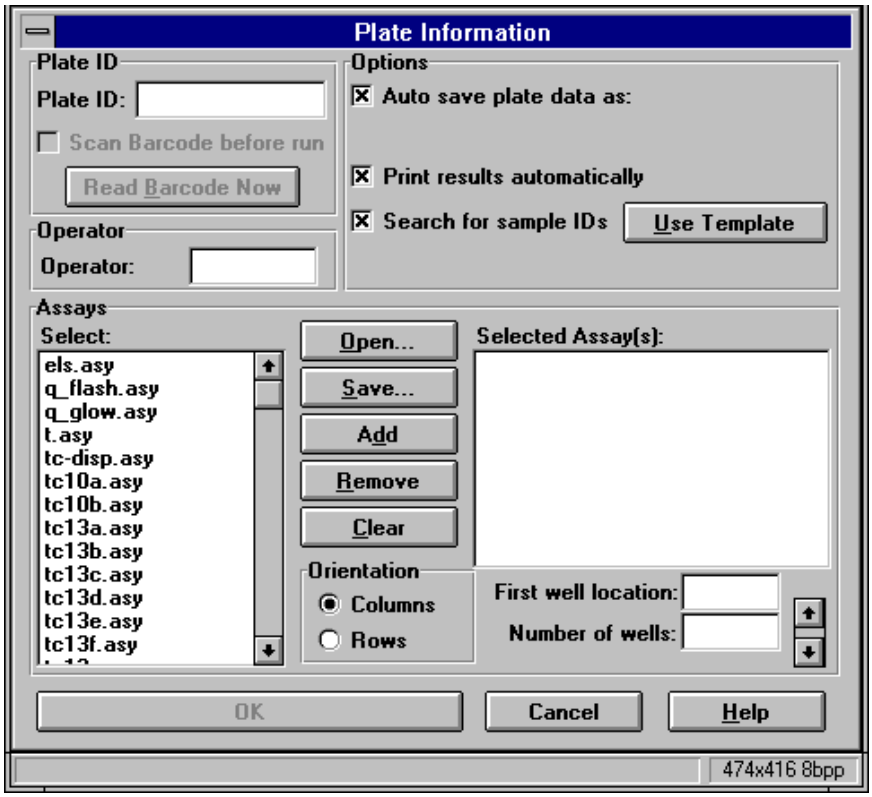
If you selected **Ask for Lot Specific Data** when you defined the Preferences for the MLX, the name of the Run Plate dialog box is changed to "Plate Information" and the **Start** button on the dialog box is labeled as **OK** (Figure 5-5). However, the dialog box is used in the same manner as the Run Plate dialog box.

The lot-specific data can be used to simply record the lot number and expiration date of the reagent kit that was used, or it can be used to specify the standard values and/or control values if standards and/or controls are included in the kit.

> **Note:** If you want to verify the RLUs obtained on controls against lot-specific control values, you must enable **Lot specific checking of control values** on the Q.C. Equations tab of the Quality Control: Raw Data dialog box.
>
> If you want lot-specific standard values to be used for preparing the standard curve, you must enable **Enter concentrations at run time** on the Standards tab of the Curve Fit dialog box.
>
> If you want to verify the results obtained on controls against the lot-specific control values, you must enable **Lot specific checking of control values** on the Calculated Q.C. tab of the Curve Fit dialog box (if quantitative results are obtained), or you must enable **Lot specific checking of control values** on the Threshold Q.C. tab of the Threshold dialog box (if qualitative results are obtained).
>
> Refer to Chapter 4 for details.

If you elect to use lot-specific data at run time, the Lot Specific Data dialog box (Figure 5-6) will be displayed after you click on the **OK** button of the Plate Information dialog box. This dialog box allows you to enter the lot number, expiration date, standard values, and the expected ranges for controls for different lots of reagents.

Figure 5-5 Plate Information Dialog Box



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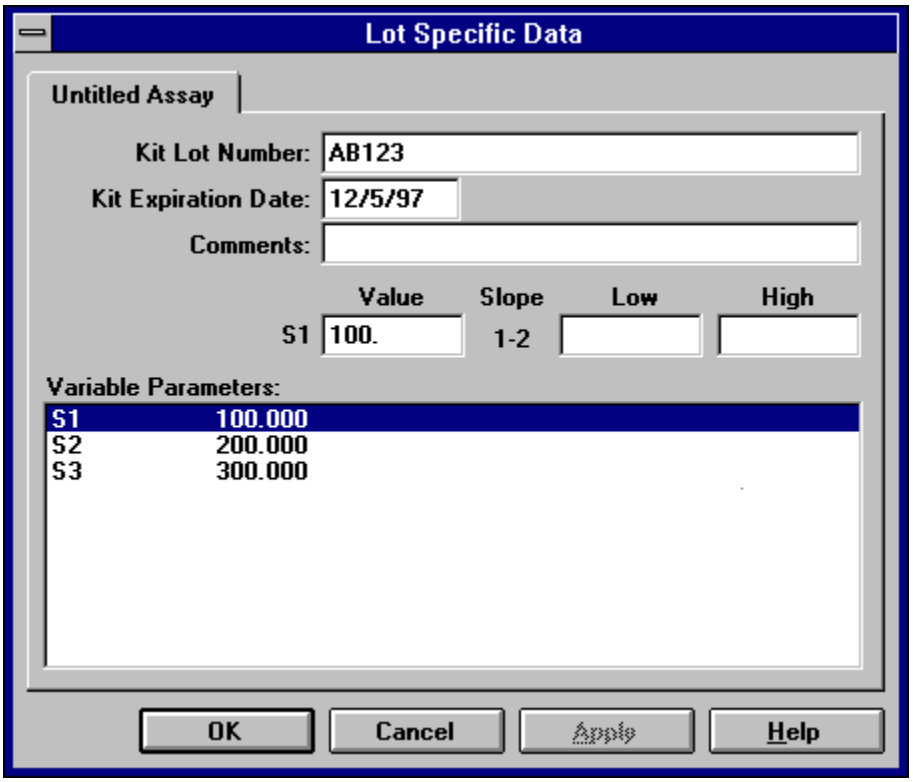
Selecting Lot-Specific Data

The data for various lots of reagents are contained on separate pages of the Lot Specific Data dialog box (Figure 5-6). At run time, you specify the lot number of the reagent kit by displaying the appropriate page.

To select lot-specific data for the run:

1. Determine the lot number of the reagent kit that you are using.
2. Select the page in the Lot Specific Data dialog box that corresponds to the lot number of the reagent kit you are using.
3. Click on **OK**.

Figure 5-6 Lot Specific Data Dialog Box



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Entering New Lot-Specific Data

If data for the reagent kit lot that you are using is not contained in Lot Specific Data dialog box, then you must enter the data by completing a new page. Once you have done this, the data can be used for subsequent runs.

To enter new lot-specific data:

1. Determine the lot number of the reagent kit that you are using.
2. Select the last page in the Lot Specific Data dialog box.
3. Type the **Kit Lot Number**, **Kit Expiration Date**, and **Reagent Lot Number** (if different from the kit lot number).
4. ***Variable Parameters:*** Click on the first lot-specific parameter. That parameter will be displayed adjacent to the **Value**, **Low**, and **High** text boxes. Then, type the appropriate quantities into each text box.

   Note: The lot-specific parameters that are displayed in the **Variable Parameters** box are established during configuration of the assay. For example, if you defined four standards and you enabled **Enter concentrations at run time** in the Standards tab of the Curve Fit dialog box, the four standards will be displayed in the **Variable Parameters** box.

5. Click on the next lot-specific parameter and type the appropriate quantities into each text box. Repeat until all of the lot-specific parameters have been entered.
6. Click on **OK**.

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Sample IDs

Entering Sample IDs at Run Time

If you selected wells for Sample ID Setup when you defined the assay, you will be prompted for the Sample ID that corresponds to each well after you click on **Start** in the previous procedure.

Note: Refer to Sample ID Setup on page 184 for details on specifying wells requiring a Sample ID at run time.

To enter Sample IDs when prompted:

1. A message box will be displayed prompting you to enter the Sample ID for the first designated well.
2. Type the Sample ID for the designated well.
3. Repeat Step 2 for each well as prompted.
4. When you have entered the last Sample ID, the assay will automatically proceed.

Linking Sample IDs to a Separate File

The Sample ID that corresponds to each well ID can be specified in a separate file. Then, if **Search for Sample IDs** is enabled in the Run Plate (or Plate Information) dialog box, that Sample ID file will be automatically linked to the plate at run time.

To prepare a Sample ID file:

1. Click on Utility and then click on Sample ID Entry. The Sample ID Entry dialog box (Figure 5-7) will be displayed.
2. ***Plate ID:*** Type the plate ID (up to 8 characters) for the plate that the file will be used with. Plate IDs may consist of any character you can enter using the keyboard.

   Note: If you have Barcode hardware and software installed, the Barcode option will be available. This allows you to read the barcode on the side of the plate as the plate ID instead of entering it manually.

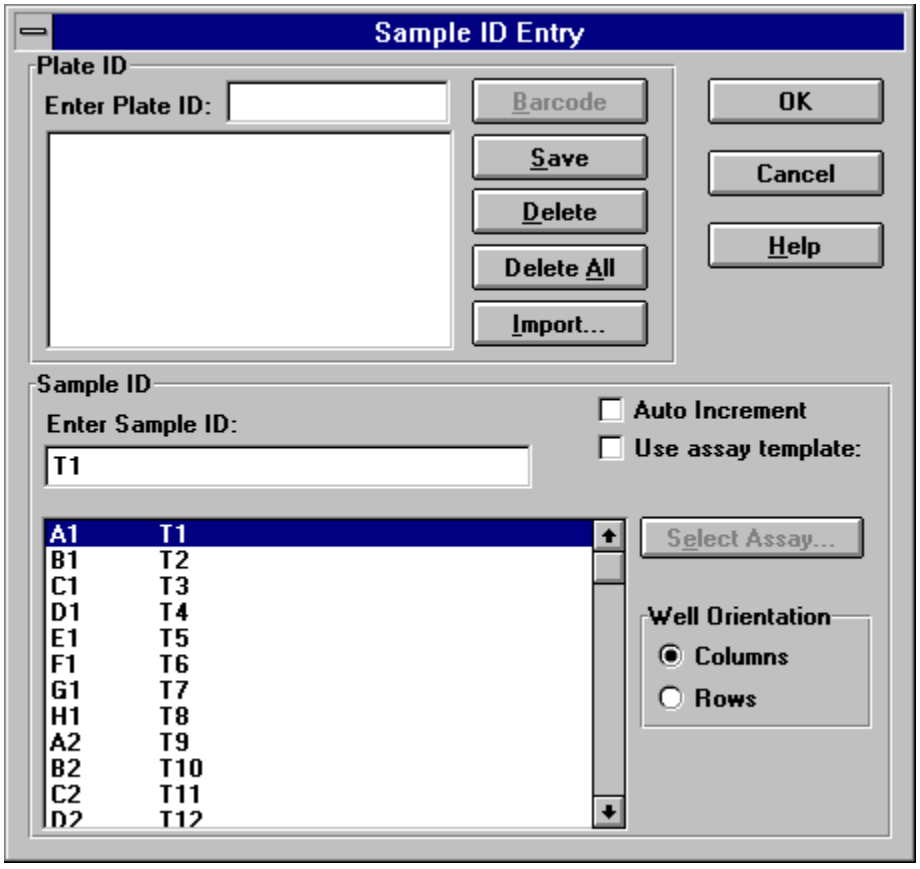
3. An existing Sample ID file can be imported and copied. As each file is imported, its name will be displayed in the Plate ID window.

   Note: You can import a Sample ID (SID) file from another software package. Revelation offers you a list of SID file types. Select the SID file type and click on Import. See “Importing Data” on page 202.

   To delete the currently selected Sample ID file, click on **Delete**. To delete all of the Sample ID files that are displayed, click on **Delete All**.

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Figure 5-7 Sample ID Entry Dialog Box



4. ***Sample ID:*** To enter a Sample ID for a well, click on that well location and then type the corresponding Sample ID in the **Enter Sample ID** box. Repeat for every Sample ID that you want to enter.

   Note: If you want to automatically increment Sample IDs, place an **x** in the **Auto Increment** check box and use a Sample ID with a number at the end. Then, when you advance to the next well location, the sample for that well will be labeled with the same ID incremented by one number.

   Note: If you want to use the well ID information that you have specified in the assay template, place an **x** in the **Use assay template** check box.

5. Designate the well orientation by clicking on the **Columns** or **Rows** button.

6. Click on **OK**.

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Linking Sample IDs after the Plate is Run

The Sample ID file that corresponds to a plate can also be linked to the plate after the plate has been run.

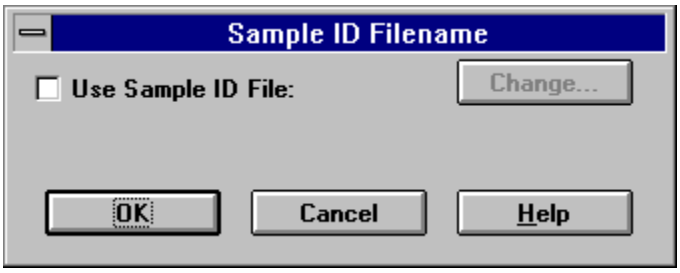
To specify that a Sample ID file is used:

1. Click on Data and then click on Sample ID Filename. The Sample ID Filename dialog box (Figure 5-8) will be displayed.
2. Place an **x** next to **Use Sample ID** Files.

   Note: If you want to use a different Sample ID file, click on **Change** to display a list of Sample ID files. Then, select the file you want to use.

3. Click on **OK**.

Figure 5-8 Sample ID Filename Dialog Box



Displaying Sample IDs in the Data Matrix

Sample IDs can be displayed in the data matrix for a run. The steps to take for enabling this feature are summarized below.

To display Sample IDs in the data matrix:

1. Click on **Settings**, click on **Assay Options**, and select the Sample ID Setup tab. In the Sample ID Setup dialog box, place a check mark in each well for which a Sample ID should be displayed.
2. Click on **Settings** and click on **Report Options**. The Report Layout dialog box will be displayed. Ensure that **Data Matrix** is in the **Check list** box.
3. Select the Data Matrix tab. In the Data Matrix dialog box, click on **Calculated Data with Template**.
4. Save the assay.

   Note: To display Sample IDs in the data matrix, the Sample IDs must be entered at run time or linked to a separate file. Refer to page 199.

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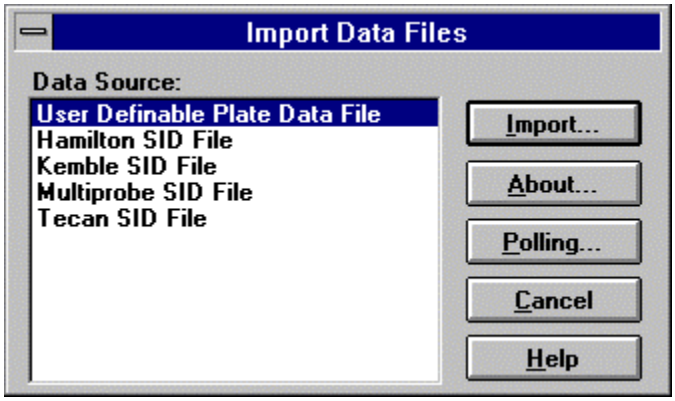
Importing Data

When you import a Sample ID file or a data file from an external software package, Revelation displays an Import Data Files dialog box (Figure 5-9).

Note: Click on **About** to view information about the software import filter in use.

If your system is connected to a sample preparation device (via a network, for example) and you want to interrogate the device software periodically, you can set Revelation up to poll for new information at regular intervals. Click on **Polling** to access the Polling dialog box.

Figure 5-9 Import Data Files Dialog Box



Note: Revelation may not be able to import files from external software if they do not follow certain file format conventions. These conventions are described in the Appendix for Tecan, Kemble, Packard Multiprobe and Hamilton.

User Definable Data File (Flexible File Import)

Click on user definable plate data file to assign different fields in the data file to different Revelation data topics. The Flexible File Import box offers you a list of the fields in the data file you are importing. The fields are defined by the delimiter box. By default, the delimiter is a comma, so Revelation defines a field as a unit of data separated from other units of data by a comma.

To assign a field from the data file to a Revelation data topic, click on the field and then click on the topic in the **Fields in Section** list box. Revelation will show the link by joining the two fields with a line.

Flexible File Import Dialog Box Command Buttons

Command	Description
Map All	Assign Field 1 to the first topic, Field 2 to the second topic and so on.
Clear	Clear all current field-topic assignments.
Import	When you have assigned the fields to topics, click on **Import** to import the file.

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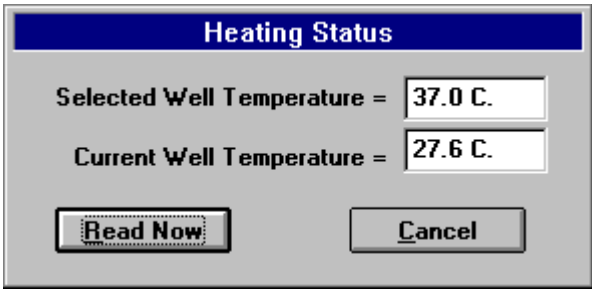
Reading of the Plate

Status Messages

When actual reading of the plate commences, certain messages may be displayed. These messages indicate the status of the MLX Luminometer and provide you an opportunity to abort the assay if conditions are not appropriate.

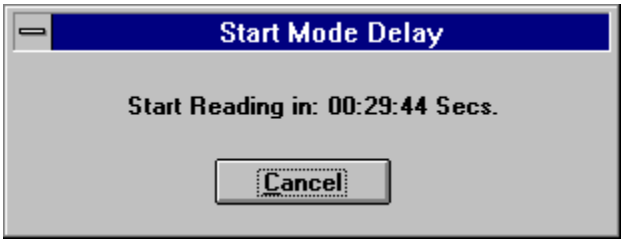
For example, if the temperature of the well is not consistent with the temperature specified in the assay parameters, a Heating Status message box (Figure 5-10) will be displayed. This message box will describe the selected and current temperatures and provides choices to **Read Now** or **Cancel**.

Figure 5-10 Heating Status Message Box



If the assay configuration specifies a delay in the start of the reading, a message box will indicate the time remaining for the reading to start (Figure 5-11).

Figure 5-11 Start Mode Delay Message Box



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Display of Results

The information that is displayed while a plate is being read depends upon the assay type. For a glow-endpoint assay, status information is displayed. For kinetic and flash assays, results are displayed and updated as each well is read.

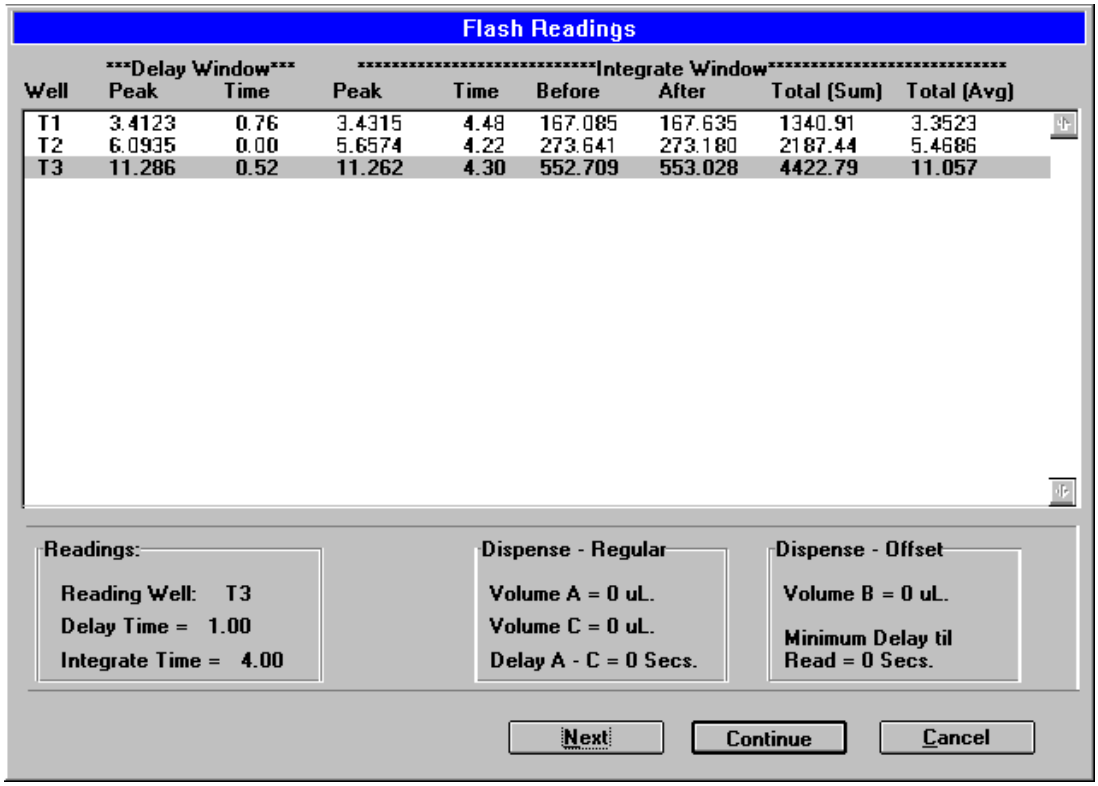
The Readings window that is displayed as wells are read contains a **Cancel** button. Clicking this button causes the remaining readings on the plate to be terminated after reading of the current well is completed.

For a flash assay, the readings that are obtained for each well are displayed in tabular form while the plate is being read (Figure 5-12) or in graphical form (Figure 5-13).

For flash assays, you can choose to examine the plotted results on each well before the next well is read. To activate this option, access Reader Control on the Settings menu, select the Data Graphs tab, and place an x in the Graph Wells on Screen box. After the first well is read, a plot of the results is displayed (Figure 5-13). Clicking on the **Next** button causes the next well to be read and the plotted results for that well to be displayed. Clicking on the **Continue** button causes the readings on the remainder of the plate to be displayed in tabular form. Clicking on the **Cancel** button causes the remaining readings on the plate to be terminated.

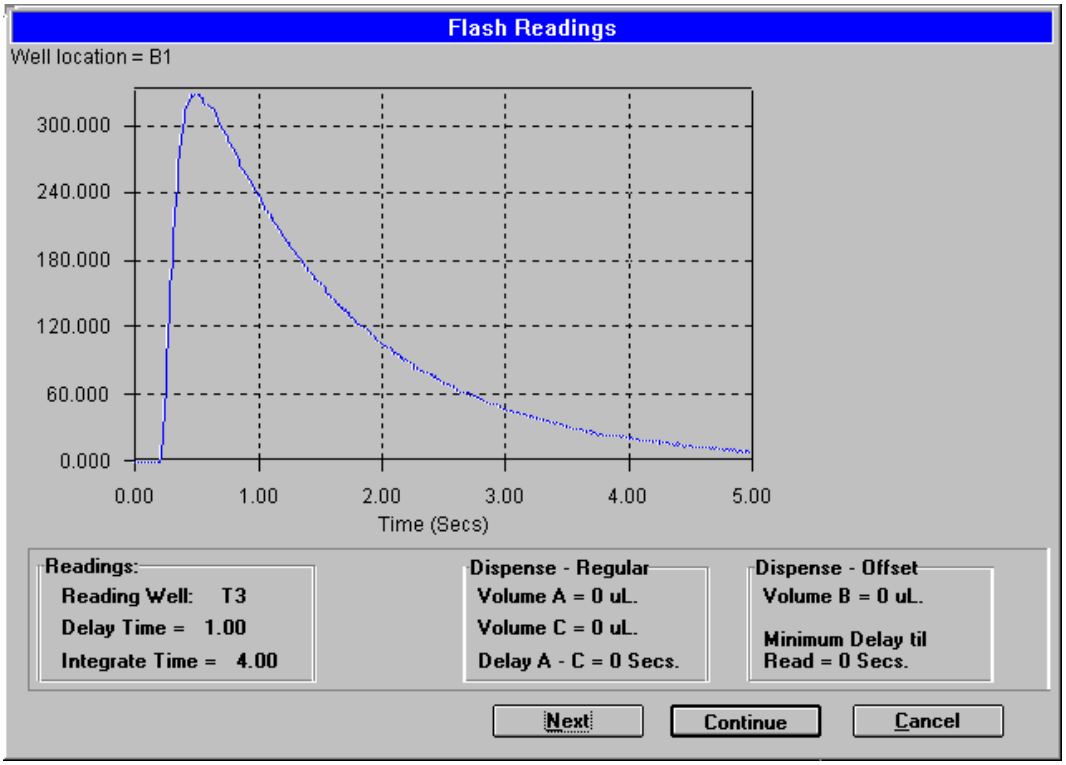
Finally, the results on all of the wells are displayed in a separate window after the assay is completed. The information that is contained in the results report and the format of the display is defined in the Settings menu for the assay.

Figure 5-12 Tabular Display of Flash Readings



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Figure 5-13 Plotted Flash Readings



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Results Presentation if QC Fails

If you enabled **Suppress results if Q.C. Fails** when you defined the Output Format during assay definition, results will not be displayed or printed if any of the Q.C. expressions that are defined for the assay are failed.

If you enabled **Full Q.C. Report** when you defined the Output Format during assay definition, the quality control results will be displayed or printed. Otherwise, only the Failure Description that you entered when you defined Quality Control: Raw Data, Calculated Q.C., or Threshold Q.C. will be displayed or printed.

Analysis of Results

Once the plate has been read, you can either utilize the results from the assay used when the plate was read, you can re-analyze the results using a different assay, you can re-analyze the results after removing outliers, or you can re-analyze the results after changing one or more assay parameters.

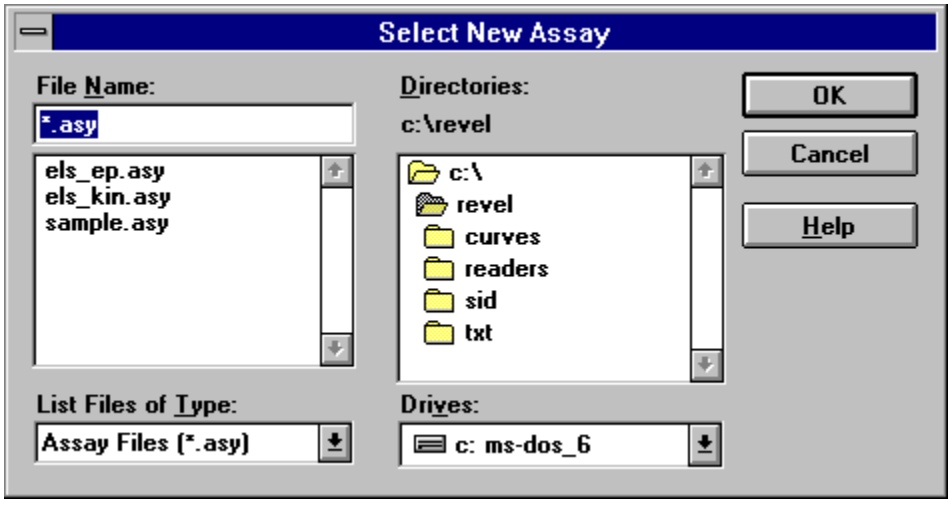
Analyzing Results Using a New Assay

You can re-analyze the data using a different assay.

To analyze results using a new assay:

1. Click on Data and then click on Select New Assay. The Select New Assay dialog box (Figure 5-14) will be displayed.
2. If necessary, select the appropriate directory. Select the new assay that you want to use for analyzing the data.
3. Click on **OK**. The data will be re-analyzed using the assay you selected.

Figure 5-14 Select New Assay Dialog Box



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Removing Outliers and Re-Analyzing Results

You can also remove outlier readings and then re-analyze the data using either the same assay or a different assay. The results for any replicate well can be removed, or one or more outlier points in a kinetic reading can be removed and the results for the well recalculated.

Usually, removal of outliers is done if you suspect that the outlier is caused by an artifact. If you remove any outliers, the values that you removed will be listed at the beginning of the results printout.

Note: This option should be used carefully, as removing readings may affect the interpretation of results.

To remove outliers and re-analyze results:

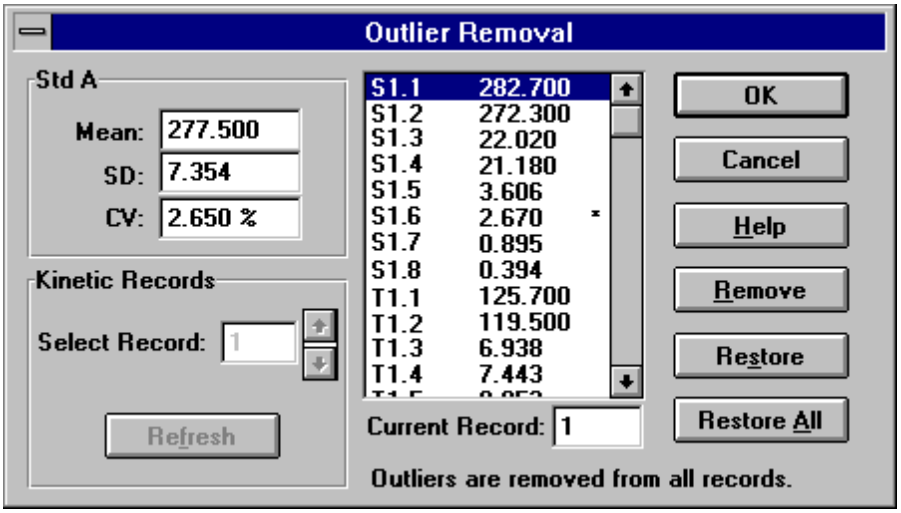
1. Click on Data and then click on Outlier Removal. The Outlier Removal dialog box (Figure 5-15) will be displayed. Each replicate well and the mean RLU for that replicate will be displayed in the text box. Use the scroll arrows to view a replicate value that does not appear in the box.

   The mean RLU value and the SD and CV of the individual RLU values that were used to compute the mean are displayed in the window to the left. The window is labeled with the well ID (for example, **T1** for the first unknown sample).

   If the plate was read using a kinetic assay, the **Kinetic Records** window will be available. The effect of successively removing outlier RLUs from the kinetic curve one reading at a time is determined by clicking on the up arrow of the **Select Record** box. Each successive kinetic record reflects the value obtained with one less RLU point, and the resulting mean value, SD, and CV for the replicate can be viewed by clicking on **Refresh**.

2. Click on the first replicate that you want to examine. Examine the Mean, SD, and CV values for that replicate to determine whether you want to either remove the replicate or remove the replicate from all plate readings if a kinetic assay was used.

Figure 5-15 Outlier Removal Dialog Box



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3. To remove a replicate reading, click on **Remove** while the corresponding well ID is highlighted. The replicate well ID will be then displayed with an asterisk (*), indicating that you have removed the replicate.

   Note: To restore a replicate reading that you have removed, highlight the corresponding well ID and click on **Restore**. To restore all replicate readings, click on **Restore All**.

4. To remove one or more replicates from a kinetic reading, click on the up arrow in the **Kinetic Records** box. Highlight the well ID of the replicate to be removed and click on **Remove**. The replicate well ID will then be displayed with an asterisk (*), indicating that you have removed the replicate.
5. Click on **OK**. The data will be reanalyzed using the outlier removals that you specified.

Refresh

You can examine the effect of modifying an assay parameter on results at any time. When you do this, the results using the original assay will be saved in the data file. The results from the new assay will be displayed on the screen but not saved.

To re-analyze results:

1. Click on **Data** and then click on **Refresh**. The data will be reanalyzed using the current assay parameters.

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Fit Information

When reviewing results, you can examine how different curve fitting algorithms will affect the results that were obtained.

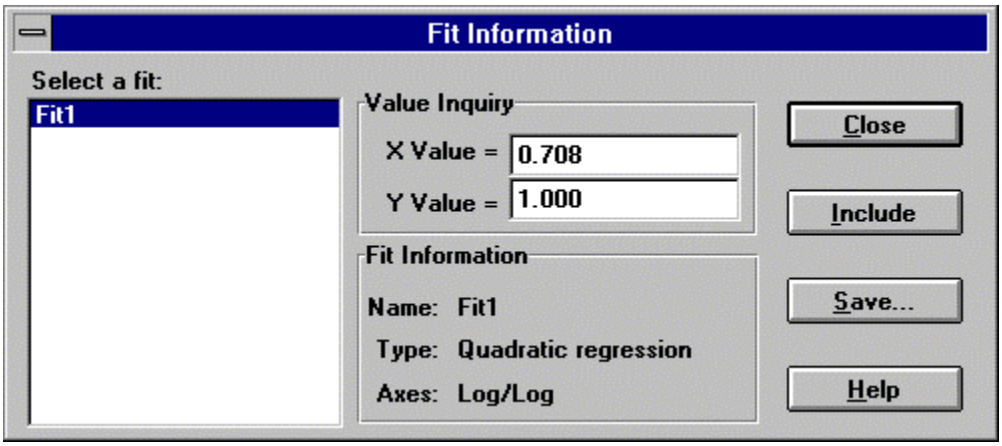
To analyze fit information:

1. Click on Data and then click on Fit Information. The Fit Information dialog box (Figure 5-16) will be displayed. The names of all of the available curve fitting algorithms will be displayed in the **Select a fit** box.
2. Click on a curve fitting algorithm that you want to examine. Information about the algorithm is displayed in the **Fit Information** box, and corresponding **x** and **y** values are displayed in the **Value Inquiry** box.

   Note: To determine a **y** value that corresponds to an **x** value, type in the **x** value (or vice versa).

3. Repeat **Step 2** for other curve fitting algorithms, if needed. When you are finished reviewing the algorithms, click on the algorithm that is to be used.
4. Click on **Close**. The data will be reanalyzed using the algorithm you selected.

Figure 5-16 Fit Information Dialog Box



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Kinetic Graphs

For kinetic assays, you can specify those wells for which a kinetic graph will be displayed or printed.

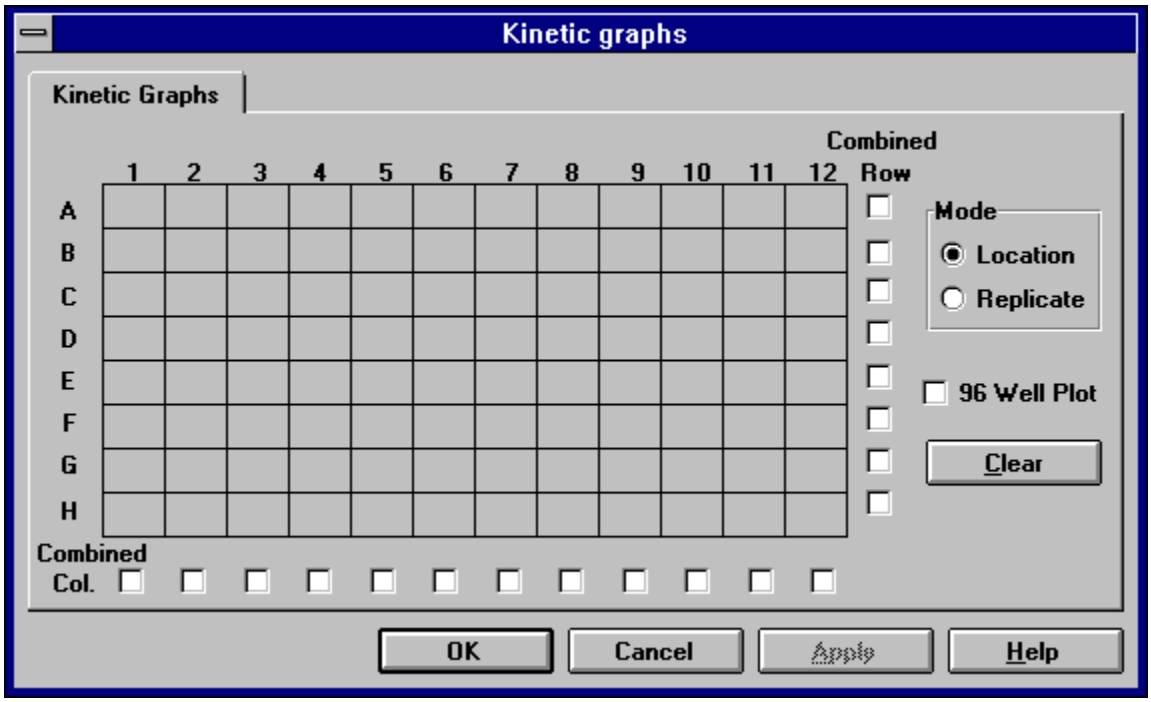
To specify kinetic graphs:

1. Click on Data and then click on Kinetic Graphs. The Kinetic Graphs dialog box (Figure 5-17) will be displayed.
2. **Mode:** Click on the **Average** radio button if you want to automatically select all replicates of a designated sample and graph the average. Click on the **Individual** radio button if you want to separately graph each designated well.
3. Click on each well for which you want a graph to be prepared. To graph an entire plate, place an **x** next to **96 Well Plot**. To graph all of the wells in a row, place an **x** in the corresponding box under **Combined Row**. To graph all of the wells in a column, place an **x** in the corresponding box under **Combined Column**.

   Note: Click on **Clear** to remove all graphs.

4. Click on **OK**. The data will be reanalyzed using the algorithm you selected.

Figure 5-17 Kinetic Graphs Dialog Box



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Titration Multiplier

For titration assays, you can specify a multiplier to apply to a plate.

To specify a titration multiplier:

1. Click on Data and then click on Titration Multiplier. The Titration Multiplier dialog box (Figure 5-18) will be displayed.
2. **Titration Multiplier:** Type the numeral corresponding to the titration multiplier.
3. Click on **OK**. The data will be reanalyzed using the titration multiplier you selected.

Figure 5-18 Titration Multiplier Dialog Box

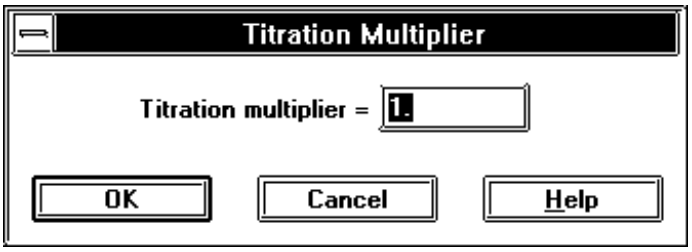


Plate-Plate Statistics

The Plate-Plate Statistics dialog box allows you to calculate the grand mean and the total SD, the total CV, and the total SEM across multiple plates on areas that you have designated within each of the plates.

Before you run Plate-Plate statistics on multiple plates, you must define the Area Statistics (see page 162) for each of the plates being analyzed. The individual areas that are to be analyzed across plates must have the same area definition. For example, if you wish to run Plate-Plate Statistics on a particular control sample for which wells are designated as **2** in Area Statistics for the first plate, the wells for that control must also be designated as **2** in the Area Statistics for all of the other plates.

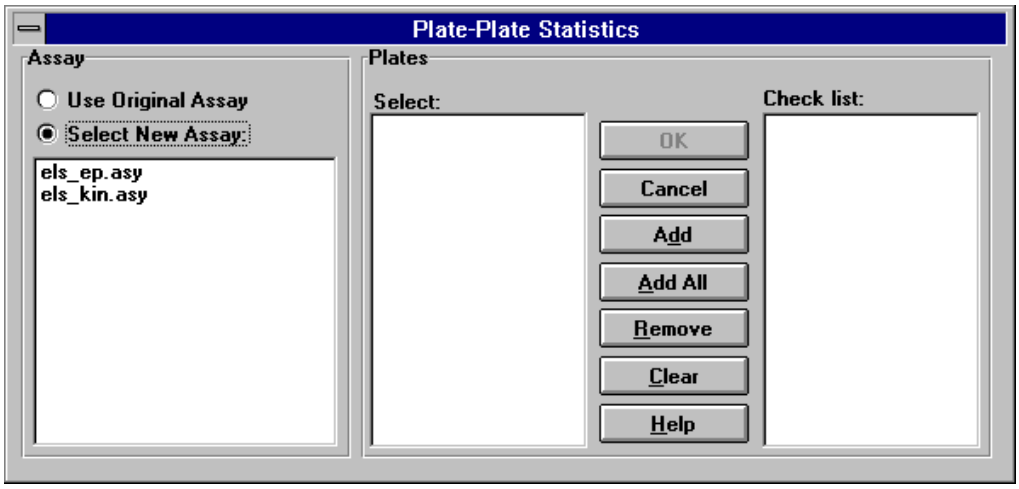
To analyze plate-to-plate statistics:

1. Click on Plate-Plate Statistics in the Utilities menu. The Plate-Plate Statistics dialog box (Figure 5-19) will be displayed. The plate data files that are stored in memory will be listed in the Select**:** window.
2. Specify the first plate data file that you want to include in the plate-to-plate statistical analysis by clicking on it in the **Select:** window. Then, click on **Add**. The sample you specified will be displayed in the **Check list:** box.

> **NOTE:** If you want to include all of the plate data files, click on **Add All**.

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Figure 5-19 Plate-Plate Statistics Dialog Box



3. Repeat Step **2** to specify additional plate data that you want to include in the plate-to-plate statistical analysis. As you add data from each plate, it will be added to the list in the **Check list:** box.

   NOTE: If you want to remove a plate data file from the **Check list:** box, highlight the data file in the box and then click on **Remove**. If you want to remove all of the plate data files from the **Check list:** box, click on **Clear**.

4. Click on the **Use Original Assay** button if you want to compare the plates analyzed with the assay they were originally measured with. If you want to compare the plates analyzed with a different assay, click on the **Select New Assay** button.

5. Click on **OK**.

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6 Maintenance/Troubleshooting

General Cleaning

The outside of the MLX Luminometer components should be periodically cleaned with a paper towel or cloth. The area around the system components should be checked to ensure that air flow around the components is unrestricted and that materials such as papers are not interfering with the air flow.

Note: Food and drinks should not be brought into the vicinity of the system.

Cleaning the Mouse

The area where the mouse is used should be kept clear of lint and small particulate matter, as this may build up inside of the mouse housing and interfere with proper operation of the mouse.

To clean the mouse:

1. Invert the mouse.
2. Unscrew the retaining clip of the mouse roller. Remove the mouse roller.
3. Shake any lint or dust from the roller housing.
4. Replace the mouse roller and retaining clip.

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Cleaning the Syringes

The syringes are intended for use with precision liquid handling equipment such as the MLX Luminometer system. The syringes are not intended for food or medicinal use.

In order to obtain optimal performance and life from the syringes, the following precautions must be observed:

- Do not operate the syringes for more than a few cycles without liquid in them.
- If the plunger is removed from the syringe barrel, it should be wiped with alcohol before replacing.
- If the plunger does not easily move within the barrel, remove the plunger, wet the seal with distilled or deionized water, and return the plunger to the syringe barrel.
- The syringes should be replaced periodically to ensure optimal performance. The frequency of syringe replacement will depend upon the level of usage and the reagents that are used.
- It is recommended that the syringes be cleaned on a weekly basis, using one of the syringe cleaning procedures outlined below.

To clean the syringes using weak detergent or 10% beach:

1. Fill the syringe with a weak detergent or a solution of 10% bleach.
2. Allow the solution to remain in the syringe for 30 minutes.
3. Flush the syringe at least ten times with distilled or deionized water.

To clean the syringes using acid and base:

1. Fill the syringe with 0.1N NaOH solution.
2. Allow the solution to remain in the syringe for 20 minutes.
3. Flush the syringe with distilled or deionized water.
4. Fill the syringe with 0.1N HCl solution.
5. Allow the solution to remain in the syringe for 20 minutes.
6. Flush the syringe at least ten times with distilled or deionized water.

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Cleaning the PMT Lens

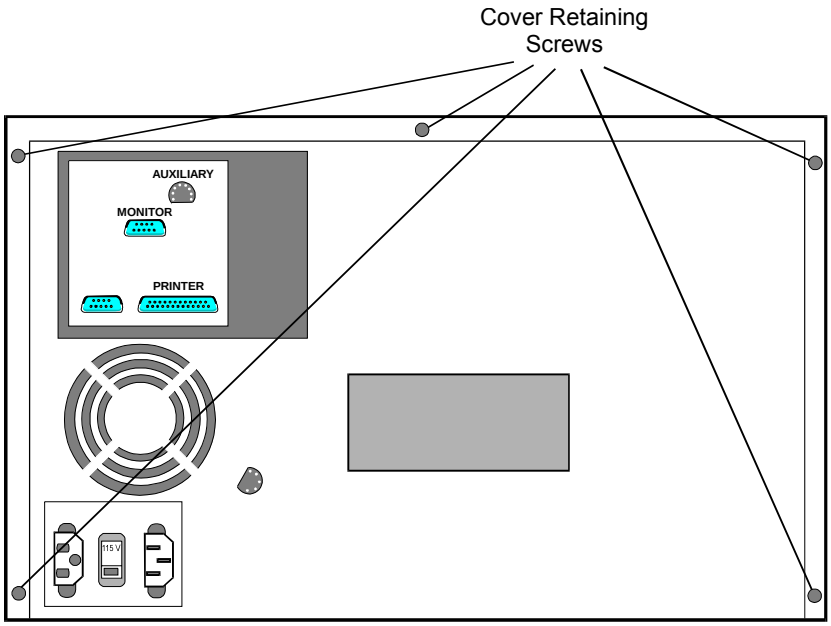
If fluids are being dispensed, it is recommended that the Photomultiplier Tube (PMT) lens be cleaned on a weekly basis.

CAUTION: The power cable of the MLX Luminometer must be disconnected from the electrical outlet before commencing with this procedure. Failure to do this may result in electrical shock.

To clean the PMT lens:

1. Disconnect the power cable of the MLX Luminometer.
2. Remove the five screws from the back of the MLX Luminometer (Figure 6-1). Slide the cover back approximately one inch and then lift the cover off.

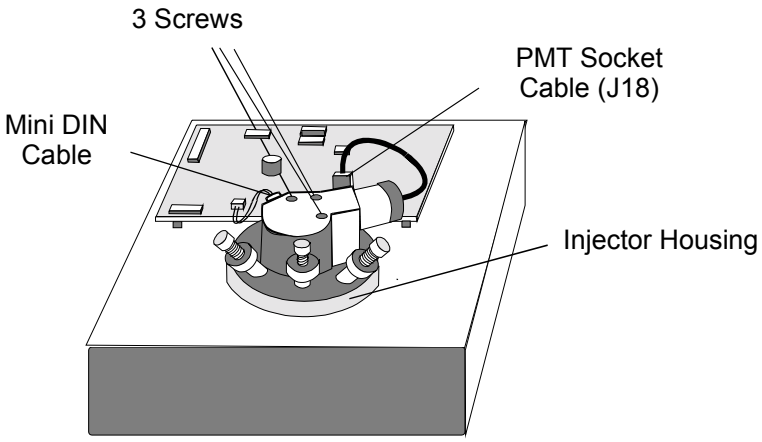
Figure 6-1 Removing Instrument Cover



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3. Disconnect the PMT Socket Cable from the J18 connector on the circuit board (Figure 6-2).
4. Disconnect the Mini DIN cable from the PMT tube housing (Figure 6-2).
5. Remove the three M3x45 socket head cap screws from the PMT tube housing (Figure 6-2).
6. Lift the PMT housing clear of the injector housing assembly.
7. Wipe the PMT lens clean using a damp cloth. Dry the lens using soft tissue paper.
8. Replace the PMT lens housing assembly.
9. Secure the PMT lens housing assembly using the three M3x45 socket head cap screws (Figure 6-2).
10. Connect the Mini DIN cable to the PMT tube housing (Figure 6-2).
11. Connect the PMT Socket Cable to the J18 connecter on the circuit board (Figure 6-2).
12. Replace the cover on the MLX Luminometer. Then, connect the power cable.

Figure 6-2 Removing the PMT Housing



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General Precautions

Certain general precautions are necessary during operation of the MLX Luminometer to ensure that data are not lost or that the system is not damaged. These precautions are listed below.

Power ON/OFF

Power switches to all of the components must be switched OFF before any of the cables that interconnect the components such as the mouse are disconnected. Failure to follow this procedure may cause damage to the MLX Luminometer components.

IMPORTANT: Never shut off the power to the MLX Luminometer while the system is being operated, as loss of data may result.

The power switch on the computer must not be turned OFF while the diskette drive indicator light is illuminated. If the power is turned off while the diskette drive is active, the drive may be damaged or the data contained on the diskette may be damaged.

Software Modification

The MLX Luminometer software and software settings are password protected in order to ensure that they are not updated. These settings must not be modified in any way. Modification of the software may cause some functions of the MLX Luminometer not to work properly.

System Rebooting

Rebooting of the system software must only be done while the diskette drive indicator light is not illuminated, to prevent damage to the drive or loss of data.

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Hazards

Certain precautions are necessary during operation of the MLX Luminometer to ensure that personal injury does not occur. These precautions are listed below.

Spills

Liquid spills on the MLX Luminometer components must be avoided, as they may lead to electrical shock or electrical problems.

Note: If a liquid spill occurs, turn OFF the power to all of the components and disconnect the power cords from their source before attempting to clean the liquid spill.

System Covers

The system must be operated with covers and panels in place and secured, since they protect the operator and others from exposure to electrical hazards, as well as protect the internal parts of the MLX Luminometer components from damage.

IMPORTANT: Do not remove any covers or panels from the MLX Luminometer components.

Data Recovery

Whenever a plate is read, the data is saved in the *temp.tmp* file and remains there until the data is overwritten when the next plate is read. The data can be recovered in the event of a system failure.

To recover data from the *temp.tmp* file:

1. Quit Revelation (using the **Exit** command).
2. Access File Manager in Windows® Program Manager.
3. In the directory REVEL, highlight the file *temp.tmp*.
4. Rename *temp.tmp* to *temp.dat*.
5. Exit File Manager.
6. Start Revelation.
7. Open the *temp.dat* file that you created in Step 4.

Note: If necessary, consult your Windows® documentation for instructions on accessing File Manager and renaming a file.

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Operational Problems

This section describes problems occurring during normal operation that may not generate an error message. The symptoms are listed along with probable causes of the problems. The probable causes are listed in order, the first being the most likely, or the simplest one to check.

Symptom	Probable Cause
No Reading Indicated	1. Wrong well selected 2. Plate inserted backwards 3. Solution bottles empty 4. No solution being delivered to the well 5. PMT not plugged in @ J18 6. Bad connection to J18 on microinstrument board 7. J17 not jumpered 1 to 2 8. Bad PMT 9. X/Y position calibration incorrect 10. Bad microinstrument board 11. Shutter failure 12. PMT housing not seated
High buffer solution readings	1. Light leaks 2. Lower door not closed 3. Cover not installed 4. Open injector port 5. Black tubing not in use
High CV levels (CV = Coefficient of Variance)	1. Light leaks 2. Lower door not closed 3. Cover not installed 4. Injector not plugged 5. Black tubing not in use 6. Number of readings per cycle is low 7. Improper gain level selected 8. Shake mode selected when not intended 9. Poor microinstrument grounding 10. Bad J18 connection to microinstrument board 11. Bad A/D converter @ U19 on microinstrument board 12. Bad PMT 13. Bad microinstrument board
Inaccuracy of RLU values (RLU = Relative Luminescent Unit)	1. Unit not recalibrated after part replacement 2. Selection of test/blanking wells incorrect 3. Improper amount of solution injected 4. Light leaks 5. Lower door not closed 6. Cover not installed 7. Injector not plugged 8. Black tubing not in use 9. X/Y position out of calibration 10. Bad microinstrument board
Temperature reading fluctuation	1. Poor microinstrument grounding 2. PMT not plugged in @ J18 3. Intermittent internal connections to microinstrument board 4. Bad microinstrument board

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Symptom	Probable Cause
Improper well temperature	1. Temperature sensors not calibrated 2. Bad internal connections 3. Ambient temperature extremes 4. Bad temperature sensors 5. Bad microinstrument board 6. Bad heater pad

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Troubleshooting

The following is a list of error messages that are displayed by the instrument. Listed with each error message is a list of probable causes and solutions. The probable causes are listed in order, the first being the most likely, or the simplest one to check. Any other error messages or error codes not listed here should be reported immediately to the nearest service center.

Message	Probable Cause	Solution
Device not detected	1. J6, J7, and/or J8 improperly jumpered 2. Bad connection from J1 to the main computer board 3. ±12 volts bad at microinstrument board 4. Bad DSR signal on microinstrument board 5. Bad main computer board	1. Call Technical Service.
Command retry error	1. Bad connection from J1 to the main computer board 2. Bad power up sequence 3. Software conflicts with other software 4. Incompatible revisions between software and firmware 5. Bad microinstrument board	1. Call Technical Service.
EEPROM Checksum error	1. Microinstrument board out of calibration 2. Bad microinstrument board 3. Incompatible revisions between software and firmware	1. Call Technical Service.
Recalibration HV Limit exceeded	1. Full scale ranges for each gain level set improperly in FAT_CaP 2. Bad connections to the PMT 3. Bad PMT 4. Bad microinstrument board 5. Incompatibility between software and firmware	1. Call Technical Service.
Unable to create MLX OLE driver	1. Software update not installed correctly 2. OLE module corrupted	1. Reinstall Revelation software. 2. Call Technical Service.
The Micro Instrument and Dispensers may not use the same Port	1. Microinstrument and dispensers using the same COM port	1. Reconfigure the dispensers to a different port
Data Collection Error	1. Available data reads does not equal requested reads 2. Bad microprocessor 3. Bad microinstrument board	1. Reinstall Revelation software. 2. Call Technical Service.

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Message	Probable Cause	Solution
A Volume exists for a Non Available Injector	1. Dispenser Configuration/ Assay definition problem	1. Check hardware configuration and assay description
Micro Instrument Reports Software Error or *Unknown Error Code:___*	1. Bad microinstrument board	1. Call Technical Service.
A/D Circuit Failure	1. Bad A/D converter @ U19 on microinstrument board 2. Bad microinstrument board	1. Reboot the system. 2. Call Technical Service.
Micro Instrument Busy or *Unknown Command* or *Parameter Out of Range* or *Unknown Command Variant* or *Incorrect Number of Parameters* or *Command Variant missing*	1. Bad connection from J1 to the main computer board 2. Bad power up sequence 3. Bad microinstrument board 4. Incompatible revisions between software and firmware	1. Switch off the unit and switch it back on 2. Call Technical Service.
EEPROM Operation Error or *EEPROM Image Write Failure*	1. Microinstrument board out of calibration 2. Bad microinstrument board 3. Incompatible revisions between software and firmware	1. Call Technical Service.
Heater Circuit Failure	1. Bad flex cable connections 2. Bad microinstrument board 3. Temperature sensor failure 4. Heater pad failure	1. Call Technical Service.
Plate Position is unknown	1. X-motor or Y-motor has lost steps 2. X or Y encoder failure 3. Motor circuit failure on microinstrument board	1. Reboot the system 2. Examine X/Y positioner path for obstructions. 3. Call Technical Service.
Plate mask door not closed	1. Operator error 2. Bad mask switch	1. Close the mask 2. Call Technical Service.
Plate positioning error	1. Plate homing error	1. Reboot the system 2. Examine X/Y positioner path for obstructions. 3. Call Technical Service.

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Message	Probable Cause	Solution
Overflow of 20-bit ADC	1. Light leak 2. Lower door is not closed 3. Injector is not plugged 4. Measured well brighter than set range allows 5. PMT is out of calibration 6. Bad A/D converter @ U19 on microinstrument board 7. Bad microinstrument board	1. Check for light tightness 2. Close the lower door 3. Plug in the injectors 4. Call Technical Service.
Bar-Code Reader is not responding	1. Bar code reader not installed 2. Bad connection at J13 on microinstrument board 3. Bar code reader failure 4. Bad microinstrument board	1. Call Technical Service.
X position failure or *Y position failure*	1. Possible obstruction 2. Bad flex cable connections 3. X or Y encoder failure 4. Bad microinstrument board 5. X/Y mechanism failure	1. Remove any obstructions 2. Call Technical Service.
20-bit ADC Test Failure	1. Bad A/D converter @ U19 on microinstrument board 2. Bad microinstrument board	1. Reboot the system 2. Call Technical Service.
ADC Read Buffer Overflow	1. Bad microinstrument board	1. Reboot the system 2. Call Technical Service.
ADC Verification Failed	1. Bad microinstrument board 2. Bad A/D converter @ U19 on microinstrument board	1. Reboot the system 2. Call Technical Service.
Home Position Sensor (X) Failure or *Home Position Sensor (Y) Failure*	1. Home sensor failure 2. Bad internal connections 3. Bad microinstrument board	1. Call Technical Service.
Self-Test Fails	1. Bad internal connections 2. Bad microinstrument board	1. Call Technical Service.

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7 Quality Control

Overview

The following procedures are used for quality control of measurements that are obtained on the MLX Luminometer:

Procedure	Description
Light Intensity Quality Control	Light intensity measurements are monitored during each run by measuring the light intensity of an internal reference and comparing it with its expected value.
Light Leak Testing	The absence of light leaks and the stability of the photomultiplier are verified weekly by measuring the light intensity obtained when no plate is present.
Light Intensity Precision Verification	Precision of light intensity measurements can be verified as needed by measuring the light intensity of an external Reference Plate and comparing the results with the expected values for the Reference Plate.
Light Intensity Accuracy Verification	Accuracy of light intensity measurements can be verified as needed by measuring the light intensity of an external Reference Plate and comparing the results with the expected values for the Reference Plate.
Assay Quality Control	Assay results should be monitored by the user during each run to verify that the results fall within expected criteria. In addition, appropriate control materials should be run by the user and the results compared with expected values.
Reagent Dispensing Accuracy Verification	Accuracy of reagent dispensing volume can be verified as needed by weighing the amount of deionized water that is dispensed from each dispenser.

Each of these quality control procedures is described in detail on the following pages.

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Light Intensity Quality Control

The MLX electro-optical system contains an internal light source which is factory-calibrated to match the output of a reference standard. The internal light source in the MLX Luminometer is a reference LED (Light Emitting Diode). The LED intensity setting for calibrating each gain range (Low, Medium, and High) is determined at the factory and the value stored in the non-volatile memory located in the instrument.

Before a plate is read, the MLX Luminometer performs an internal PMT (Photo Multiplier Tube) re-calibration. First, a shutter closes to isolate the PMT from the chamber containing the sample test plate and multiple readings of the Dark Current (PMT noise) are taken. The LED intensity value is then recalled from the non-volatile memory and the LED set to the proper intensity for use in the internal calibration. A correction factor (Calibration Factor) is computed based on the known amount of light emitted from the LED as compared to the amount of light as seen by the PMT.

When the sample Microplate is read, the MLX Luminometer reads each well, subtracts the noise, and multiplies by the correction factor to calculate the RLU (Relative Light Units). This is shown in the following equation:

Measured well value = (raw PMT well reading - PMT noise) x Calibration Factor

If the light measurement system does not operate within set QC limits, the user is warned of the problem and the plate read is aborted. Refer to Chapter 6 for a listing of the various error messages that may be encountered.

Note: Contact Technical Service if one of these error messages is displayed.

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Light Leak Testing

The MLX electro-optical system should be tested on a monthly basis for light leaks and/or a noisy signal from the photomultiplier tube. More frequent testing using this procedure is not necessary unless the Light Intensity Quality Control testing indicates an error.

The procedure for performing light leak testing is outlined below.

To test for light leaks:

1. Configure a glow-endpoint assay using the parameters described below. Do not place a Microplate on the plate carrier.

Parameter	Setting
Start Mode	Immediate.
Shake	Disable shaking.
Temperature	Disable heating.
Reader Options	Select High Gain. Select 1 for Scaling Factor. Select Average All Readings. Enter 0.64 secs for Read Time/Well.
Template	Use default value of Test Wells 1 through 96.
Report Options	Select the Raw Data option from the Data Matrix dialog box.

2. Run the assay.
3. Examine the results. The RLU for each well should ≤ 0.0008.

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Light Intensity Accuracy and Precision Verification

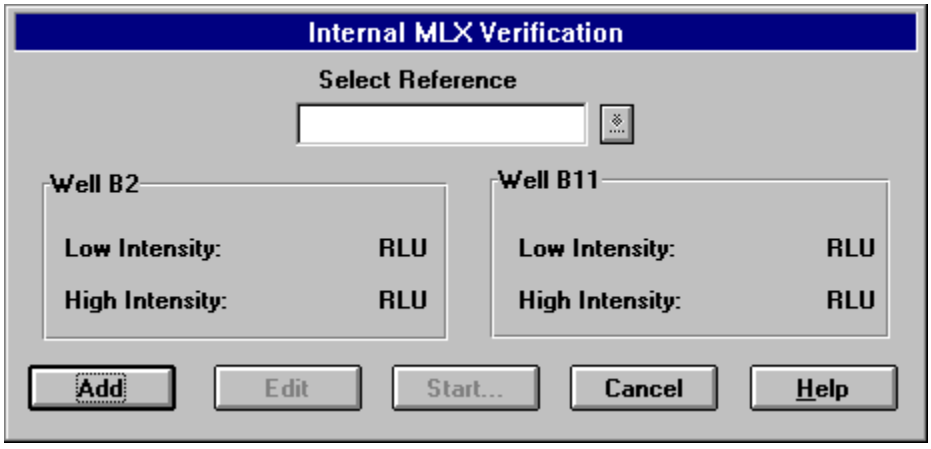
Attention: This MLX has been factory calibrated with the injector(s) in place. When running the Verification Plate, ensure the injector(s) are in place. This does not apply to No Injector units.

The accuracy and precision of Relative Light Units (RLU) measured by the MLX can be tested as needed, using a **Reference Plate**. The Reference Plate contains two or more "wells" which emit a predetermined amount of light. The allowable range of expected values for RLU measurements on each well is provided on labeling that accompanies the Reference Plate. The Reference Plate can be obtained from DYNEX TECHNOLOGIES, INC.

To test the accuracy and precision of RLU measurements:

1. Select **Verification** from the **Utility** pull-down menu. The Internal MLX Verification dialog box (Figure 7-1) will be displayed.
2. ***Select Reference:*** Select the identification number of the Reference Plate that you will use. The **Low Intensity** and **High Intensity** values for the two plate wells will be displayed.
3. Place the Reference Plate on the plate carrier.
4. Click on **Start**. Then follow the prompts that are displayed. The accuracy and precision results will be automatically calculated at the end of the test. The resulting report can be printed or saved.

Figure 7-1 Internal MLX Verification Dialog Box



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To enter data for a new Reference Plate:

1. Select **Verification** from the **Utility** pull-down menu. The Internal MLX Verification dialog box (Figure 7-1) will be displayed.
2. Click on **Add**.
3. Enter the identification number of the new Reference Plate.
4. Enter the labelled **Low Intensity** and **High Intensity** values for Well 1 and Well 2 of the new Reference Plate.
5. Click on **OK**.

To edit data for a Reference Plate:

1. Select **Verification** from the **Utility** pull-down menu. The Internal MLX Verification dialog box (Figure 7-1) will be displayed.
2. Click on **Edit**.
3. Select the identification number of the Reference Plate for which you are changing values .
4. Enter the new **Low Intensity** and **High Intensity** values for Well 1 and Well 2 of the Reference Plate.
5. Click on **OK**.

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Assay Quality Control

Monitoring of Results

The MLX Revelation software includes a number of options for automatic analysis and verification of data obtained from a run. Using these options, you can define various quality control criteria for acceptance of the results. Whenever any of the criteria that you establish for a run are not met, an error message will accompany the results for that run.

For example, you may wish to apply one or more Raw Data Quality Control equations to automatically verify that results from duplicate assays agree within preset limits. Or, you may want to automatically check that the results obtained on control materials during a run fall within a preset range of values and/or have an SD or CV that does not exceed an certain level.

In addition, you may wish to apply one or more Curve Fit Quality Control equations to verify that the expected response curve is obtained when you run a series of calibrators for an assay.

The various options that you can use for automatic analysis of data from a run are described in Chapter 4.

Use of Control Materials

Each assay that is run on the MLX Luminometer should be verified on a routine basis by the use of multi-level control samples. If you are using a commercial test kit, refer to the product labeling accompanying the test kit for recommendations concerning the control materials to use and the frequency of their usage. If you are running a test that you have developed, you must determine the appropriate control materials to use and their frequency of usage.

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Reagent Dispensing

For most flash chemistry applications, substrate reagent is in excess and the actual amount dispensed is not critical. However, if the actual amount dispensed is critical in your application, the amount of reagent that is dispensed from each dispenser can be periodically verified. The procedure for doing this is outlined below.

To test the accuracy of reagent delivery:

1. Fill dispenser bottles with deionized water. Place the dispenser bottles in position and initialize and prime the dispensers as outlined in Chapter 3.
2. Configure a flash assay using the following parameters:

Parameter	Setting
Shake	Disable shaking.
Temperature	Disable heating.
Dispensing Options	Select 100 µL for Dispenser A. Select 0 µL for Dispensers B and C. Select Dispense Without Reading
Template	Select Wells A1 through H1 (one column of 8 wells).

3. Weigh an empty Microplate to the nearest milligram. Record the weight.
4. Place the weighed Microplate on the plate carrier.
5. Run the assay.
6. Remove Microplate from the plate carrier and weigh the plate to the nearest milligram. Record the weight.
7. Subtract the weight obtained in Step 2 from the weight obtained in Step 5. The result should fall between 784 and 816 milligrams.
8. Reconfigure the Dispensing Options for the assay, selecting 100 µL for Dispenser B and 0 µL for Dispensers A and C.
9. Repeat Steps 3 though 7 for Dispenser B.
10. Reconfigure the Dispensing Options for the assay, selecting 100 µL for Dispenser C and 0 µL for Dispensers A and B.
11. Repeat Steps 3 though 7 for Dispenser C.

The amount of reagent that is dispensed from each dispenser is verified when the MLX fluidics option is installed. Repeat testing using this procedure is not necessary unless other quality control testing indicates an error that may be due to an incorrect amount of reagent being dispensed.

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Appendix

Revelation can have problems when importing files if certain file format conventions are not followed. This appendix describes the correct file formats to use for:

- **Hamilton**
- **Kemble**
- **Tecan** (DD1 and APL)
- **Packard Multiprobe**

Hamilton

Hamilton plate data are stored in AT_BARCO.xxx files, where xxx identifies a record in the associated REGISTER.INF file. Later versions of Hamilton's Sunrise software allow you to place these files on a network for access by Revelation. Earlier versions can not do this and, therefore, should not be used.

AT_BARCO.xxx Format:

These files take the form:

Line	Description
1	Not used by Revelation
2	Used as a check against the Plate ID in REGISTER.INF
3	Not used by Revelation
4	Not used by Revelation
5	Not used by Revelation
6+	Body of file (96 lines)

Each line in the file ends with carriage return and line feed characters. Each line in the body of the file must contain, in the following sequence, the following fields:

Size	Field	Extended Description
3	Position	Well location (i.e. A1 to H12)
2	Length of Sample ID	These two characters indicate the length of the Sample ID string
See previous field	Sample ID	—
2	Unused	—

Size indicates the size of the field in characters.

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REGISTER.INF Format:

These files take the form:

Line	Description
1	Not used by Revelation
2+	Body of file (96 lines)

Each line in the file ends with carriage return and line feed characters. Each line in the body of the file must contain, in the following sequence, the following fields:

Field	Extended Description
Plate ID	Revelation uses the first eight characters of this field
Not used	—
Test name	Not used by Revelation
Not used	—

Fields are separated from each other with an ASCII code 255 character. Fields not used by Revelation to create .SID files are identified as “not used”.

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Kemble

These files take the form:

Line	Description
1	Not used
2+	Body of file (96 lines)

Each line in the file ends with carriage return and line feed characters. Fields must be separated by space characters.

Each line, of 96, in the body of the file must contain, in the following sequence, these fields:

Field	Extended Description
Not used	—
Destination position	Must be in plate position format (i.e. left-justified and, if necessary, padded out to six characters with spaces). Used by Revelation to determine well location (i.e. A1 to H12)
Sample ID	—
Not used	—

Fields must be separated by comma characters.

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Tecan

Tecan DD1 files must take the form:

Line	Description
1	Date of pipetting (in dd-mm-yyyy format). Not used
2	Time of pipetting (in hh:mm:ss format). Not used
3	Body of file (96 lines), see below

Each line in the file ends with carriage return and line feed characters. Each line in the body of the file must contain, in the following sequence, the following fields:

Size	Field	Extended Description
3	Not used	—
20	Test name	Not used
12	Sample ID	—
8	Not used	—
8	Plate ID	Not used
4	Position	Well location (i.e. A1 to H12)
6	Not used	—

Fields not used by Revelation are identified as “not used”.

Note: Tecan APL files are identical to DD1 but without the date/time header (the first two lines).

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Packard Multiprobe

Packard files consist of 96 lines containing, in sequence, the following fields:

Line	Description
Not used	—
Not used	—
Not used	—
Not used	—
Assay name	Not used
Not used	—
Sample ID	Revelation uses this field to create a sample ID
Not used	—
Well location	That is, A1 to H12
Not used	—
Not used	—

Fields are separated from each other with the "|" character. Each line in the file ends with carriage return and line feed characters. Fields not used by Revelation to create .SID files are identified as "not used".

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Examples of Templates

Single Well Standards in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
B	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
C	T1	T1	T1	T1	T1	T1	T1	T2	T2	T2	T2	T2
D	T2	T2	T2	T2	T2	T3	T3	T3	T3	T3	T3	T3
E	T3	T3	T3	T4	T4	T4	T4	T4	T4	T4	T4	T4
F	T4	T5	T5	T5	T5	T5	T5	T5	T5	T5	T5	T6
G	T6	T6	T6	T6	T6	T6	T6	T6	T6	T7	T7	T7
H	T7	T7	T7	T7	T7	T7	T7	T8	T8	T8	T8	T8

Single Well Standards in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	T1	T9	T1	T2	T3	T4	T4	T5	T6	T7	T8
B	S2	T2	T1	T1	T2	T3	T4	T5	T5	T6	T7	T8
C	S3	T3	T1	T1	T2	T3	T4	T5	T5	T6	T7	T8
D	S4	T4	T1	T2	T2	T3	T4	T5	T6	T6	T7	T8
E	S5	T5	T1	T2	T2	T3	T4	T5	T6	T6	T7	T8
F	S6	T6	T1	T2	T3	T3	T4	T5	T6	T7	T7	T8
G	S7	T7	T1	T2	T3	T3	T4	T5	T6	T7	T7	T8
H	S8	T8	T1	T2	T3	T4	T4	T5	T6	T7	T8	T8

Double Well Standards in Rows; Replicates in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
C	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
D	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
E	T1	T1	T1	T1	T1	T1	T1	T2	T2	T2	T2	T2
F	T1	T1	T1	T1	T1	T1	T1	T2	T2	T2	T2	T2
G	T2	T2	T2	T2	T2	T3	T3	T3	T3	T3	T3	T3
H	T2	T2	T2	T2	T2	T3	T3	T3	T3	T3	T3	T3

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Double Well Standards in Columns; Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	T1	T1	T9	T9	T1	T1	T2	T2	T3	T3
B	S2	S2	T2	T2	T1	T1	T1	T1	T2	T2	T3	T3
C	S3	S3	T3	T3	T1	T1	T1	T1	T2	T2	T3	T3
D	S4	S4	T4	T4	T1	T1	T2	T2	T2	T2	T3	T3
E	S5	S5	T5	T5	T1	T1	T2	T2	T2	T2	T3	T3
F	S6	S6	T6	T6	T1	T1	T2	T2	T3	T3	T3	T3
G	S7	S7	T7	T7	T1	T1	T2	T2	T3	T3	T3	T3
H	S8	S8	T8	T8	T1	T1	T2	T2	T3	T3	T4	T4

Triple Well Standards in Rows; Replicates in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	S1	S1
D	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
E	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
F	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T1	T1
G	-	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Triple Well Standards in Columns; Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	T1	T1	T1	T9	T9	T9	T1	T1	T1
B	S2	S2	S2	T2	T2	T2	T1	T1	T1	T1	T1	T1
C	S3	S3	S3	T3	T3	T3	T1	T1	T1	T1	T1	T1
D	S4	S4	S4	T4	T4	T4	T1	T1	T1	T2	T2	T2
E	S5	S5	S5	T5	T5	T5	T1	T1	T1	T2	T2	T2
F	S6	S6	S6	T6	T6	T6	T1	T1	T1	T2	T2	T2
G	S7	S7	S7	T7	T7	T7	T1	T1	T1	T2	T2	T2
H	S8	S8	S8	T8	T8	T8	T1	T1	T1	T2	T2	T2

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Alternative Format: Single Well Standards in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	*	*	*	*	*	*	*	*	*
B	*	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1	*
C	*	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	*
D	*	T1	T1	T1	T1	T1	T1	T1	T1	T1	T2	*
E	*	T2	T2	T2	T2	T2	T2	T2	T2	T2	T3	*
F	*	T3	T3	T3	T3	T3	T3	T3	T3	T3	T4	*
G	*	T4	T4	T4	T4	T4	T4	T4	T4	T4	T5	*
H	*	*	*	*	*	*	*	*	*	*	*	*

Alternative Format: Quadruple Well Standards in Columns; Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	*	*	*	*	*	*	*	*	*
B	*	S1	S1	S1	S1	T1	T1	T1	T1	-	-	*
C	*	S2	S2	S2	S2	T2	T2	T2	T2	-	-	*
D	*	S3	S3	S3	S3	T3	T3	T3	T3	-	-	*
E	*	S4	S4	S4	S4	T4	T4	T4	T4	-	-	*
F	*	S5	S5	S5	S5	T5	T5	T5	T5	-	-	*
G	*	S6	S6	S6	S6	T6	T6	T6	T6	-	-	*
H	*	*	*	*	*	*	*	*	*	*	*	*

- Denotes Unused Wells

* Denotes Unused Wells in Alternative Format

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Curve Fit Equations

For all curve fitting routines, data are always plotted in the following way:

X axis represents concentration

Y axis represents measured variables (i.e. optical density, time, or rate of change with time)

Linear Regression

Linear Regression is used to put a "best fit" straight line through a set of data points. The equation for a straight line is:

$$y = mx + c$$

where:

m = the slope
c = the y-intercept defining the line
x = the value for concentration
y = the value for the measured variable.

The linear regression formulae for determining the best straight line through a series of data points are:

$$m = \frac{N\sum x_i y_i - \sum x_i \sum y_i}{N\sum x_i^2 - \left(\sum x_i\right)^2}$$

$$c = \bar{y} - m \cdot \bar{x}$$

Where $\bar{x}$ and $\bar{y}$ are the mean values of x and y, i.e.:

$$\bar{x} = \frac{\sum x_i}{N}$$

The linear regression coefficient, **R**, is defined by:

$$R^2 = \frac{\left(N\sum x_i y_i - \sum x_i \sum y_i\right)^2}{\left(N\sum x_i^2 - \left(\sum x_i\right)^2\right)\left(N\sum y_i^2 - \left(\sum y_i\right)^2\right)}$$

When $\mathbf{R^2=1}$, all data points lie along the line of best fit. When $\mathbf{R^2=0}$, the data points are highly scattered.

Once a line of best fit has been established for a set of known Standards, the concentration of an unknown sample can be determined for a measured sample as follows:

$$\text{concentration} = \frac{y - c}{m}$$

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Polygon Fitting

The polygon fitting procedure joins successive data points using straight lines.

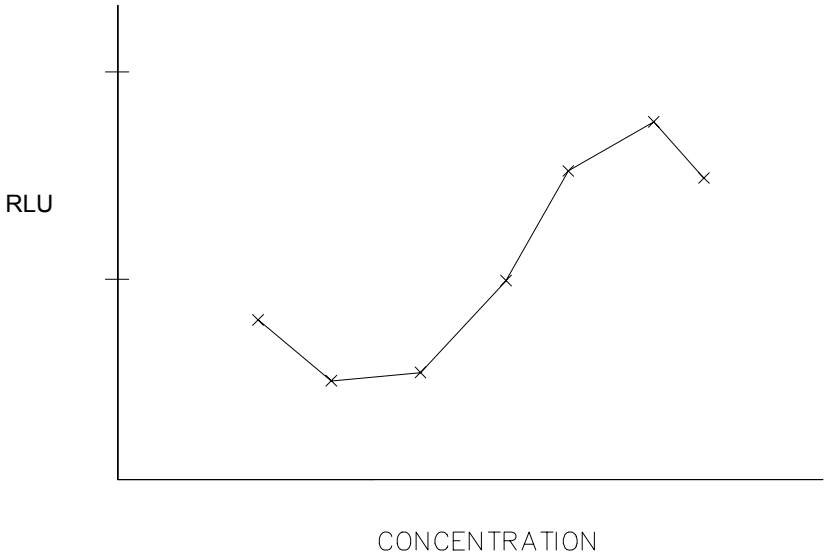


Figure A-1 Polygon Fitting

Non Linear Curve Fitting

The remaining fitting routines all produce curves rather than straight line plots.

The Reader will only calculate one concentration value for each measured variable. The user should examine the printed graph to determine if another curve would provide a better fit for the data points.

Some curves fit functions may not behave as expected if extrapolated beyond the range defined by the data points.

For example, in a particular test, the readings for a set of Standards increase in a non-linear fashion with increasing concentration. The user determines that a quadratic curve fit might give the best fit for such data. However, at concentrations lower than the concentration of the lowest Standard, the curve might turn sharply upwards as shown in Figure A2.

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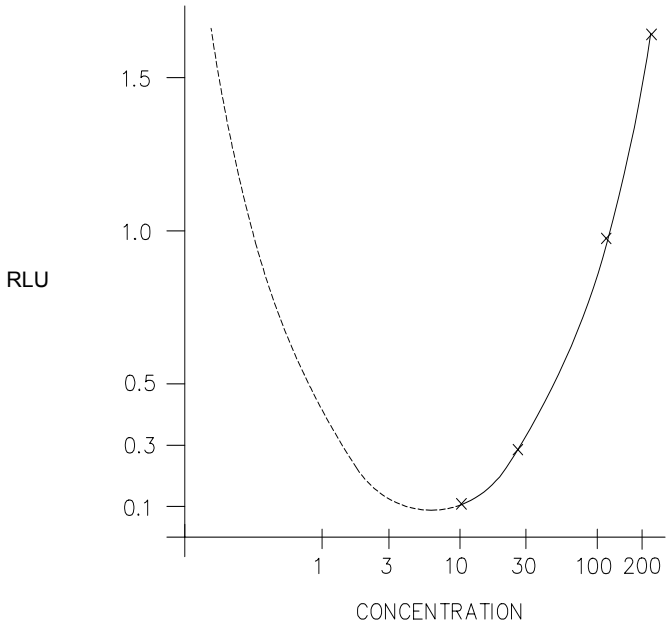


Figure A-2 Using Tails

Quadratic, Cubic and Quartic Regression

Polynomial (quadratic, cubic and quartic) regression is best explained in parallel with linear regression, where the function to fit to experimental data can be written as:

$$y = a + bx^1$$

where:

b = the slope (corresponding to m in straight line equation)
a = the y-intercept of the line (corresponding to **c** in the equation for a straight line)

A quadratic equation is defined as:

$$y = a + bx + cx^2$$

A cubic equation as:

$$y = a + bx + cx^2 + dx^3$$

And a quartic equation as:

$$y = a + bx + cx^2 + dx^3 + ex^4$$

The procedures to fit such functions to experimental data are closely related to linear regression and have a similar mathematical derivation. Polynomial regression minimizes the deviations of the data points from the polynomial equation (the square of the data point deviations are minimized since positive and negative deviations would otherwise cancel each other out).

There is an analogous regression coefficient **R**, which when $\mathbf{R^2=1}$ indicates that all the data points have been fitted to the curve; when $\mathbf{R^2=0}$, the data points are highly scattered.

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Cubic Spline Curve Fitting

Cubic spline curve fitting passes a curve through all the data points. This is not a best fit algorithm since it assumes that the data set is perfect. The relevant equations are given below without explanation.

$$y = a_0 + a_1 x + \sum_{j=1}^{n-2} b_j \Phi_j(x)$$

Where:

$$\Phi_j(x) = \varnothing_j(x) - \left(\frac{x_n - x_j}{x_n - x_{n-1}}\right)\varnothing_{n-1}(x) + \left(\frac{x_{n-1} - x_j}{x_n - x_{n-1}}\right)\varnothing_n(x)$$

$$\Phi_j(x) = \begin{cases} 0 & x < x_j \\ (x - x_j)^3 & x \geq x_j \end{cases}$$

Difficulties can arise with spline fitting, if data are highly erratic or the curve bends sharply several times. The curve may break up at high concentrations (see Figure A-3). The Reader will not be able to perform the calculations because of numerical rounding errors generated when attempting to fit the curve. Using a semi-log or log-log axis fit rather than a linear fit, will reduce this problem.

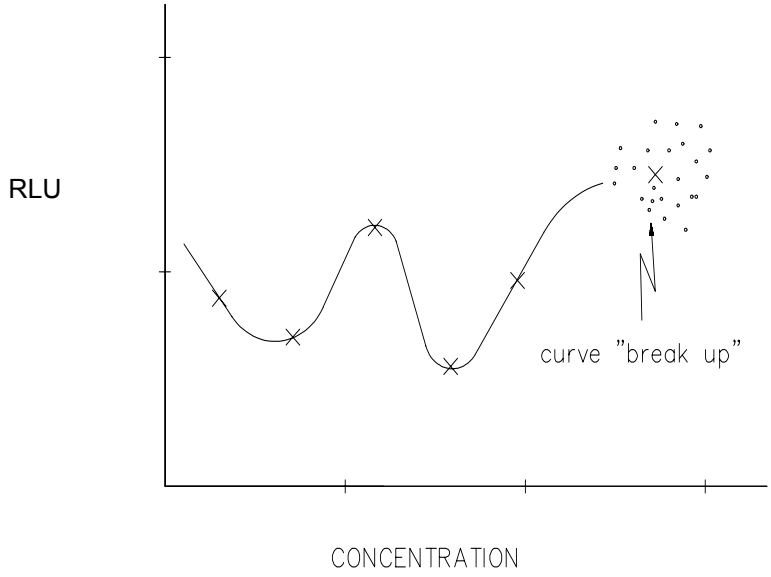


Figure A-3 Spline Curve Break Up

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Michaelis- Menten

The Michaelis-Menten equation is used to describe enzyme kinetics, but its rectangular hyperbolic shape is useful for describing other experimental responses (see Figure A-4).

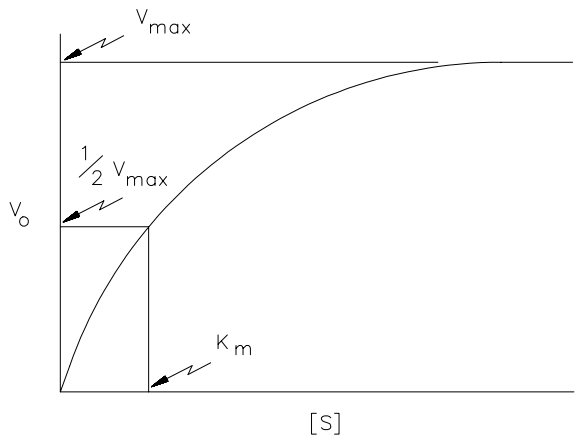


Figure A-4 Michaelis Menten Curve

The relationship between RLU and concentration, **[x]**, can be written in the following general form, where **a** and **b** are constants:

$$RLU = \frac{a[x]}{[x]+b}$$

It can be shown that **a** equals the limiting $\mathbf{RLU_{max}}$ as $\mathbf{[x]\rightarrow\infty}$, and that **b** is the concentration at half $\mathbf{RLU_{max}}$. The equivalent formula applied to enzyme reaction rates is:

$$V_0 = \frac{V_{max}[S]}{[S]+K_m}$$

Where:

[S] = the enzyme concentration
$\mathbf{V_{max}}$ = the maximum reaction velocity
constant $\mathbf{K_m}$ = the enzyme concentration at which the reaction velocity is half $\mathbf{V_{max}}$.

The constant $\mathbf{K_m}$ is known as the Michaelis constant.

For practical applications it is best to transform the Michaelis-Menten equation into a linear form, known as the Lineweaver-Burk equation.

$$\frac{1}{RLU} = \frac{1}{RLU_{max}} + \frac{K_m}{RLU_{max}} \cdot \frac{1}{[x]}$$

If **1/RLU** is plotted against **1/[x]**, a straight line is obtained whose slope is $\mathbf{K_m/RLU_{max}}$, with an intercept on the **1/RLU** axis $\mathbf{1/RLU_{max}}$ and an intercept on the **1/[x]** axis of $\mathbf{1/K_m}$. The Reader performs this calculation automatically and determines the best straight line fit by linear regression. $\mathbf{RLU_{max}}$ (printed as $\mathbf{V_{max}}$) and $\mathbf{K_m}$ are evaluated and printed out.

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Sigmoid Equation

Many immunological reactions are characterized by an S-shaped or sigmoid curve.

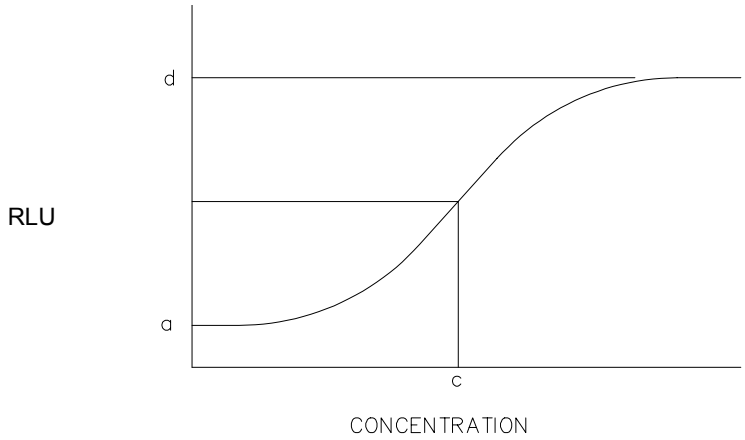


Figure A-5 Sigmoid Curve

The Sigmoid equation can be written as:

$$Y = \frac{[a-d]}{\left[1+\left(\frac{X}{c}\right)^{b}\right]} + d$$

Note: This equation is also known as Rodbard's Four Parameter equation.

The four parameters are:

- **a** the minimum response
- **b** the shape factor (determines the gradient of the curve)
- **c** the response midway between the maximum (d) and the minimum (a) responses
- **d** the maximum response

This definition assumes a positive value for **b**. Where **b** is negative, the definitions for parameters **a** and **d** are reversed.

The Reader contains an efficient algorithm for estimating the four parameters and determining the best fit for the sigmoid curve. The curve requires at least four Standards (data points): one for each of the four parameters. It is recommended that at least eight Standards are defined to ensure satisfactory statistical significance. The Reader will allow a maximum of 24 Standards.

The sigmoid curve algorithm is iterative, requiring many complex calculations and the Reader may take some time to process the results. If the Reader has difficulty fitting the data to the curve, processing will continue, either until the Reader has processed the data, or until you select **ABORT**. If you have aborted the data processing, examine the data carefully, to see if the number of Standards could be altered to better define the curve.

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Akima Curve Fit

The Akima option constructs a smooth curve through the data points. The fitted curve will appear smooth and natural and approximates a manually drawn curve.

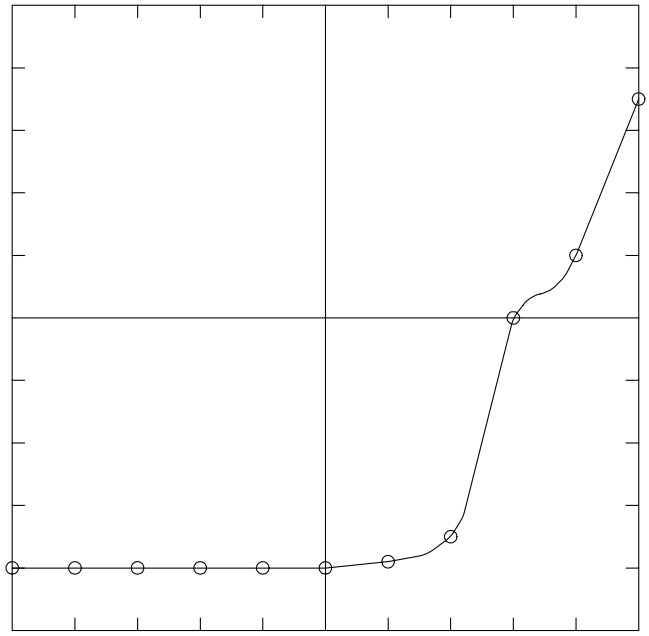


Figure A-6 Points Joined by the Akima Curve Fit Method

The Akima curve fit consists of a set of polynomials which are applied to the set of data points. The slope of the curve at each point is determined by these polynomials. The Akima fit is based on the assumption that each data point is determined locally by five points: The point of interest and two points on either side of it.

Calculation of the Slope of the Curve

Numbering the points 1, 2, 3, 4, 5, the slope of the curve at the point of interest (point 3) is assumed to be determined by:

$$t = \frac{\left(|m_4 - m_3|m_2 + |m_2 - m_1|m_3\right)}{\left(|m_4 - m_3| + |m_2 - m_1|\right)}$$

where **t** is the slope of the curve, and m_1, m_2 m_3, and m_4 are the slopes of the following line segments:

$$\overline{12},\ \overline{23},\ \overline{34},\ \overline{45}$$

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Interpolating Between a Pair of Points

With the slopes of the points determined, the portion of the curve between two points is calculated so that the curve:

- passes through both points
- the slopes at the data points are the same as previously determined.

The portion of the curve is expressed as a uniquely determined polynomial in the form:

$$y = p_0 + p_1(x - x_1) + p_2(x - x_2)^2 + p_3(x - x_1)^3$$

where

$p_0 = y_1$ and $p_1 = t_1$

$$p_2 = \frac{3(y_2 - y_1)/(x_2 - x_1) - 2t_1 - t_2}{x_2 - x_1}$$

$$p_3 = \frac{t_1 + t_2 - 2(y_2 - y_1)/(x_2 - x_1)}{x_2 - x_1}$$

Axis Fitting

The normal method of fitting data to the axes of a graph is to use linear scales for the x (concentration) and y (measured variable) axes. Two other methods are available:

- **Semi-Log Fit**
- **Log-Log Fit**

For semi-log fit, all x are transformed in the following way:

$$x = \log(\text{concentration})$$

Thus, to obtain the concentration of a sample using linear regression:

$$\text{concentration} = \text{antilog}\left(\frac{y - c}{m}\right)$$

For log-log fit, all x and y values are transformed as follows:

$$x = \log(\text{concentration})$$

$$y = \log(\text{measured variable})$$

Thus to obtain the concentration of a sample using linear regression:

$$\text{concentration} = \text{antilog}\left(\frac{y - c}{m}\right)$$

Note: These examples use linear regression, but Log-Log and Semi-Log Axes Fits may be used with other curve fitting routines as well.

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Examples of Q.C. Expressions

The expressions that are used are similar whether the expression is prepared for Raw Data: Q.C., Threshold Q.C., or Curve Fit Q.C. Examples of typical expressions and their use are shown below.

1. Reject any RLU value for the Negative Control which is more than 50% higher or lower than the mean of the other two values:

   0.5*(NC-NCi)/2<=NCi<=1.5*(NC-NCi)/2

   Valid(NC)>=2

   or

   Verify(NC,50%,1)>=2

2. Calculate the mean RLU of the two replicates of the Positive Control (Px). (These should have an RLU of more than 0.25 above the Negative Control:

   PC>NC+0.25

3. The mean RLU of the Negative Control should be less than 0.25 but values up to 0.3 may be accepted as long as the Positive Control is giving a value greater than 0.55:

   if PC>0.55 then NC<0.3 else NC<0.25

4. Calculate the mean RLU of the 4 replicates of the Negative Control serum. If any of the Negative Control wells has an RLU greater than 2 times the mean Negative Control, discard this value and calculate the new mean RLU.

   NCi<=2*NC

   Valid(NC)>=3

5. The mean RLU for the Negative Control serum is less than 0.4.

   NC<0.4

6. The RLU values of both the Positive Control sera are more than 0.5 above the RLU for the Negative Control:

   PC1>NC+0.5

   PC2>NC+0.5

   or

   PCi>NC+0.5

   Valid(PC)==2

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Index

A

Area Statistics 182
Assay Options command 31, 181
Assay Title command 31, 75
Assays
  Creating 74
  Entering title 75
  Dual Flash 1, 12, 106
  Flash 1, 12, 92
  Glow-Endpoint 1, 11, 72
  Kinetic 1, 11, 76
  Modifying 74
  Overview 73
  Running 191, 203
  Selecting new 206-208
  Setting options 181
Assigning Well Type Samples 69

B

Blank Mode command 31, 122
Buttons
  Using 191
  Defining 61-62

C

Calculation Mode
  For Dual Flash Assay 107
  For Glow-Kinetic Assay 82
  For Flash Assay 93
Cleaning
  General 213
  Mouse 213
  PMT Lens 215
  Syringes 214
Colours
  Selecting for report 65
Commands
  Assay Title 31, 75
  Blank Mode 31, 122
  Configure Reader 35, 49
  Curve Fit 31, 138
  Dispensers 42, 99
  Fit Information 138
  Heater Control 58
  Help Menu 38
  Import 202
  Kinetic Graphs 89, 210
  Options 36, 60
  Outlier Removal 207
  Plate In 35
  Plate Out 35
  Plate-Plate Statistics 182, 211
  Print Setup 30
  Q.C. - Raw Data 31, 125
  Ratio 31, 162
  Reader Control - Dual Flash Assays 106
  Reader Control - Endpoint Assays 76
  Reader Control - Flash Assays 92
  Reader Control - Kinetic Assays 81
  Reagent Lot Number 196-198
  Refresh 208
  Results Font 59
  Run Plate 29, 191
  Sample ID Entry 184, 199
  Sample ID Filename 201
  Select Language 48
  Select New Assay 32, 206
  Spreadsheet 31, 168
  Template 31, 69, 118
  Threshold 31, 131
  Titration Multiplier 32, 120, 211
  Window Menu 37
Components, placement of 16
Configure Reader command 35, 49
Connecting components 17
Control Materials 230
Creating or Modifying an Assay 74
Curve Fit command 31, 138
Curve Fit Types
  Descriptions of 142
  Quality Control 150-154
  Results Flagging 157
  Selecting 142

D

Data Conversion 155
Data File Commands
  Fit Information 209
Data Menu
  Description of 32
Data Menu Commands
  Kinetic Graphs 32
  Outlier Removal 207
  Reagent Lot Number 196
  Refresh 208
  Sample ID Filename 199-201
  Select New Assay 206
  Titration Multiplier 32, 120, 211
Data Recovery 218
Dilutions 120, 148, 211

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Directories
  Viewing Revelation 66
Dispenser
  Initializing and priming 44
  Preparing of 21
  Setting options in assay 96
Dispensers command 35, 42-47
Dispensing Options 98
Dual Flash Assays
  Description of 1, 12, 106
Drain Plate 43

E

Equation Components 128, 132, 150, 152, 163
Error Messages 221
Exporting Files 178

F

File formats
  for importing Hamilton data 233
  for importing Kemble data 235
  for importing Packard Multiprobe data 237
  for importing Tecan data 236
File Menu
  Commands 29
  Description of 4, 29
File Menu Commands
  Import 29, 202
  Print Setup 29
  Run Plate 29, 195
Fit Information command 209
Flash Assays
  Description of 1, 12, 92
Flexible File Import 202
Fluidics 13, 98

G

General Precautions 217
Glow-Endpoint Assays
  Description of 1, 11, 76

H

Hamilton import file formats 233
Hardware Components 2
Hazards 218
Heater Control command 58
Help Menu
  Commands 37
  Description of 37

I

Import Command 29, 202
Injectors
  Initializing and priming 44
  Installing optional 21
  Installing syringes on 23
  Washing or purging 46
Installation 15
Intended Use 1

K

Kemble import file formats 235
Kinetic Assays
  Description of 1, 11, 81
Kinetic Graphs command 32, 210

L

Lab Information 67
Lot Specific Data command 32, 196-198

M

Maintenance/Troubleshooting 213
Menu Bars 4, 28
Micro-instrument 3
MLX Microtiter Plate Luminometer
  Connecting components of 19
  Installation of 15
  Intended use of 1
  Maintenance and Troubleshooting 213
  Operational problem solving 219
  Software 4
  Specifications 9
  Starting of 25
  Subsystems of 2
  Unpacking and Inspection 15
Monitoring of Results 206, 232

O

Operational Problems 219
Options command 35, 60-67
Outlier Removal command 32, 207

P

Packard Multiprobe import file formats 237
Password
  Entering system 68
Plate In command 35
Plate Out command 35

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Plate-Plate Statistics command 35, 182, 211
PMT Lens
  Cleaning 215
Power Cables 19
Precautions 217
Preferences
  Selecting system 35, 63
Print Setup Command 30
Processing Order
  Defining 183

Q

Q.C. - Raw Data command 31, 125
Quality Control
  Control Materials 230
  Expressions and Examples 128, 132, 150, 152, 163
  Light Intensity 226
  Light Intensity Accuracy 228
  Light Intensity Precision 228
  Light Leak Testing 227
  Monitoring of Results 206, 230
  Overview 225
  Reagent Dispensing 231
Quick Operation Buttons 61, 191

R

Ratio command 31, 162
Ratio Equations 162
  Examples 162
  Components of 162
Reader Control command
  for Dual Flash Assays 106
  for Flash Assays 92
  for Glow-Endpoint Assays 76
  for Kinetic Assays 81
Reagent bottles
  Positioning of 23-34
Reagent lot number 32, 196-198
Reagents
  Changing lot numbers 196-198
  Recovering 47
Rebooting 217
Recovering Data 218
Refresh Command 32, 208
Report Options 175
Results
  Display of 204
  Exporting 31, 178
  Output Format 136, 159, 165, 172
  Report Options of 174
Results Flagging
  Curve Fit 157
  Ratio 163
  Spreadsheet 170
Results Font command 35, 59
Revelation Menus
  Description of 4, 28
Revelation Window
  Description of 27
  Menu bars 4, 28
Run Plate Command 29, 195

S

Sample ID Entry command 35, 199-201
Sample ID Filename command 32, 201
Sample ID Setup 184
Select Language command 35, 48
Select New Assay command 32, 206
Settings Menu
  Description of 30
Settings Menu Commands
  Assay Options 31, 181
  Assay Title 31, 75
  Blank Mode 31, 122
  Curve Fit 31, 138
  Q.C. - Raw Data 31, 125
  Ratio 31, 162
  Reader Control - Dual Flash Assays 106
  Reader Control - Endpoint Assays 76
  Reader Control - Flash Assays 93
  Reader Control - Kinetic Assays 81
  Spreadsheet 31, 168
  Template 31, 118
  Threshold 31, 132
Specifications 9
Spreadsheet command 31, 168
Standards
  Selecting 140
Starting the MLX Luminometer 25
Status Messages 203
Subsystems 2
Syringes
  Cleaning of 214
  Installation of 21
System Description 2-9
System Overview 1-13
System Rebooting 217

T

Tecan import file formats 236
Template Command 31, 118
Threshold command 31, 131
Threshold Equations 132
Threshold Output Format 136
Titration Multiplier command 32, 120, 211
Toolbars

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Settings Toolbar 40
Starting Toolbar 39

U

Unpacking and Inspection 15
User Definable Data File 202
Utility Menu Commands
Barcode Reader 35
Configure Reader 35, 49
Description of 5
Dispensers 35, 98
Heater Control 35, 58
Options 35, 60
Plate In 35
Plate Out 35
Plate-Plate Statistics 35, 182, 211
Remote Control 35
Results Font 35, 59
Sample ID Entry 35, 199–201
Select Language 35, 48
Verification 35, 228

V

Verification Command 35, 228
View Menu
Commands 34
Description of 4, 34

W

Well Types
Assigning to samples 69-71, 118
Window Menu
Commands 36
Description of 36
Wizard
Use of 185

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