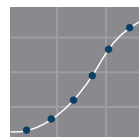


Microplate Manager® 6 Software

Instruction Manual | Version 6.1



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Table of Contents

Table of Contents	ii
Introduction	1
Microplate Manager 6 Overview	1
Menu Bars and Icons.....	2
File Menu	3
Window Menu	4
Installation and Instrument Setup	5
Minimum Requirements.....	5
iMark Reader Instrument Setup.....	6
xMark Instrument Setup	8
Quick Start.....	10
Setting Up an Experiment.....	10
Instrument Controls.....	11
Incubator Controls.....	12
Shaker Controls	12
Reading Microplates.....	13
Data and Protocol Files	14
File Storage by Operating System.....	14
Opening Files	15
Saving Files	15
Data AutoSave	16
Importing and Exporting Data	16
Importing Data.....	16
Entering Data Manually.....	17
Exporting Data or Reports	18

Exporting Data	18
Exporting Reports	19
Exporting Custom Screening Reports (BSE, TSE, TSE, NSP)	20
Procedure for Custom Export Reports	20
BSE Custom Screening Report	21
Setting up a Protocol	24
Setting Up A Template	25
Entering Equations	26
Entering and Formatting Experimental Info	27
Select and Customize Curve Fit Plot	28
Setting up the Report Contents	30
Saving the Protocol	31
Analyzing Data Using a Standard Curve	32
Standard Curve Analysis Options	33
Linear Fit	33
Zero-intercept Linear	34
Semi-log Fit	35
Log/log Fit	35
Log/log Fit	36
Quadratic	36
Log/Logit and 4-Parameter Fit	37
Point to Point	39
Cubic Spline	39
Interpolation Using a Previous Standard Curve	40
Customizing Standard Curve Plots	41
Equations and Calculations	42
Analyzing Data Using Cutoffs	45
Using Equations for Setting Cutoffs	46
Cutoff Examples for Assays with Controls	47
Setting Up a Percent Control Cutoff Analysis Report	48

Running Kinetic Assays	52
Reading a Kinetic Assay.....	52
Analyzing Kinetics Data.....	53
Kinetic Zoom Plots	54
Viewing Raw Data at Kinetic Time Points.....	55
Defining Kinetic Parameters	56
 Running Spectra Assays (xMark only).....	 57
 Reading Terasaki Plates (xMark only).....	 57
 Producing Reports.....	 58
Setting Up Reports	58
Printing Reports.....	61
Customizing Reports	63
 Appendix.....	 63
 Appendix.....	 64
References	64
 Quantitative ELISA Assay Tutorial	 65
Defining the Template	65
Setting up the Equations	66
Selecting and Customizing a Curve Fit Plot	67
Entering Experimental info	67
 Dilution Data Assay Tutorial	 68
1 Defining the Template	68
2 Setting up the Dilutions.....	70

3 Selecting Curve Fit and Customizing Curve Fit Plot.....	73
4 Entering Experimental Info	77
5 Setting up the Report.....	78

Percent Control Assay Tutorial 82

1 Defining the Template	82
2 Setting up the Equations	83
3 Setting up the Screening Report	84
4 Entering Experimental info	87
5 Setting up Report.....	88

Percent Max Binding Assay Tutorial..... 92

1 Defining the Template	92
2 Setting up the Equations	93
3 Selecting Curve Fit and Customizing Curve Fit Plot.....	94
4 Entering Experimental Info	99
5 Setting up the Report.....	100

Introduction

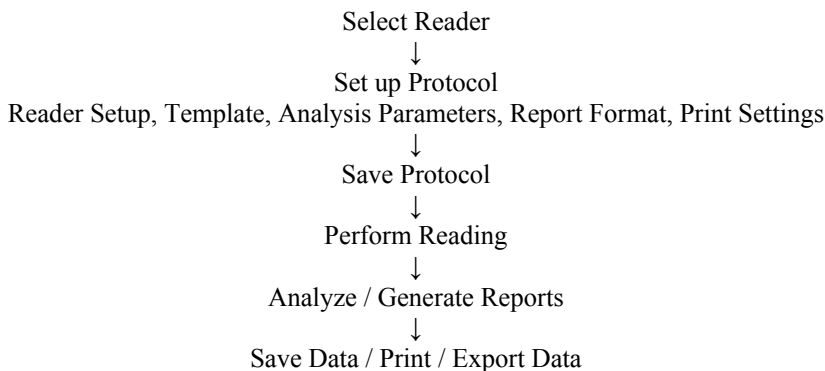
This manual assumes that you are familiar with the standard commands and functions associated with the Windows® or Mac® operating system, such as opening, closing, and saving files, and moving and clicking your mouse.

Some of the features and functions in Microplate Manager 6 will be slightly different depending on which microplate reader you are using (**xMark** or **iMark**). These differences are described in the text.

Microplate Manager 6 Overview

MPM 6 is designed to collect, analyze, and output absorbance data from Bio-Rad's microplate readers. It runs on a Windows® or Mac computer that is directly connected to the reader. It features a standard user interface, with pulldown menus, toolbars and keyboard shortcuts.

Using Microplate Manager 6, you first specify the type of reader you are using, the type of reading you want to perform, and the layout of your microplate. Then you can perform a read using the microplate reader. You can then display your results in a variety of reports. And finally, you can print, save and/or export your data.



Microplate Manager 6 can perform four types of microplate readings.

- **Endpoint Reads** are used to acquire a single absorbance reading from each well.
- **Kinetics Reads** are used to acquire a series of absorbance readings from each well over a user-defined *time* interval to calculate reaction rate etc.
- **Plate 2-1 Reads** are endpoint data from two plates with data from the second plate subtracted from the data from the first plate.
- **Spectra Reads (xMark only)** are a series of absorbance readings for each well over a user-defined *wavelength* interval.

Select the test protocol that you want to open from the File Menu sample protocols, or create your own. Specify the settings for your Protocol and define or select a microplate template. Then take the reading. If you want to use a particular Protocol again in the future, save it as a Protocol file.

After the absorbance values have been read, you can save them as a Data File and display them in a variety of reports. The data can be viewed, printed, or exported to other applications.

Menu Bars and Icons

Icons and pulldown menus are both available to use the commands and functions of Microplate Manager 6 software.

The File, Window, Raw Data and Results menus and icons are described in the following *Introduction* section.

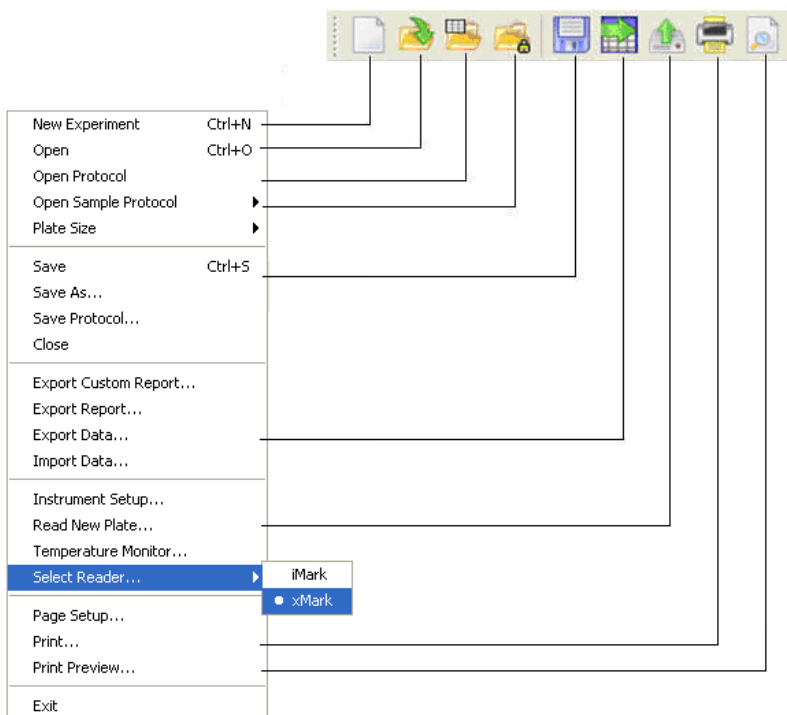
The Template icons are described in the *Setting Up A Template* section on page 25.

The Curve Fit Plot icons are described in the *Customizing Standard Curve Plots* section on page 41.

File Menu

After creating a **New Experiment** or opening an existing data file, the File Menu will list the main program functions.

The nine icons grouped at the left end of the Main Toolbar correspond to the same function items listed under the File Menu.



Full File menu descriptions are below:

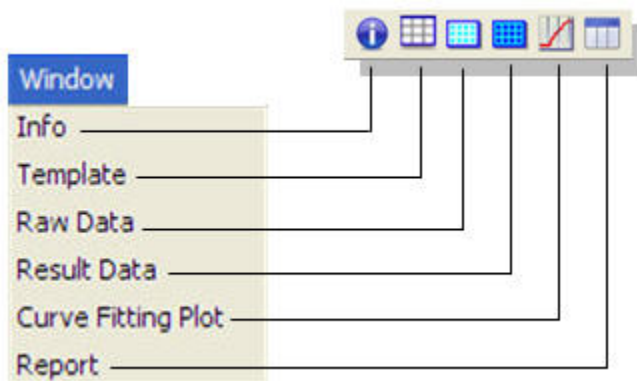
- Create a **New Experiment** or Protocol
- **Open** an Existing File
- **Open** an Existing Protocol
- **Open** a Sample Protocol
- **Save** the Experiment

- **Export** Data to Excel
- **Read** a Plate
- Print **Report**/active window
- **Print Preview** of Report

Window Menu

After creating a **New Experiment** or opening an existing data file, the **Window Menu** will list the display functions for raw data, results and reports.

The six icons grouped at the right end of the Main Toolbar correspond to the same function items listed under the **Window Menu**.



Full Window menu descriptions are below:

- Open the **Info** Window to enter experiment information.
- Open **Template** window to create/view template.
- View **Raw Data** window.
- View **Result Data** window.
- **View** Standard Curve Fit Plot
- View **Report** (data, plot tables, etc.)

Installation and Instrument Setup

Minimum Requirements

Computer

- Windows XP, Windows Vista or Mac OS 10.4 or higher
- For Windows Pentium III (or compatible) Processor (500 MHz or higher)
- For Macintosh Power PC or Intel Core 2 Duo Processor (700 MHz or higher)
- 1 GB RAM (512 MB RAM)
- 1 GB Hard drive space
- CD-ROM-readable Drive
- Keyboard
- Mouse
- Monitor XGA resolution (1024 x 768 or higher)
- Bio-Rad xMark Spectrophotometer or iMark Microplate Reader

Cables and Connections

USB 2.0 is required to connect from the computer to xMark or iMark. Use the USB cable provided, or purchase from any computer store.

For Windows® Users:

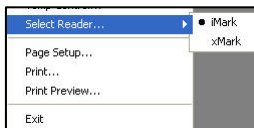
1. Insert the software CD into the CD-ROM drive. The installer user interface displays.
2. Click on the software installation icon to launch the Microplate Setup Wizard.
3. Follow the on-screen instructions.
4. When the installation is complete, click **Finish**.

For Mac Users:

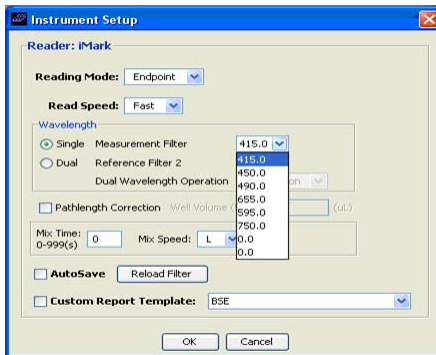
1. Insert the software CD into the CD-ROM drive. The installer user interface displays.
2. Drag the Microplate Manager 6 icon from the CD folder to the Applications folder on your computer hard drive.

iMark Reader Instrument Setup

1. Turn the **iMark** reader on.
2. Enter a five-digit password on the **iMark** keypad to enable remote control for iMark.
3. Select **New Experiment** from the File menu. An empty template displays, and the File Menu displays expanded choices.
4. Select **iMark** from File > Select Reader.



A list of filters installed in the iMark are automatically sent to the computer when iMark is selected. The available filters display in the Wavelength area.



- If filters are added, removed, or their positions changed, the changes must be entered on the **iMark reader keypad**, so that filters are displayed properly in **MPM 6**.

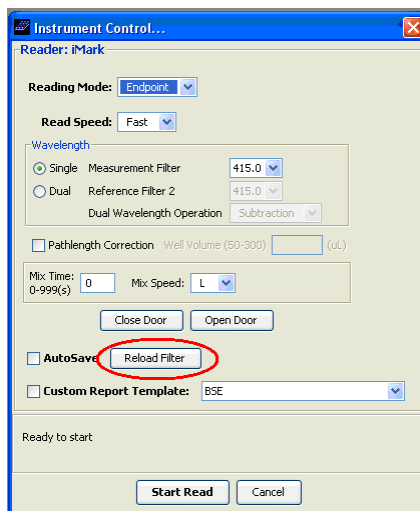
Entering Filters

On the iMark Keypad:

1. Click the **Edit** button.
2. Press down arrow button 2 times to select **Filters**, and press the **Enter** button.
3. Using the keypad, select numbers by using arrow buttons to move through positions 1-8.
4. Press the **Enter** button to save settings.
5. Press **Main** button to put reader back into ready mode.

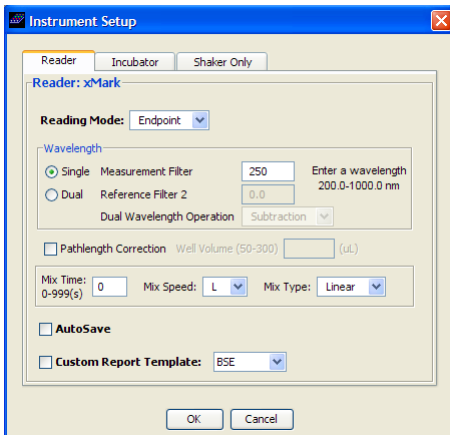
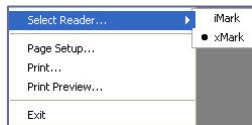
In Microplate Manager 6:

6. Click **Reload Filter** on the Instrument Setup dialog.

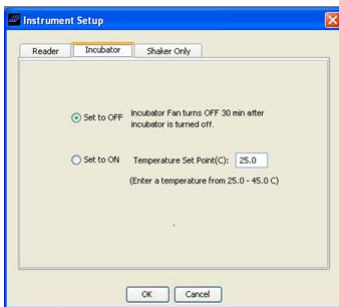


xMark Instrument Setup

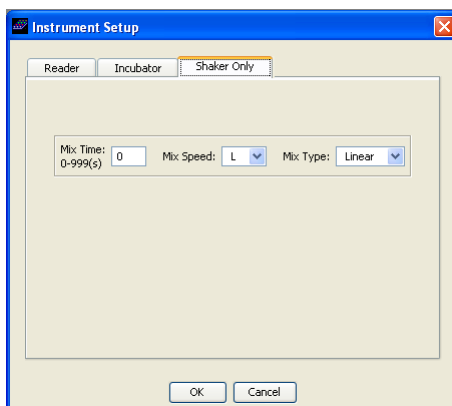
1. After opening MPM 6 for the first time, choose **New Experiment** from the File menu.
2. Select xMark from **Select Reader**.
3. Select the **Reader** tab. In the Wavelength area, set up spectral values from 200.0 – 1000.0 nm.



4. Set up temperature parameters in the **Incubator** tab, if the incubator is required.



5. Select the **Shaker Only** tab to set up mixing parameters (xMark only).



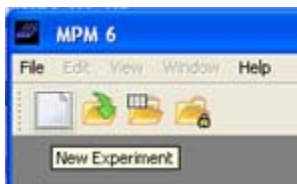
Quick Start

Setting Up an Experiment

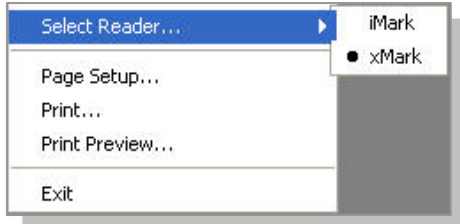
1. Launch software from the desktop shortcut.



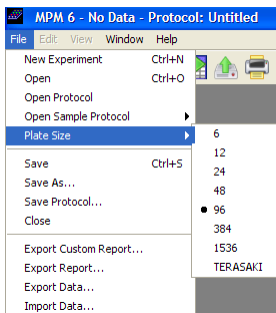
2. Click on the **New Experiment** icon or choose **File > New Experiment** to set up a New Experiment.



3. The program title bar displays “No Data - Protocol : Untitled”.
4. Select the available instrument under **Select Reader**.



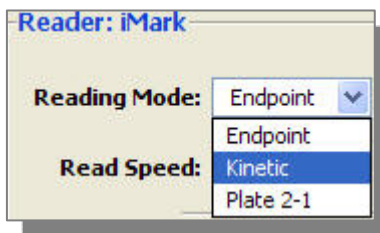
5. Choose **Plate Size** (xMark only). The default is 96.



Open a saved protocol or set up a new protocol (by setting up a template, and selecting the instrument, assay info, analysis options and print options). Refer to the

Instrument Controls

1. Click **File > Read New Plate**. The Instrument Controls dialog displays.
2. Under the Reader tab, set the **Reading mode**: Endpoint, Kinetic, or Plate 2-1.



If you are using an xMark, Spectra is another Reading mode option.

3. Select the other Reading options:
 - **Read speed** (iMark only)
 - Wavelength (nm) and single / dual
 - Mix time & speed, and mix type (xMark only)
 - Pathlength Correction¹

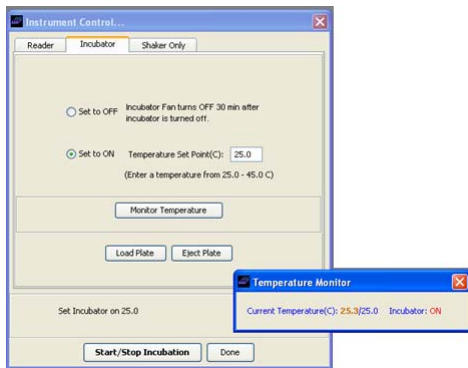
¹Pathlength correction is used to calculate the OD (or % transmission) for each well, normalized to 1 cm fluid depth (d) in each well.

$$OD_{1cm} = \frac{1cm}{d(cm)} \times OD_{sample}$$

Incubator Controls

Under the Instrument Control dialog box, select the **Incubator** tab (xMark only)

1. Click the **Set to On** button.
2. Enter a Temperature Set Point.
3. Click the **Monitor Temperature** button if you want to monitor the temperature continuously.



You can also activate the Temperature monitor from the File menu. This window regularly monitors the xMark temperature status, listing the current temperature & set temperature and whether the incubator is on or off.

Shaker Controls

In the xMark, the shaker can be set to mixing mode, with no plate read.



Reading Microplates

After all Instrument settings have been completed, press the **Start Read** button to read the plate.

Data analysis results can be viewed, changed, saved, and printed after raw data are received from the instrument. See the *Analyzing Data Using a Standard Curve* and the *Analyzing Data Using Cutoffs* sections.

Data and Protocol Files

The following table explains how protocol and data files differ, and describes the file types MPM 6 uses to save each one.

Protocol	Data
.pro is the extension for a protocol file.	.mpl is the extension for a data file.
A protocol file includes all the settings for reading a plate (measurement wavelength, shake, runtime, ...) and all the settings for analyzing the data (template, data transformations, type of curve fit, plot options, cutoffs, custom report options, ...).	A data file contains endpoint or kinetic data for a single plate and the description of the assay (read mode used, measurement wavelength, run time, plate ID, data, conc. unit, other info) and a protocol and template. For Dual Wavelength, the data file contains the data for both wavelengths.
When protocol files are defined and saved, they become an independent file and can be used as a guide for many assays.	When a data file is created, a protocol can be defined and saved within the assay, or an existing Protocol File can be used, modified and saved as part of the Data file.
Since a protocol file contains no data, it cannot be used alone to generate data analysis results.	A template must always be present in a data file order to see any Result Data or Data in the Table.
Changes to a protocol file are not reflected in any previous data files based on that protocol. A new Data file must be created using the revised protocol.	Within a data file , you can choose "Save Protocol" from the file menu to save changes made to a modified protocol or created a new protocol file.

File Storage by Operating System

For pre-Vista Windows PC computers:

The program files are stored in the default location

C: \ Program Files\ Bio-Rad \ Microplate Manager 6

For Vista PC Computers:

The files installed in Program Files cannot be modified. This includes the Sample Data & Sample Protocols folder. Therefore, user-generated data & protocols are stored in a different location.

The Sample Data files are stored in the following default location:

C: \ Bio-Rad \ Microplate Manager 6 \ Sample Data

The Sample Protocol files are stored in the default location

C: \ Bio-Rad \ Microplate Manager 6 \ Sample Protocols

Newly-generated Protocol and Data files are stored in the default location:

C: \ Bio-Rad \ Microplate Manager 6

For Macintosh Computers:

The Data, Protocols, Sample Data & Sample Protocols folders are all installed in the program application folder on the Hard Drive. These Data & Protocol file folders can be moved, if desired.

Opening Files

To open an existing Protocol File or Data File, click on the **Open** icon in the Main tool bar or Select **Open** from the File Menu on the Main Toolbar. Select the name of the file you want to open, and click on the **Open** button.

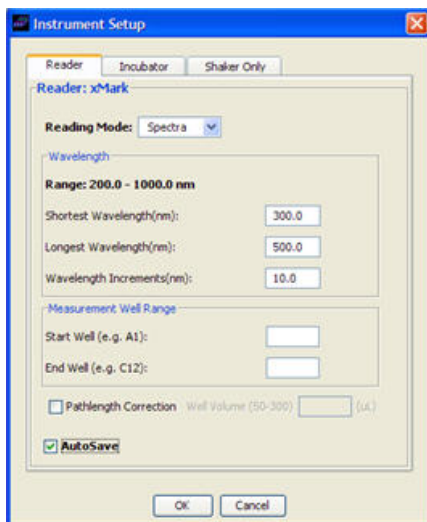
Saving Files

To save a Protocol File, select **Save Protocol or Save As...** from the File menu. To save a Data File, select **Save or Save As...** from the File menu. If you are saving a new file or renaming an old file, enter the new file name and click on the **Save** button.

The Microplate Manager 6 program opens and saves files from the last-used directory location.

Data AutoSave

When the **AutoSave** feature is selected in the **Instrument Setup Window**, the data will be saved automatically after each read (and will not be displayed). The file name will automatically have the date/time and number of the read. This file name can be changed later.



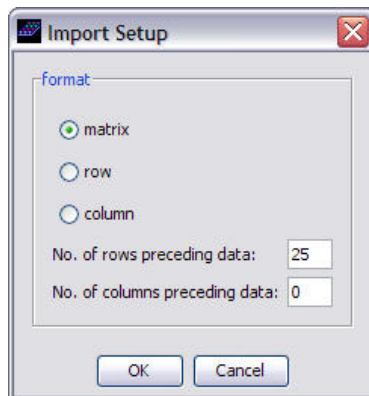
Importing and Exporting Data

Microplate Manger can import or export data to other programs which accept.xls, .csv, or .txt file types.

Importing Data

In order to view data from previous versions, data must be imported into MPM 6.

1. Open file in Microplate Manager version 5.2.1 or lower.
2. Choose **File > Export** "Name of file", destination, and the suffix ".epc" . Click **Save**.



3. Change file Extension from ".epc " to ".csv "

In Microplate Manager 6,

1. Choose **New Experiment**.
2. Select **File > Import Data**
3. Select File > Excel file type (*.xls,*.csv,*.txt)“ , and click **Open**.
4. In the Import Setup dialog, click on **Matrix**.
5. Enter the # Rows preceding data.
6. Enter the # Columns preceding data. Click **OK**.
7. The Raw Data displays. Save file in Microplate Manager 6.

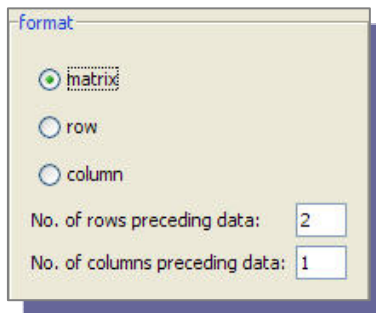
Importing Data from Excel

MPM 6 will import Excel files using *.txt, *.csv, or *.xls format.

1. Choose **File > New Experiment**
2. **File > Import**
3. Specify the format of the Excel data:
Matrix, Row or Column
4. Specify the number of rows and/or the
number of columns preceding the data.
5. Click **OK**.

(No data will be imported if incorrect number of preceding rows and columns are entered.)

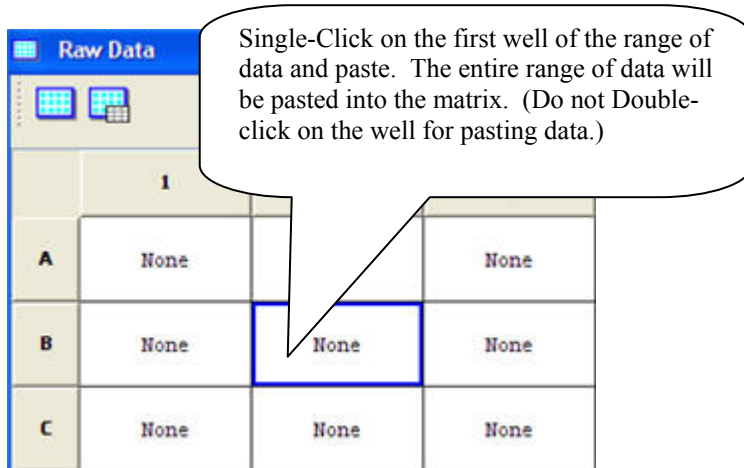
Data will be imported and displayed in the Raw Data window.



Entering Data Manually

Click on the **New Experiment** icon on the main toolbar. A window showing a plate with "None" in all the wells appears.

You can copy and paste from another program such as Excel. This is the easiest way to manually enter data.



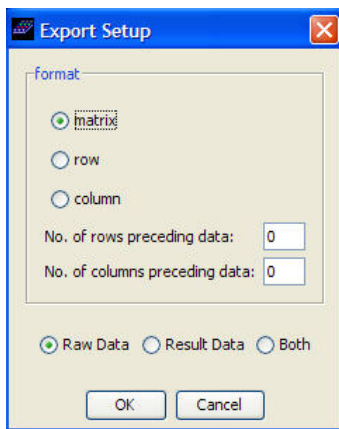
Data can also be entered into the program by typing from the keyboard.

Exporting Data or Reports

Both raw data and result data can be exported to Excel from Microplate Manager 6.

Exporting Data

1. Click on the **Export Data** icon on the main toolbar and name the file to be exported. The Export Setup window will appear:
2. Select the type of data and format to be used in exporting and click **OK**.



The exported file will be saved as an Excel (.xls) file.

Exporting Reports

1. To export data in a report format, including table or matrix headings, select the desired items such as Info, Plate Data, Standard List, Result List, etc. from the list in the **Content Selector** window under the Edit menu.
2. Choose **File > Export Report**, name and save the report.

An example of an exported standards table from a Percent B/Bo Assay is shown below:

	A	B	C	D	E	F	G
1	Standards Report:						
2	Std #	Conc (ng/r	Well	Replicates	Mean % B	SD	%CV
3	1	7.8	E3	90.766	90.871	0.148	0.163
4			F3	90.976			
5	2	15.6	C3	88.877	89.087	0.297	0.333
6			D3	89.297			
7	3	31.3	A3	87.618	87.723	0.148	0.169
8			B3	87.828			
9	4	62.5	G2	84.47	84.68	0.297	0.35
10			H2	84.89			
11	5	125	E2	77.754	78.279	0.742	0.948
12			F2	78.804			
13	6	250	C2	69.36	69.675	0.445	0.639
14			D2	69.99			
15	7	500	A2	61.175	60.965	0.297	0.487
16			B2	60.756			
17	8	1000	G1	54.879	54.669	0.297	0.543
18			H1	54.46			

Exporting Custom Screening Reports (BSE, TSE, TSE, NSP)

(Windows PC only, using Bio-Rad custom Excel templates BSE, TSE, TSE(NSP)...)

The Custom Export feature under File menu is for automatically exporting data and generating a Screening Report for a series of Bio-Rad Platelia BSE and related detection kits.

This Custom Export feature is used together with the BSE (and related) Protocols in the Sample Protocol folder and the “Custom Templates” folder in the Microplate Manager 6.2 folder. The BSE Custom Template is included with the Microplate Manager 6.1 software, and Custom Templates for other kits can be purchased separately.

These Custom Templates are for a Microsoft Excel workbook, with built-in calculation functions for reporting the results from these kits. Microplate Manager 6.1 automatically generates a Screening Report using the data from a microplate reading.

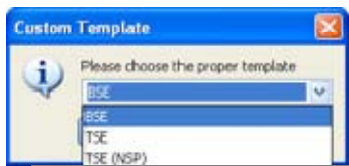
Procedure for Custom Export Reports

1. Prepare a 96-well microplate according to the instructions in the BSE (or related) detection kit manual.
2. Open the corresponding BSE (or other) protocol (located in the Protocols folder) in order to perform a dual wavelength (450-620 nm) read as specified in the protocol.
3. If creating a new protocol, enter the requested label information (i.e. Technician, Plate ID, etc.) in the window that appears. This information will appear in the BSE report.



4. Read the Plate or Open a previously-read BSE plate.

5. Select File >Custom Export.
6. Select desired Template (which contains the built-in calculation functions for reporting the results for this kit).



7. Once the Template is selected, the program will automatically open Excel and display the correct Screening Report. The BSE (or other) Screening Report will be saved automatically with the name that was entered.

BSE Custom Screening Report

This BSE (or other) Screening Report contains two worksheets; *Analysis Settings* and *Report*. Click on the navigation bar at the bottom of the workbook to display the different worksheets.



The *Analysis Settings Worksheet* contains settings for control validation and cutoff criteria.

The *cutoff criteria constants*, k_1 , k_2 , k_3 can be changed to modify these cutoff values. These are the only constants that can be changed in the Workbook. All other data fields and calculation functions are password-protected and unchangeable.

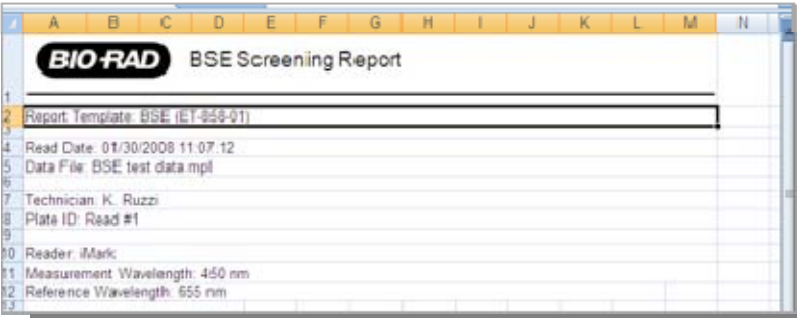
PC Validation		Cutoff Criteria	
Valid(PC) >=	2	POS Cutoff = (NC + k1) * k2	
PCi >	1.00 OD	NEG Cutoff = (NC + k1) * k3	
		k1 = 0.210	
		k2 = 1.00	
		k3 = 0.90	
NC Validation		Note: The values for k1, k2 and k3 can be changed. The default values for k1, k2 and k3 are 0.210, 1.00 and 0.90 respectively.	
Valid(NC) >=	3		
NCi <	0.15 OD		
NCi <=	1.40 * NC		
NCi >=	0.60 * NC		

The *Report Worksheet* contains the raw test data from the microplate reader. The Report is a single sheet printable format. It has four sections: the Information

Setting Up a Protocol

Section, the Control Validation Section, the Cutoff Values Section, and the Matrix Data including the Sample info.

In the Information section, the report template, technician, plate, reader, and reading parameters are given.



The custom report template is uniquely identified with a name, part number and revision number. Each custom report template is released as a controlled document with its own part number and revision level. All validation procedures and results are included with each release.

In the *Control Validation Section*, the results for the positive and negative controls are given, as specified in the Analysis Settings Worksheet. The number of valid controls and the mean absorbance value for each control group (positive and negative) are shown. These values are highlighted in red, if the control group has failed validation.

Control Validation	
Negative Controls	
Criteria:	Valid(NC) >= 3 NCi < 0.15 AND NCi <= 1.4 * NC AND NCi >= 0.6 * NC NCi < 0.15 AND NCi <= 0.1862 AND NCi >= 0.0798
Result:	Valid(NC) = 3 Mean(NC) = 0.1330
Positive Controls	
Criteria:	Valid(PC) >= 2 PCi > 1
Result:	Valid(PC) = 2 Mean(PC) = 3.8530

The *Calculated Cutoff Values* for identifying the positive, negative and indeterminate samples are shown in the third section. These are calculated as specified in the Analysis Settings Worksheet.

Cutoff Criteria			
POS	POS Cutoff = (NC + 0.21) * 1	POS Cutoff >=	0.343
NEG	NEG Cutoff = (NC + 0.21) * 0.9	NEG Cutoff <	0.309
???	Cutoff [POS Cutoff, NEG Cutoff]		

The matrix of the 96-well absorbance data, including the well identifiers, is shown in the fourth section. Positive control wells (PC1 and PC2) and negative control wells (NC1 through NC4) and their absorbance values are shown. Controls that do not pass validation are flagged in red with strikethrough marks. Each sample well shows the result (POS, NEG or ???) and its absorbance value. Wells flagged with a *.*** are out of range, and blank wells are unformatted.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.134	0.244	0.483	0.853	0.302	0.556	0.630	0.191	0.173	0.162	0.154	0.200
	NC1	NEG	POS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG
B	0.126	0.237	0.721	0.849	0.415	0.802	0.992	0.650	0.186	0.150	0.148	0.171
	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	NEG
C	0.120	0.264	0.360	0.513	0.778	0.891	1.236	0.472	0.161	0.202	0.202	0.188
	NC3	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	NEG
D	0.145	0.173	0.625	0.419	0.424	0.802	0.579	0.238	0.226	0.194	0.262	0.185
	NC4	NEG	POS	POS	POS	POS	POS	NEG	NEG	NEG	NEG	NEG
E	3.889	0.209	0.714	0.819	0.489	0.203	0.792	0.340	0.290	0.188	0.278	0.278
	PC1	NEG	POS	POS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG
F	3.837	0.189	0.365	0.863	0.400	0.161	0.660	0.548	0.214	0.231	0.262	0.204
	PC2	NEG	POS	POS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG
G	0.202	0.195	0.262	0.339	0.283	0.235	0.699	0.412	0.223	0.191	0.218	0.188
	NEG	NEG	NEG	???	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG
H	0.181	0.188	0.490	0.931	0.198	0.176	0.477	0.367	0.177	0.142	0.160	0.220
	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG

Setting up a Protocol

Setting up protocols automates your test processes. Parameters for frequently-run assays can be set up and saved once, and used whenever the assay is performed.

To set up a assay protocol, follow these steps:

1. Set up a Template
2. Select Instrument Setup settings
3. Select Instrument Controls
4. Enter Assay Info
5. Select an Analysis Option
6. Select Report Options
7. Name and Save the Protocol

The screenshot shows a 'Template' window with a toolbar and a data table. The table has columns labeled 1, 2, 3, 4, and 5. Rows are labeled A through H. Callouts provide the following information:

- Standard:** Points to the '#' symbol in column 1 of rows A through H.
- Sample:** Points to the 'S01' through 'S07' labels in column 5 of rows A through G.
- Blank Definition:** A callout box states: "Define blank as # on the template to have it automatically subtracted from raw data. In Result Data view the blank is subtracted." This points to the '#' symbols in column 1.
- Table Requirement:** A callout box states: "A template must always be present in order to see any Result Data or Data in the Table." This points to the entire table structure.

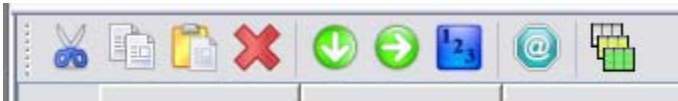
	1	2	3	4	5
A	#	500	31.3	S01	S05
B	#	500	31.3	S01	S06
C	#				
D	#	250	15.6	S02	S07
E	#	125	7.8	S03	S08
F	#	125	7.8	S03	S07
G	#	2.5	3.9	S04	
H	#		3.9	S04	

Setting Up A Template





The template defines the identifier locations for your plate data. A template must always be present in order to see any Result Data or Report window.

The template controls allow you to edit your template, as well as to enter equations and dilutions. The controls are available as menu items, or as icons under the Template Window toolbar.

Click on the **Template** icon in the Main toolbar or choose **Template** under the Window menu to open a template.



Blanks, standards or sample identifiers are entered in the template for each well.

	Samples	Begin Sample IDs with an alphanumeric letter, for example, POS or NEG (positive or negative control), or MAX (well with a maximum sample value). Any long sample ID or barcode can be entered. Sample wells are shown in blue.
	Standards	Enter standard concentration values as positive numbers. Standard wells are shown in red.
	Blanks	Enter the number sign, # (shift 3) for each blank well. Blank wells are green with #. The average of all blank wells with “#” will automatically be subtracted from the raw data. A second blank can be defined as BLK, if desired, and equations can be set up to do the subtraction. (see Percent Control Assay Tutorial on page 82)
	Unused Wells	Leave unused wells empty.

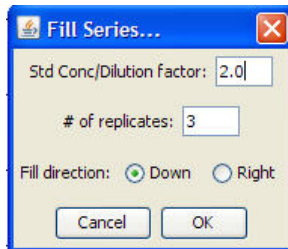
To fill in a template, click on a well and enter identifier for that well. To move from well to well, use the arrow keys or the tab key.

To use the Fill features, highlight a section of the template and select Fill Down, Fill Right, or Fill Series.

To highlight the entire plate, choose Select All under the Edit menu. To clear the template, press the delete key.

To re-size Template columns, in order to display long ID names, position the cursor in the column number at the top of the window and drag it to the right.

To AutoFill a series of identifiers that begin with a number, highlight the area to be filled in, click on fill a series icon. In the Fill Series window, enter the desired concentration or dilution factor. Enter the number of replicates and select the fill direction. Click **OK** to fill the template.



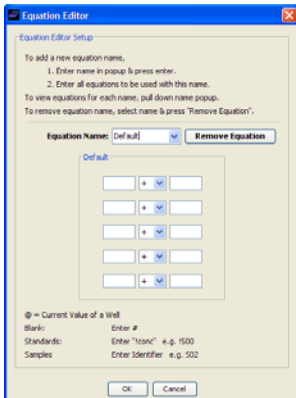
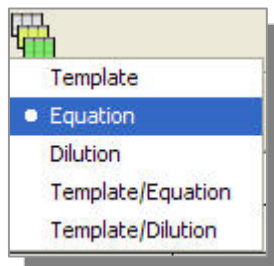
Entering Equations

To enter Equations:

click **Template View** icon. Select **Equations.**

or choose **View > Equations** in Main menu.

See the *Quantitative ELISA Assay Tutorial* section for more information on how to enter equations.

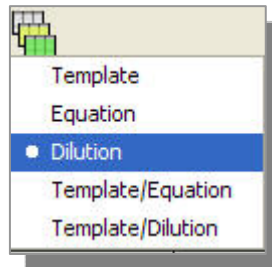


Entering Dilutions

Dilutions can be entered for any sample well (except for blanks or standards).

To enter Dilutions:

1. Click the **Template View** icon.
2. Select **Dilutions**, or choose **View > Dilutions** in the Main menu.



In the Dilution window, the default is all the wells are filled in with 1.0, indicating that the dilution is one (no dilution).

The dilution factor is the ratio of 1 to the value entered. For 1:2 dilution, enter the dilution factor as 2. For a 1:100 dilution, enter a factor of 100.

In the **Template/Dilutions** view, the dilutions appear in the **lower part of each well**.

Entering and Formatting Experimental Info

1. Click on the *Info* icon.
2. Enter the Experimental Info for each plate read (title, operator, concentration unit, or comments) to save this info with the protocol.
3. Add Labels or Customize the Label fields in the Info window by clicking on the Format Info icon in the Info window.

The 'run #23 Info' dialog box contains the following fields and text:

- Title:** % B/Bo
- Protocol:** B-Bo Assay.pro
- Date/Time:** 10/02/2007 10:48:29
- Plate ID:** run #23
- Detection Plate:** (empty field)
- Technician:** Kristin A. Ruzzi
- Detection Lot:** (empty field)
- Conc Unit:** ng/ml
- Comments:**

```

NSB is non-specific binding well
MAX is maximum binding well
purpose: to compare all wells to the maximum binding well
           and convert data to percent of maximum binding well

```

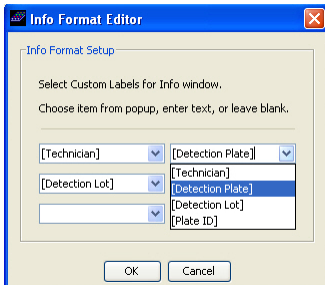
Buttons: OK, Cancel

Setting Up a Protocol

Select up to 6 Custom Labels from a pop-down menu by selecting one of the items in the list, or by entering a new Label ID in the edit field.

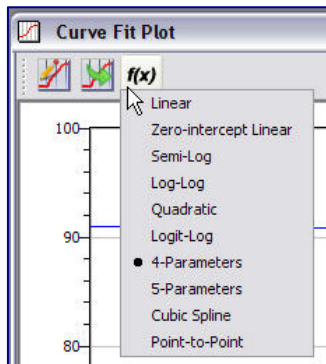
The selected Labels will appear in the Info Window and will be saved in a Protocol.

Custom Labels can also be set up and used for Custom BSE (or other) Screening Reports.

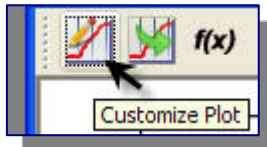


Select and Customize Curve Fit Plot

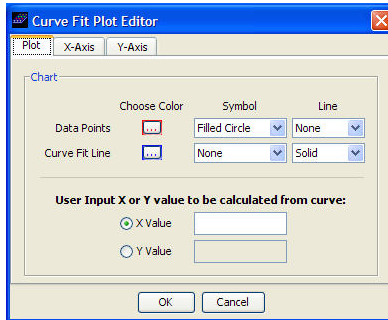
1. Click **Curve Fit** icon to select desired Curve fit.



2. Click on the **Customize Plot** icon.



3. In the Curve Fit Plot Editor, select Auto/ Manual Scaling, Number Format, Axes labels, etc. for Curve Fit Plot.



Setting up the Report Contents

1. Click **Report** Icon in Main Toolbar.

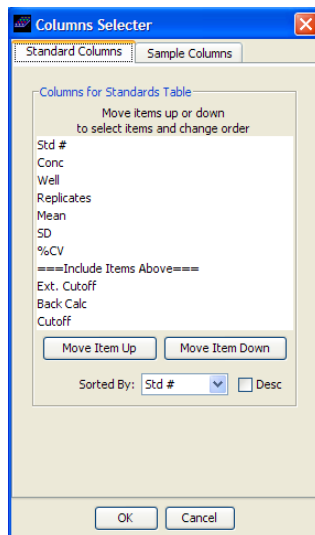
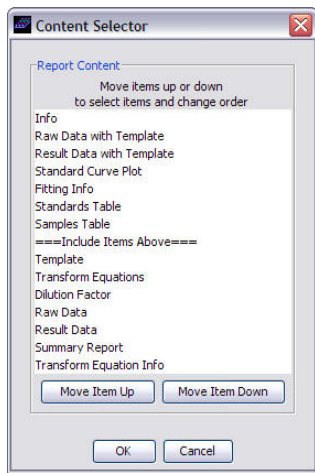
2. Click the **Select Report Contents** icon,




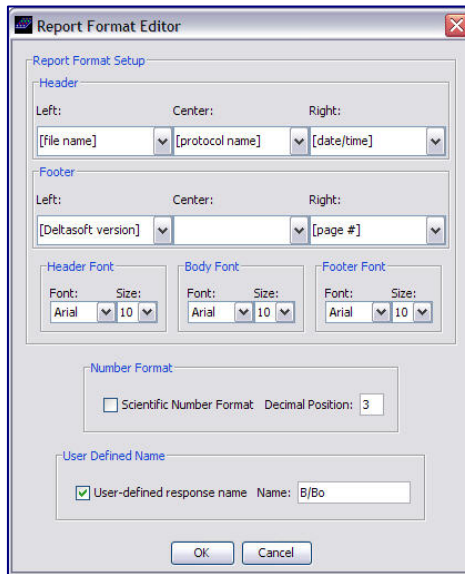
and then, select the **Select Table Columns** icon



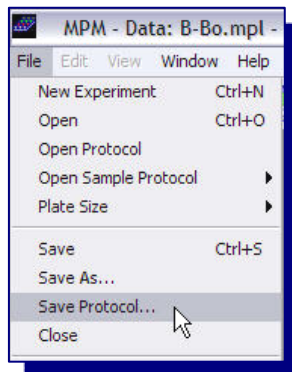
3. Select contents (Raw Data, Results Data, Plot, Info, Samples Table...) to include in the report by moving items up to the top section of the list and arranging them in the desired order.
4. Select columns (Standard #, Well ID, Concentration, Absorbance, etc.) to be included in the Standards and Samples Report Tables, and arrange in the desired order. Select Sort parameters and Sorting direction.



5. Click **Format Report Editor** icon  in the Report toolbar to format the report with titles, number format, headers and footers.



Saving the Protocol



1. Choose **File > Save Protocol**.
2. Name the protocol and click **Save**.

Analyzing Data Using a Standard Curve

The standard curve is a plot of the concentration of the standards you have designated in the template (on the x axis) versus the readings from the instrument (in OD units for an endpoint experiment) (on the y axis).

Microplate Manager 6 provides the following types of curve fit options:

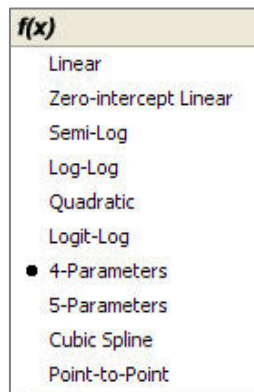
- Linear
- Zero-intercept Linear
- Semi-Log
- Log-Log
- Quadratic
- Log-Logit
- 4-Parameters
- 5-Parameters
- Cubic Spline
- Point-to-Point

To perform a curve fit and display the curve fit plot on the screen click on the **Curve Fit Plot** icon and select the desired curve fit by clicking on the **Choose Fitting Function** icon.



If a transformation has been applied to the template, then the values plotted in the standard curve will be the transformed values.

The mean value of the replicates of the standards, and not the individual values, are used in the calculation of the standard curve.



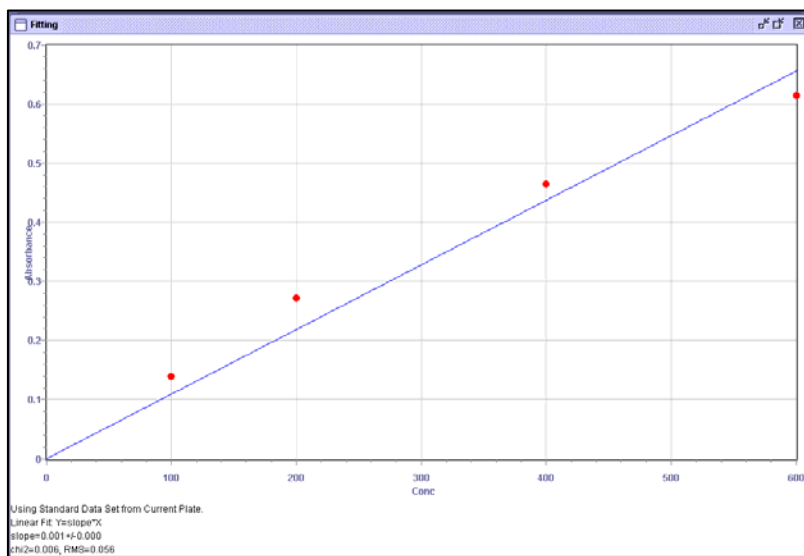
Standard Curve Analysis Options

Linear Fit

For a linear fit, the best straight line is fitted to the data points, OD's (response) (y-axis) vs. the standard concentration (x-axis). The equation of the line has the form

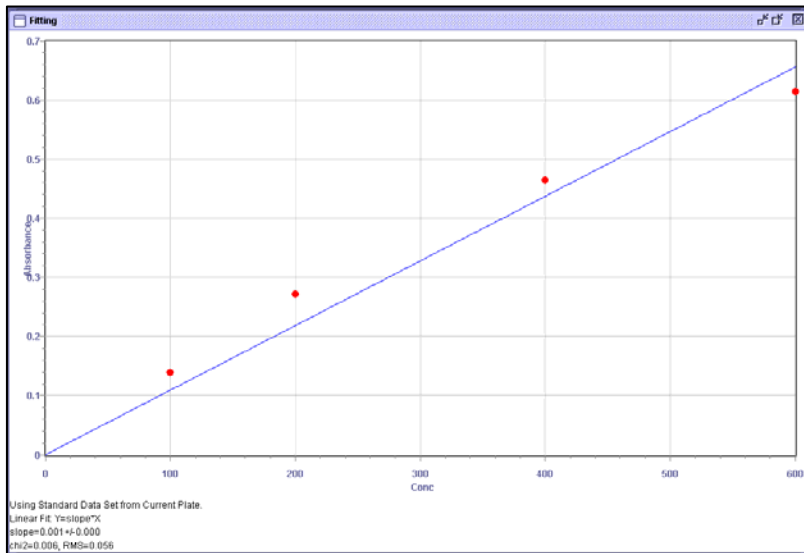
$$y = \text{Intercept} + \text{Slope} * \text{Conc}$$

The calculated intercept, the slope, the correlation coefficient (r) and χ^2 values of the line are shown below the plot.



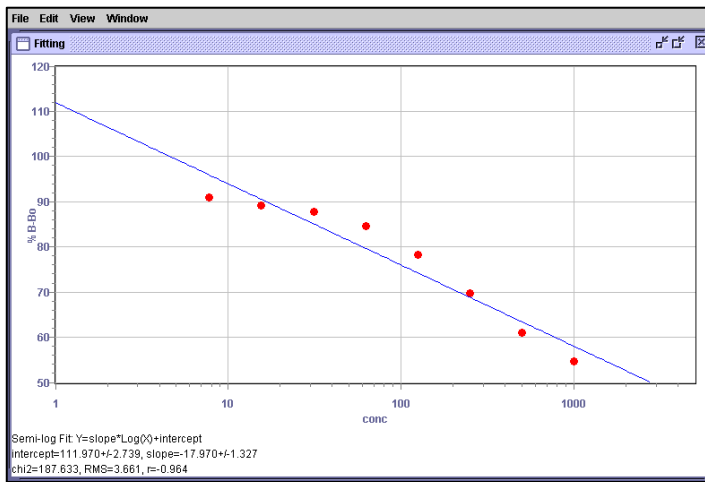
Zero-intercept Linear

In the Zero-intercept of analysis a linear fit is used, but the intercept is set to Zero. This method of analysis is useful for assays which have a linear response to the concentration such as protein assays or other assays.



Semi-log Fit

When Semi-Log is selected in the Curve Fit dialog box, a plot of the response (y axis) vs. the logarithm of the concentration (x-axis) is obtained as shown below:



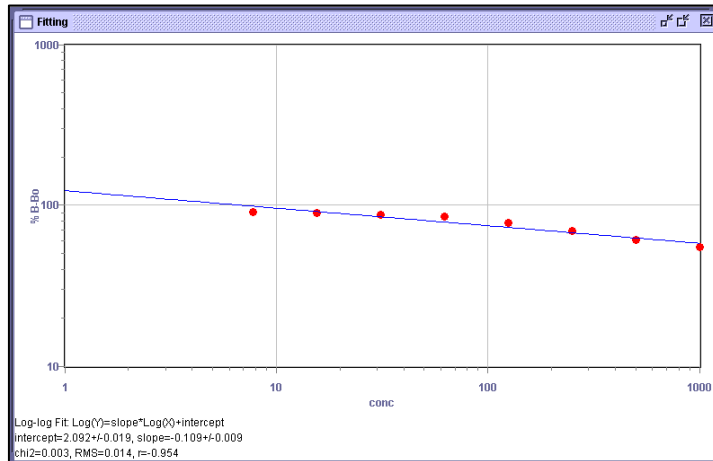
For the semi-log fit, the equation is:

$$y = \text{Slope} * \text{Log}_{10}(\text{conc}) + \text{Intercept}$$

The calculated intercept, slope, the correlation coefficient (r) and χ^2 values of the line are shown below the plot.

The semi-log standard curve fitting option should be used when a linear plot of the data exhibits a strong curvature up or down.

Log/log Fit



The Log/Log curve fit option fits the best straight line to the data consisting of the logarithm of the OD (response) vs. the logarithm of the concentration.

For the Log/log method, the equation for the standard curve is:

$$\text{Log}_{10}(y) = \text{Intercept} + \text{Slope} * \text{Log}_{10}(\text{conc})$$

In the plot, the concentration and the OD axes are displayed on the log scale. The calculated intercept, slope, the correlation coefficient (r) and the χ^2 values of the standard curve are shown below the plot.

The log-log option should be used when a linear plot of the data exhibits a strong curvature up or down.

Quadratic

The quadratic function fits the best parabolic curve according to the equation

$$Y = A + Bx + Cx^2$$

where A is the intercept, B is the slope of the curve at the intercept, and C is the measure of curvature of the parabola.

The quadratic fit is most appropriate when the standard curve curves up or down.

Log/Logit and 4-Parameter Fit

Both the Log/Logit and the 4-Parameter curve fit methods are based on the same equation:

$$y = \frac{A - D}{1 + (\text{conc} / C)^B} + D$$

which can be expressed in the equivalent form:

$$\text{logit } y = a + b * \log_{10}(\text{conc})$$

where

$$\text{logit } y = \ln(y'/1-y'), \quad B = -b/\ln_{10} = \text{slope at inflection point of curve}$$

$$y' = (y-D)/(A-D) \quad C = \text{EXP}(a/B) = \text{concentration at midpoint between A \& D}$$

The difference between the Log/Logit and the 4-parameter curve fit options is in how the parameters A and D (the asymptotes for $\text{conc} \rightarrow 0$ and $\text{conc} \rightarrow \infty$, respectively) are calculated.

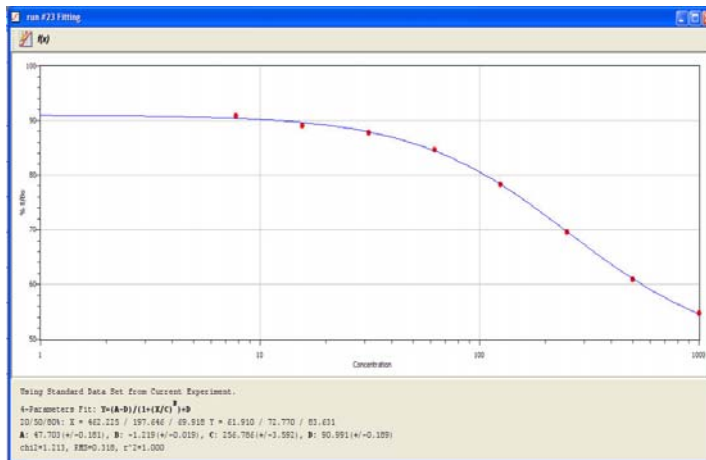
In the Log/Logit method, the parameters A and D are fixed to the response of the lowest and highest standards, respectively, and only the B and C values are fitted. The Log/Logit method requires that at least one standard lies within the intermediate range between the two asymptote portions of the S-shaped standard curve.

In the 4-parameter method, all 4 parameters A, B, C, D are fitted. The algorithm used by the program fits the parameters A, B, C, D iteratively with non-linear regression (Levenberg-Marquardt method) as described in Ref 2. The starting values for the A, B, C, D parameters are taken from an initial Log/Logit fit. In Microplate Manager 6.0 the 4-parameter logistic curve uses 2000 iterations. If the fit does not converge in this number of iterations, the program will report fitting failed and not display a curve.

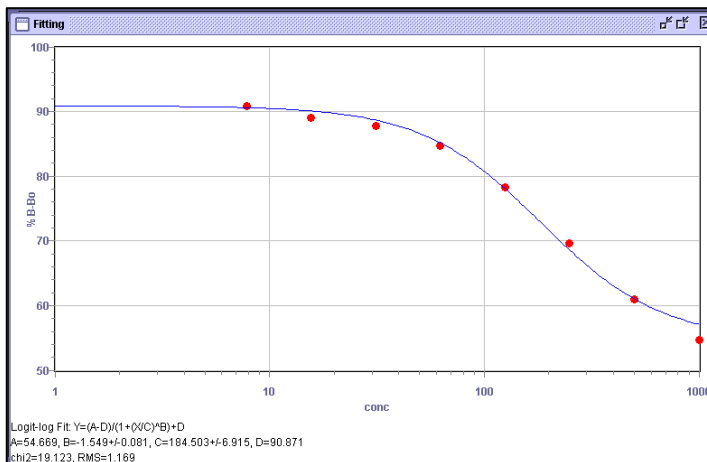
In both methods, the B value is the slope at the inflection point or half maximum of the curve. The 50% Value is the Mean Y Axis Value of the A & D asymptotes. The C value is the concentration (X value) for the 50% Y value which corresponds to the midpoint between A and D.

Analyzing Data Using a Standard Curve

The 4-parameter method is usually preferred over the Log/Logit method, because the results for the 4-parameter method are more accurate.



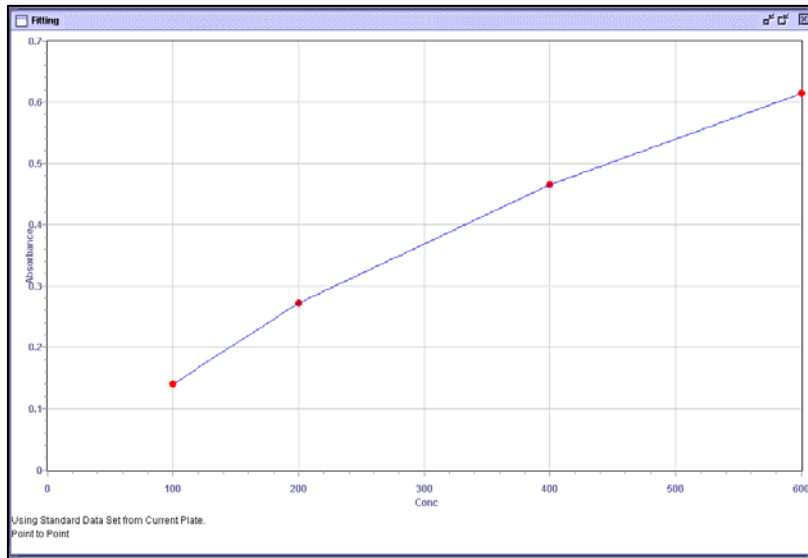
In both the Log/Logit and the 4-Parameter plots, the concentration axes are displayed on the log scale. The parameters A, B, C, D, the χ^2 values of the standard curve are shown below the plot.



Point to Point

In the Point to Point method of analysis, the data points are simply connected. No fitting of the data points to a line or curve is done.

For reactions where there is a lag time or a plateau, it is often convenient to look at the data using this plot in order to see the true linear range of the assay.



Cubic Spline

Cubic spline is a piecewise polynomial approximation in which a set of data points are joined by a series of curve fits. These multiple curve fits are smoothed by using a cubic fit

$$y = Ax + Bx^2 + Cx^3 + D$$

This type of curve fit is less affected when any one of the standards has a poor value. Because multiple curve fits are performed with this function, the fit parameters are not shown with the graph.

A minimum number of standards that pass certain criteria are required for each type of curve fit. For the best results, always use more than the minimum number of standards required to do the calculation.

If these requirements are not met, the Fitting will Fail.

Interpolation Using a Previous Standard Curve

It is frequently convenient to run one plate containing all the standards for a standard curve and other plates having only samples. The plate may contain one or two or more standards if desired.

The **Import Curve** feature allows users to run a plate containing only samples and determine concentrations from a previous standard curve file.

To use the **Interpolate from a Previous Standard Curve** feature, read a plate containing samples.

1. Click the **Curve Fit Plot** icon on the main toolbar.
2. Click the **Import Curve** icon.
3. Choose the Standard Curve file you want to import.



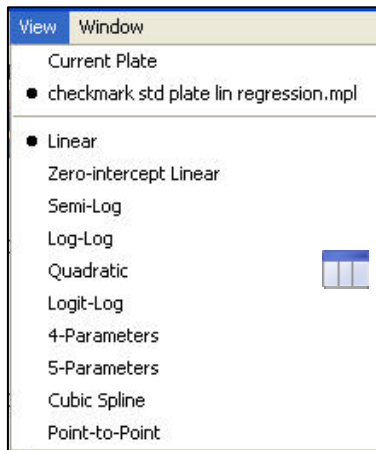
Under the **View** menu, the current plate and the imported standard curve file are displayed.

4. Select the plate with the imported standard curve.

A standard curve will be drawn and the samples for the current plate will be interpolated using this imported curve.

5. Click on the **Report** icon on the main toolbar to view the Table.

Make sure concentration is included in the Sample Table by clicking on the **Select Table Columns** icon. Enter a concentration unit in the edit field on the Info Window in order to have concentration unit included in the report.



It is possible to import more than one standard curve for easy comparison between interpolated values for several standard curves.



The printed report will show only the samples and standards (if there are any) on the current template.

To obtain a printed standard table report for the imported standard curve, print the imported standard curve file separately.

Customizing Standard Curve Plots

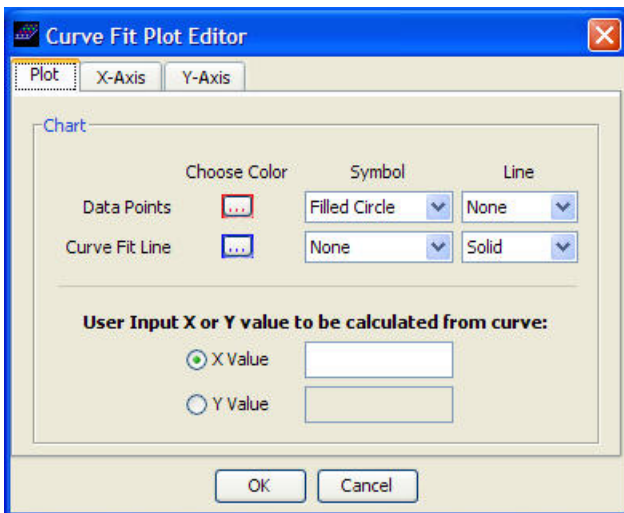
To customize the standard curve plot with axes labels or different X, Y ranges:

1. Click on the **Customize Plot** icon.
2. In the **Plot Editor** window, select the **Plot Tab**, to customize the color of the lines or point size and choose grid line on or off. In the **X-Axis** or **Y-Axis** windows, you can enter the X-Axis or Y-Axis labels, such as Concentration or choose Scaling preferences.
3. In the **Auto Scale** option, the X,Y axes cutoffs are calculated automatically by the program from the data. The axes are rounded off to provide reasonable tick marks.

In the **Manual Scale** option, the user can enter the desired X, Y axes cutoffs.

Select the same axes cutoffs in order to compare similar plots for a given assay. For log plots, the plot should span full log decades.

When **Scientific Label Format** is selected, the concentrations will be listed as 1.0E2, 1.0E3, etc. instead of 100, 1000, etc. The option is useful for very large concentration ranges.



Equations and Calculations

Definitions of the statistical parameters calculated for a set of values $\langle y \rangle$ are discussed below. For data sets with dilutions, the equations used in calculating the weighted average of dilutions are also described.

- Mean
- Standard Deviation, SD
- % Coefficient of Variation, % CV
- Root Mean Square Error, RMS
- Chi-square statistic value
- Correlation Coefficient, r
- Weighted Average of dilutions, Wt. Avg

The equations are:

- **Mean** = y value = $\langle y \rangle = \sum y_i / n$
y = measured response for measurement i
n = number of measurements
- **Standard Deviation = SD** = $\text{SQR} (\sum (y_i - \langle y \rangle)^2 / (n-1))$ where SQR = Square Root
- % Coefficient of Variation = % CV = $100 * \text{SD} / \langle y \rangle$
- **RMS** = Root Mean Square Error = Square Root of (Residual Variance of) Standard Curve, where a residual is the difference between an observed value of the response variable and the value predicted by the regression line. That is, Residual = observed y – predicted y.

Residual variance (Res. Variance) is defined as the weighted sum of the squared deviations of data points from the fitted line divided by the degrees of freedom of the fit.

- Res. Variance = $\sum W_i (y_i - Y_i)^2 / (n-2)$ for a linear curve
- Res. Variance = $\sum W_i (y_i - Y_i)^2 / (n-3)$ for a 4-parameter curve
- W_i = normalized weight of measurement (normalized so that sum of weights = number of measurements, n)

Y_i = corresponding y value on fitted curve

A residual is the difference between an observed value of the response variable and the value predicted by the regression line.

That is, Residual = observed y - predicted y.

The **chi square** value is a measure of how close the observed values are to the calculated values. A chi value of zero means that the observed values are equal to the calculated values. Likewise, small chi values indicate a good fit.

$$\chi^2 = \text{SQR}(\sum (y_i - f(x_i, p))^2 / (n - np))$$

For a set of data points (y_i, x_i) ;

$y = f(x, p)$ where p is a set of parameters.

n is total number of values; np is total number of parameters

Correlation coefficient r is a slope of least square regression line for linear plots.

The correlation coefficient, r, is calculated for data pairs (x_i, y_i) with weighting factors w_i

$$r = \sum w_i (y_i - Y) (x_i - X) / (\sum w_i (y_i - Y)^2)^{1/2} (\sum w_i (x_i - X)^2)^{1/2}$$

$$X = \sum w_i x_i / \sum w_i, \quad Y = \sum w_i y_i / \sum w_i$$

$$r = \sum w_i \sum w_i x_i y_i - \sum w_i x_i \sum w_i y_i / (\sum w_i \sum w_i x_i^2 - (\sum w_i x_i)^2)^{1/2} (\sum w_i \sum w_i y_i^2 - (\sum w_i y_i)^2)^{1/2}$$

The r^2 (coefficient of determination) is often reported instead of r.

Back calc is the calculated concentration (x value) based on the calculated y value for the curve.

OD is Optical Density (or Absorbance), the response values obtained from a reader without pathlength correction. This is the logarithm (base 10) of the ratio of the amount of incident light to the amount of light that passes through the sample. Therefore an OD of 2.0 absorbs 10 times more incident light.

Weighted Average Concentration of Dilutions

The estimated error is calculated

$$S_{y, \text{pred}} = \text{SQR variance}_y = \text{RMS} (y)$$

If a sample has been analyzed in replicates, the mean response of n replicate measurements will be more reliable than an individual measurement. The Standard Error of the Mean (SEM) of the response y is

$$\text{SEM}_y = s_y / \text{SQR } n$$

Error of the interpolated concentration (conc_y) is related to the error of the response s_y according to

$$s_{\text{conc}} = (\delta \text{conc}(y) / \delta y) s_y$$

The estimated standard error of concentration (SEM_{conc}) calculated from the mean response of n replicates is

$$\text{SEM}_{\text{conc}} = (\hat{I}' \text{conc}(y) / \hat{I}' y) \text{RMS}(y) / \text{SQR } n$$

The $(\hat{I}' \text{conc}(y) / \hat{I}' y)$ depends on the form of the standard curve.

For a linear standard curve (for example),

$$Y = A + B * \text{conc} \rightarrow \text{conc}(y) = (y - A) / B$$

$$(\delta \text{conc}(y) / \delta y) = 1 / B$$

$$\text{Wt avg conc} = \langle \text{conc} \rangle = \sum w_i \text{conc}_i / \sum w_i$$

$$\text{Weight} \quad w_i = 1 / (\text{SEM}_i)^2$$

conc_i interpolated concentration for dilution i

If there are replicate samples with dilution i , conc_i will be the mean of these replicates.

SEM_i is Standard Error of the Mean for conc_i .

Example of Weighted Mean

For two concentrations with SEM values as shown,

$$100 \pm 10$$

$$150 \pm 50$$

the mean concentration should NOT be taken as 125. The weighted mean of these two concentrations is 102, since 25 times as much weight is given to the value of 100 with the 5-fold smaller standard error value. When conc_n is calculated using the

$$\langle \text{conc} \rangle = \sum w_i \text{conc}_i / \sum w_i$$

$$\text{with } w_i = 1 / (\text{SEM}_i)^2$$

Analyzing Data Using Cutoffs

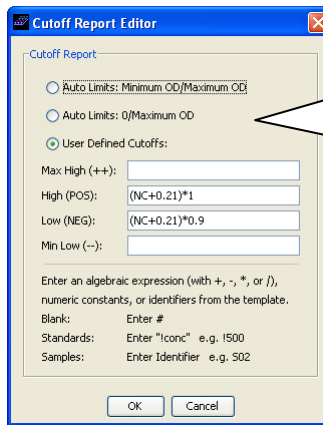
The Cutoff Result analysis options are used for assays including one or two controls (and no standard curve). All the samples are compared to the cutoffs or controls.

To set up a Cutoff Analysis Report:

1. Click on the **Result** icon on the main toolbar.
2. Click on the **Cutoff Report** icon on the Result Window.

In the **Cutoff Report Editor** window, a user defines the cutoff values, Max High (+++), High (+), Low (-), and Min Low (--), for all the screening reports.

Max High and Min Low are optional cutoffs for setting up an intermediate range beyond the High and Low values.



A template must always be present in order to see any Result Data or Data in the Table.

In the two Auto Cutoff options, the **Minimum OD/Maximum OD** or the **0/Maximum OD**, the values for the minimum and maximum ODs in the assay are used as the cutoffs.

3. Click on the **Choose Report View** icon.

The three cutoff display options in the menu are described here. Either two or four cutoffs are used based on the user definitions in the Cutoffs tab window.

- **Gray Scale:** In the Gray Scale window, the data values are shown numerically and in shades of gray. Results equal to or below the lowest cutoff are white, results equal to or above the highest cutoff are black, and results between the two cutoffs are shown in shades of gray.
- **Cutoff Report:** In the Cutoff Report chosen under the View menu, the data values are shown as --, -, *, or +, ++ if four cutoffs are defined. If two cutoffs are defined, the results are shown as -, *, or +. Intermediate values between cutoffs are shown as (*). The report is also displayed in color. High values are displayed in red, low as blue, and intermediate as white.
- **Extended Cutoff Report:** In the Extended Cutoff Report chosen in the View menu, the data values are shown as --, -, 0-9, or +, ++ if four cutoffs are defined. If two cutoffs are defined, the results are shown as -, 0-9, or +. Intermediate values between cutoffs are shown as 0-9. The report is also displayed in color. High values are displayed in red, low as blue, and intermediate as white.

Using Equations for Setting Cutoffs

High and low cutoffs can be assigned by numeric constants or sample or standard IDs defined on the template.

Cutoffs can also be defined by equations for more powerful and flexible analysis options.

Allowable arithmetic operations for cutoffs are:

- + for addition
- - for subtraction
- * for multiplication
- / for division
- Parentheses () may be used.

Numeric constants can be entered in two forms:

- decimal (e.g. -2.51)
- exponential (e.g. -2.51 E3 or 3.8 E-3)

Reference can be made to standard or sample IDs that have been defined in the Template setup

- Standards: enter ! followed by concentration
- Unknowns: enter identifier
- Blanks: enter #

In all Cutoff equations, *Microplate Manager 6.0* uses the averaged value of that group.

Examples of valid expressions:

- U01
- (U01+.1)/2
- (!100-!10)*2

Cutoff Examples for Assays with Controls

Positive & Negative Controls – positive (POS) & negative (NEG) control well(s) defined on template

- Low Cutoff: NEG
- High Cutoff: POS

% B/Bo – non-specific binding blank (NSB) & maximum or Bo well(s) (MAX) defined on template

- Low Cutoff: NSB
- High Cutoff: MAX

% Calibrator –blank (#) & calibrator (CALB) well(s) defined on template

- Low Cutoff: #
- High Cutoff: CALB

% Control - non-specific binding blank (NSB) & control well(s) (CNTRL) defined on template

- Low Cutoff: NSB
- High Cutoff: CNTRL

Data transformed – viewed as a Percent (0 - 100 %)

- Low Cutoff: 0
- High Cutoff: 100

Using values from a Standard Curve – standards 0, 5, 10, 25, 50, 100, 250, and 500 defined on template

- Low Cutoff: ! 0
- High Cutoff: ! 500

Using an equation – sample (SAMPLE2) well(s) defined on template

- Low Cutoff: 0
- High Cutoff: (SAMPLE2-0.025)*100

Setting Up a Percent Control Cutoff Analysis Report

Define the desired cutoffs in the **Cutoff Report Editor** window.

For example, the Max High is defined as $\geq 85\%$ of the control in all samples. The other high and low cutoffs are defined as shown:

Cutoff Report Editor

Cutoff Report:

☐ Auto Cutoffs: Minimum OD/Maximum OD

☐ Auto Cutoffs: 0/Maximum OD

☒ User Defined Cutoffs:

Max High (++):

High (POS):

Low (NEG):

Min Low (--):

Enter an algebraic expression (with +, -, *, or /), numeric constants, or identifiers from the template.

Blank: Enter #

Standards: Enter "!conc" e.g. !500

Samples: Enter Identifier e.g. 502

OK Cancel

Identifiers used in the Cutoff Report Editor must be used on the template.

	1	2
A		
B	BLK	BLK
C	CNTRL	CNTRL

The Control wells are defined on the template.

The resulting Extended Cutoff Report, is shown here with the high values shown in red, the low values in blue and the intermediate values in white:

Extended Cutoff Report													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	#	#	#	U06	U06	U06	U14	U14	U14	U22	U22	U22	
	--	--	--	9	9	9	8	8	8	3	3	3	
B	BLK	BLK	BLK	U07	U07	U07	U15	U15	U15	U23	U23	U23	
	--	--	--	POS	POS	POS	4	4	4	NEG	NEG	NEG	
C	CNTRL	CNTRL	CNTRL	U08	U08	U08	U16	U16	U16	U24	U24	U24	
	++	++	++	6	6	6	NEG	NEG	NEG	++	++	++	
D	U01	U01	U01	U09	U09	U09	U17	U17	U17	U25	U25	U25	
	4	4	4	0	0	0	++	++	++	6	6	6	
*To exclude an outlier well from analysis, double click on well. To re-include we *A Template must be defined in order to see Results View Blank=0.002 Max High Cutoff (++) : 85.000 High Cutoff (POS) : 75.000 Low Cut													
--	NEG	0	1	2	3	4							
<=5.000	<=15.000	<21.000	<27.000	<33.000	<39.000	<45.000							
5	6	7	8	9	POS	++							
<51.000	<57.000	<63.000	<69.000	<75.000	>=75.000	>=85.000							

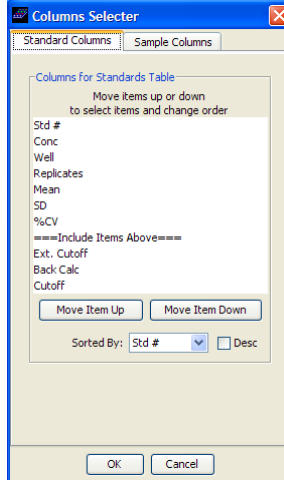
1. To view the results in tabular format, click on the **Report** icon in the main toolbar.



2. Click the **Select Table Columns** icon in the Report Toolbar..



3. In the Columns Selector window, move Ext. Cutoff Value into the included item. Move the items up or down to select which items to include in the Report and change their order. Select Sorted By Mean and Descending order.



The information entered in the Info window is automatically listed at the top of the Report.

Data Analysis Report:

Sample ID	Well	Replicates	Mean % CNTRL	SD	%CV	Ext. Cutoff Value
CNTRL	C1	99.629	100.000	0.371	0.371	++
	C2	100.371				
	C3	100.000				
U19	F7	97.032	97.093	0.283	0.292	++
	F8	97.403				
	F9	96.846				
U05	H1	93.692	94.310	0.652	0.691	++
	H2	94.991				
	H3	94.249				

Concentration unit information is displayed on the Tables and Curve fit Plot.

Running Kinetic Assays

Running assays kinetically has many advantages:

- Kinetic assays are more accurate. The errors in endpoint assays such as the time interval between substrate addition and reading in wells across the plate, differences in the optical uniformity of wells, and the substrate volume in the wells are eliminated.
- Using kinetics, assay sensitivity is greater for competitive assays.
- In kinetics the (acid) stop reagent and the decision to know when to stop the enzyme reaction are eliminated.
- Kinetics is more accurate since rate, but not necessarily endpoint absorbance, is directly proportional to the limiting reagent concentration.
- With kinetics, a broader concentration range is possible with fewer sample dilutions.
- In kinetics, the assay time and substrate incubation are shorter.

Reading a Kinetic Assay

1. Click on the New Experiment icon or choose **File > New Experiment**.
2. Choose **File > Instrument Setup**.
3. Select **Kinetic** in the Reading Mode pop-up menu.
4. Select the desired kinetic parameter, Rate or **Maximum Rate** from the Kinetic Mode popup.

In the kinetic mode, up to five different reading sets can be done successively. A reading set consists of a number of times a plate is read, the delay between each read and mix time, or before each reading.

To activate a reading set:

1. Click on the box next to the set number. Only sets that are checked will be run.
2. Enter the number of reads in the **Reads** column.

- Enter the interval between the start of two consecutive reads in the Interval column.

The interval time is the time necessary for one read plus mix time specified.

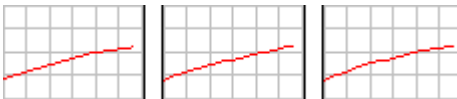
- Enter mix time, if desired.

In the above kinetic read example, the reader will do 40 readings with 30 second intervals and no mix.

- After the parameters are set, click the Read Plate icon to start the readings.

Analyzing Kinetics Data

When the raw kinetic data have been collected, a Kinetic Plots screen will appear. This shows response versus time plots for the reactions in all the wells.



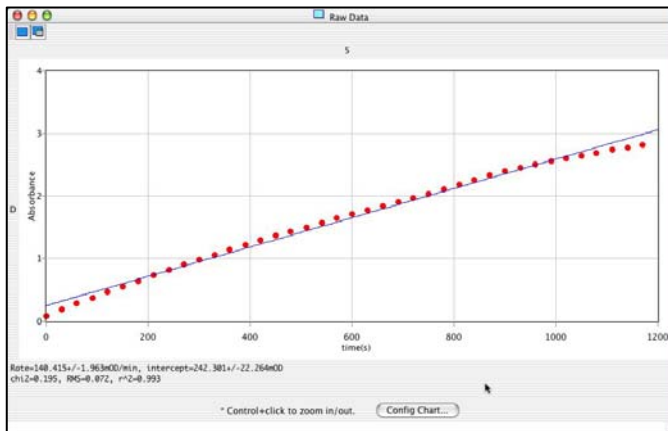
The raw data are shown in the initial displays as the total run time, beginning from time 0 to the end of the run time.

Kinetic Zoom Plots

Zoom in on a range of wells or a single well in the Kinetic Plots display to get an enlarged view of the plots.

To zoom on a single well:

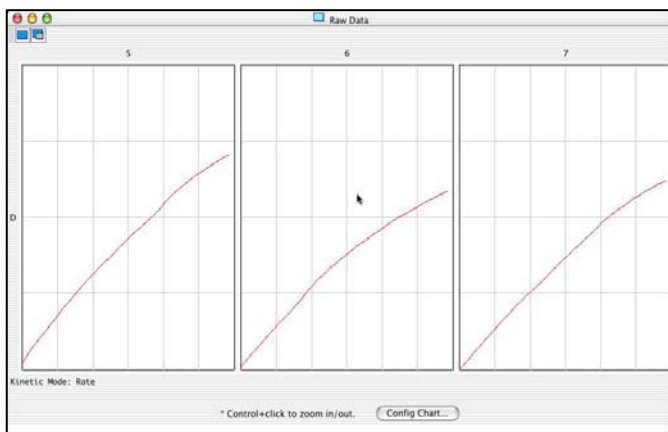
- Right-click on the desired well.



To return to the display of the whole plate:

- Right-click in the plot and click on zoom out.

To zoom in on a range of wells:

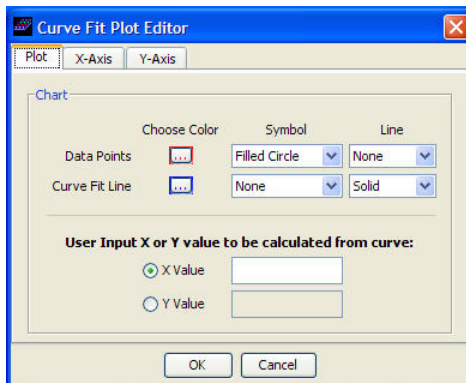


- Shift-drag over the desired range.

To zoom in on a single well or return to the display of the whole plate:

1. Right-click in the desired plot.
2. Select **Zoom In** to see large view or **Zoom Out** to return to the whole plate.

To change the plot properties, such as axes labels and scaling, click the **Customize Plot** button.



Viewing Raw Data at Kinetic Time Points

To see raw data values, go to the View menu and uncheck **Show Kinetics** to return to the Raw Data matrix.

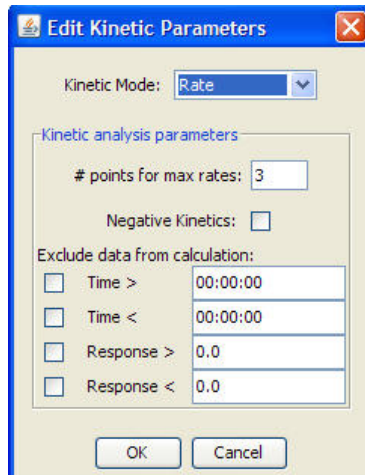
At each time point the raw data values for the entire plate can be viewed.

Click on the times pulldown menu in the right corner of the matrix and select the desired time point to view the raw data for that time.

Wavelength: 450.0		time(s): 0
10	11	0
		32
		61
0.042	0.040	81
		121
0.032	0.033	151
		181
0.037	0.033	211
		0.059

This feature is useful to check the numerical values for any of the data points in the kinetic plots.

- To edit the initial kinetic plots, click on the **Edit Kinetic Plots** icon and enter the desired time and OD range.



Minimum or maximum time or OD values can be set to exclude a lag time or other outlying data.

After the read is completed, data can be excluded from calculation by defining a new time range or new OD range. Original data is not lost, but is not included in the current calculation.

Defining Kinetic Parameters

Several alternative kinetic parameters can be used for analyzing the raw kinetics data in the program:

- **Rate** - The kinetic rate (slope of the OD vs. time curve) will be calculated by linear regression, using all the data points within the selected OD and time range.

Set time or OD cutoffs on the data, to exclude part of the data from the analysis. This is useful for excluding an initial lag time in delayed reactions or for excluding the end of a reaction where the reaction approaches equilibrium.

For reactions where the OD values decrease with time, the rates will appear as negative values (negative kinetics). To change the values to positive numbers, apply the transformation $-1 * @$ to the data.

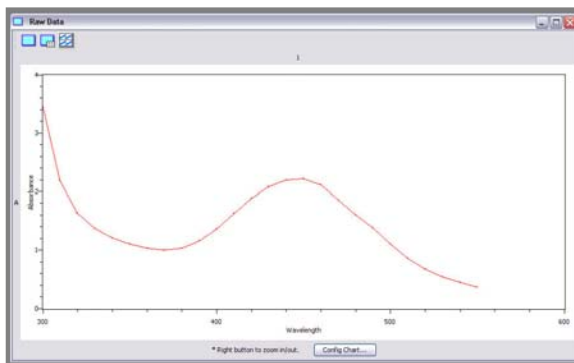
- **Maximum Rate** - The Maximum Rate option is useful, as an alternative to selecting the linear part of the curve using OD and time limits. In the Maximum Rate method, the OD vs. time curve is segmented and a series of linear curve fits are performed in order to determine the maximum rate. The first segment starts at the first data point within the selected time and OD range, the second segment starts at the second data point, etc. The maximum rate is determined by evaluating all of these rate calculations.

The number of data points included in a segment is specified by the user (# of points). If the # of points is equal to or higher than the total number of data points within the selected OD and time range, a single regression will be performed using all valid data points.

For cases where the OD values decrease with time (negative kinetics) the rate values will be negative. To change the sign of negative values, apply the transformation $-1 * @$ to the data.

Running Spectra Assays (xMark only)

1. Select **File > Instrument Setup**, Spectra Reading Mode.
2. Enter desired wavelength range and increment interval.
3. Enter desired starting and ending Well Range values.
4. Click **Start Read**.
5. Click the **Raw Data** icon on the main toolbar.
6. Click the Show Plots icon or select **Show Spectra** under the View menu to view the Spectra plots.



Reading Terasaki Plates (xMark only)

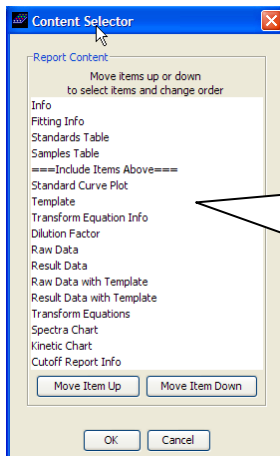
Terasaki plates (60- or 72-well formats) are used for a wide range of applications with sample volumes below 10ul, such as serological determination of HLA antigens and HLA microtests.

The plates are treated to ensure a smooth hydrophilic surface for sera and cells. The maximum well volume is 10ul. Terasaki plates are available from Greiner Bio-One company (www.greinerbioone.com)

To read a Terasaki plate, select File>Plate Size>Terasaki. Choose either 60- or 72-well size.

The selected plate size can be saved in a protocol. Alternatively, the set plate size will remain as a default until it is changed.

Producing Reports



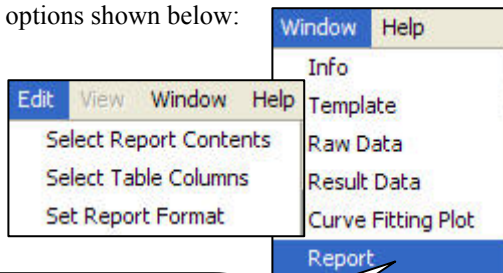
A template must always be present in order to see any Result Data or Report Tables.

Setting Up Reports

To create a Report, click on the **Report** icon in the main toolbar, or select **Report** under the **Window** menu.



The **Edit** and the **View** menus include the options shown below:



A template must always be present, in order to see any Results Data or Data in the Table.

Click the Report icon on the main toolbar.

Click the Select Report Contents icon in the Report toolbar.



Select Report Contents



Select Table Columns



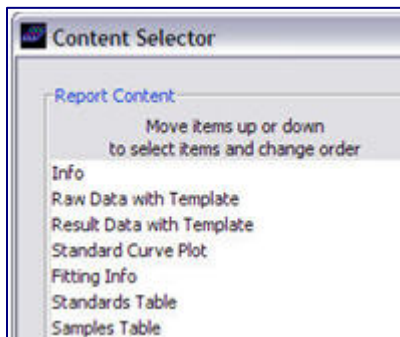
Format Report



Print Preview

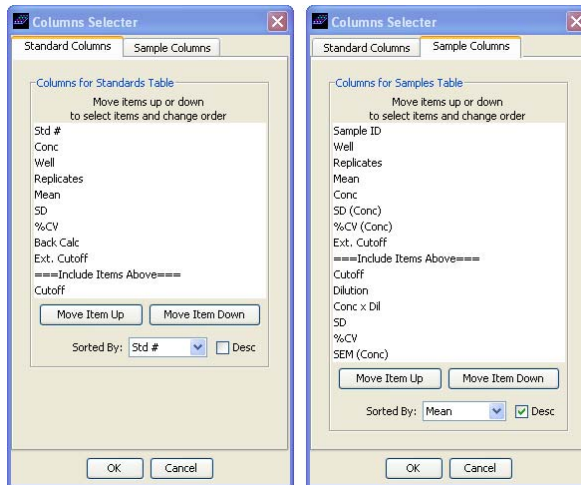
In the Contents Selector dialog, move the contents items above or below the “Include Items Above” line to select items and arrange the order.

1. Click Report icon in Main toolbar.



2. Click **Select Report Contents** icon in the Report toolbar.
3. Click the **Select Table Columns** icon in the Report toolbar.
4. Select Columns to be included for Standard and Sample Table in Report window.

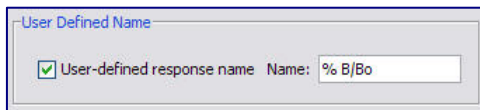
5. Select sorting option, Ascending or Descending Separate tabs for Standard and Samples.



6. Click the **Format Report** icon in the Report toolbar.

In the Report Format Editor window:

1. Check On, User-defined response name.
2. Enter % B/Bo in Name Edit field.



A section of the Report Standard and Samples Tables with user-defined name '%B/Bo' as a column header is shown here.

Standards Report:

Std #	Conc (ng/ml)	Well	Replicates	Mean % B/Bo	SD	%CV
1	7.8	E3	90.766	90.871	0.148	0.163
		F3	90.976			
2	15.6	C3	88.877	89.087	0.297	0.333
		D3	89.297			

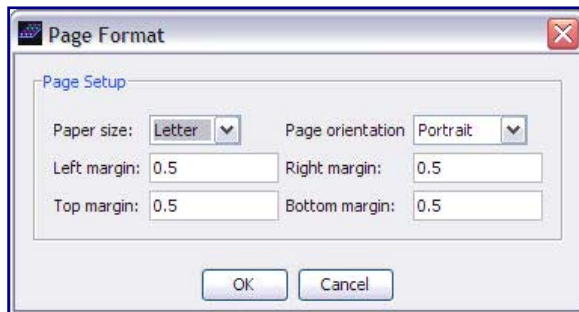
Data Analysis Report:

Sample	ID	Well	Replicates	Mean % B/Bo	Conc (ng/ml)	SD (Conc)	%CV (Conc)
MAX	C1		100.210	100.000	(+)	(*)	(*)
	D1		99.790				
S19	C8		89.717	89.507	16.596	2.822	17.018
	D8		89.297				
S11	C6		88.248	88.458	26.279	2.684	10.214
	D6		88.667				

With Report selected under the Window menu, choose **File > Print** to print the report.

Printing Reports

1. Choose **File > Page Setup**, to set margins and paper size and orientation.



2. With Report selected under Window menu & Report as the active window, Choose **File > Print** to print the report.



Or, click on the **Report icon** in the Main toolbar.

3. After setting up the report, click on **Print Preview** icon to preview the entire report.
4. Click on **Print** icon to print the Report.

The screenshot shows a software window titled "run #23 Info". It contains a tab labeled "Experiment Info". The form fields are as follows:

Title	% MAX - Percent Maximum Binding (% B / Bo)		
Protocol	Percent Max Binding Assay.pro	Date/Time	05/27/2008 12:37:32
Technician	K.Ruzzi	Plate ID	run #23
Conc Unit	ng/ml		
Endpoint Single 450.0nm Mix off Temp --			
Reader Model # iMark			
Reader Serial # TEP02			
Reader Version # 0.02.00 2007/05/15			
Comments			
MAX is maximum binding well & NSB is non-specific binding well purpose: to compare all wells to the max binding well and convert data to percent of max binding well (% B/Bo)			

At the bottom of the window are "OK" and "Cancel" buttons.

Customizing Reports

1. Click on the **Report** icon on the main toolbar.
2. Click on the **Format Report** icon on the report window.

In the **Report Format Editor** window, the mean response column heading in the Report can be customized, for Results that have been transformed by equations. For example, the heading **%B/Bo** entered here will appear in the report column heading as “Mean **%B/Bo**”.

In this window, the **Number Format** for the report is selected. Scientific number format will be used in the report tables and plot if this box is checked.

Decimal Position 3 indicates that 3 number digits will be displayed after the decimal.

The screenshot shows the 'Report Format Editor' dialog box. It has a title bar with a close button. The main area is titled 'Report Format Setup' and contains several sections: 'Header' with dropdowns for Left, Center, and Right; 'Footer' with dropdowns for Left, Center, and Right; 'Header Font', 'Body Font', and 'Footer Font' each with 'Font' and 'Size' dropdowns; 'Number Format' with a checked 'Scientific Number Format' checkbox and a 'Decimal Position' of 3; and 'User Defined Name' with a checked 'User-defined response name' checkbox and a 'Name' field containing 'B/Bo'. At the bottom are 'OK' and 'Cancel' buttons. A red oval highlights the 'Number Format' section.

Appendix

References

- [1] Channing Rodgers, R. P. (1984) "Data Analysis and Quality Control of Assays: A Practical Primer" in Practical Immunoassay W. R. Butt (ed), Marcel Dekker, NY, 253-308.
- [2] Press, William H.; Flannery, Brian P.; Teukolski, Saul A.; Vetterling, William T. "Numerical Recipes in C: The Art of Scientific Computing", Cambridge Univ. Press, NY, 1988.
- [3] Davis, S.E., Munson, P.J., Jaffe, M.L. and Rodbard, D. (1980), J. of Immunoassay, 1, 15. Radioimmunoassay Data Processing.
- [4] Finney, D.J. (1976), Biometrics 32, 721. Radioligand Assay.
- [5] Rodbard, D. and Lewald, J.E. (1970), Computer analysis of radioligand assay and radioimmunoassay data. Acta Endo. 64, Suppl. 147, 79.
- [6] Rodbard, D. and Hutt, D.M. (1974) "Statistical Analysis of Radioimmunoassays Assays" in "Radioimmunoassay and Related Procedures in Medicine", Int. Atomic Energy Agency, Vienna, 165
- [7] Rodbard, D., Lenox, R.H., Wray, H.L., and Ramseth, D. (1976), Statistical Characterization of the Random Errors in the Radioimmunometric Dose Response Variable. Clin. Chem. 22, 350
- [8] Rodbard, D., Munson, P. J., and de Lean, A. (1977) "Improved Curve Fitting, Parallelism Testing, Characterization of Sensitivity and Specificity, Validation, and Optimization for Radioligand Assays" in "Radioimmunoassay and Related Procedures in Medicine", Int. Atomic Energy Agency, Vienna, 469
- [9] Malan, P. G., (1982) "Immunoassay Data-processing and Quality Control Error Analysis in RIA Design", Pergamon Press France, 45-58.

Quantitative ELISA Assay Tutorial

A tutorial showing how to set up an ELISA Assay, including a standard curve and equations to transform the absorbance data into percentage values of the maximum bound well, is given here.

1. To set up the protocol, Choose **File > Instrument Setup**.
2. Select **Endpoint Reading Mode** and the desired wavelength.
3. Open sample data file (Percent Max Binding Assay) with its protocol & template.

C:\Program Files\Bio-Rad\Microplate Manager 6\Sample Data

Defining the Template

Click the **Template** icon in the Main Toolbar.

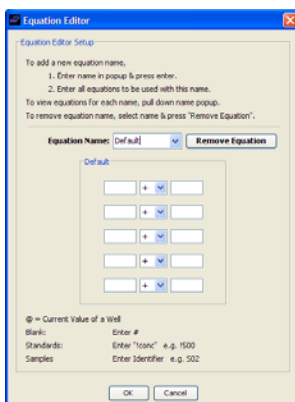
Blanks are defined as #. The average of these # wells will be subtracted from all the wells.

NSB are non-specific binding wells.

MAX is for the maximum binding well.



	1	2	3	4
A		500	31.3	502
B		500	31.3	502
C	MAX	250	15.6	503
D	MAX	250	15.6	503
E	NSB	125	7.8	504
F	NSB	125	7.8	504
G	1000	62.5	501	505
H	1000	62.5	501	505



Equation Editor

Equation Editor Setup

To add a new equation name,
 1. Enter name in popup & press enter.
 2. Enter all equations to be used with this name.
 To view equations for each name, pull down name popup.
 To remove equation name, select name & press "Remove Equation".

Equation Names: [Default] [Remove Equation]

Default:

Blank: Enter #
 Standards: Enter "name" e.g. 1500
 Samples: Enter Identifier e.g. 502

OK Cancel

Setting up the Equations

1. Click Template View icon, select Equation from popup.
2. Click Equation Editor icon. Choose % Max from Equation Name popup to view equations to be applied sequentially to data.

@ - # subtracts mean of blank (#) wells

@ - NSB subtracts mean of non specific binding (NSB) wells

@ / MAX divides by mean of maximum binding (MAX) wells

@ * 100 multiplies by 100 to convert to % bound

Equation Editor Setup

To add a new equation name,
 1. Enter name in popup & press enter.
 2. Enter all equations to be used with this name.
 To view equations for each name, pull down name popup.
 To remove equation name, select name & press "Remove Equation".

Equation Name: % MAX Remove Equation

% MAX

Blank @ - #

@ - NSB

@ / MAX

@ * 100

+

+

@ = Current Value of a Well

Blank: Enter #

Standards: Enter "Iconc" e.g. 1500

Samples: Enter Identifier e.g. S02

OK Cancel

Selecting and Customizing a Curve Fit Plot

1. Click **Curve Fit Plot** icon in Main Toolbar.
2. Choose **Fitting Function** icon in Curve Fit Plot toolbar.
3. Select 4-Parameters from list.
4. Click **Customize Plot** icon in Curve Fit Plot toolbar.

In the Curve Fit Plot Editor window:

X-axis tab: enter: **Concentration**

Y-axis tab: enter: **% B / Bo** in the Axes label edit fields

Entering Experimental info

run #23 Info

Experiment Info

Title: % MAX - Percent Maximum Binding (% B / Bo)

Protocol: Percent Max Binding Assay.pro Date/Time: 05/27/2008 12:37:32

Technician: K.Ruzzi Plate ID: run #23

Conc Unit: ng/ml

Endpoint: Single 450.0nm Mix off Temp --:-

Reader Model # iMark

Reader Serial # TEP02

Reader Version # 0.02.00 2007/05/15

Comments

MAX is maximum binding well & NSB is non-specific binding well
purpose: to compare all wells to the max binding well and
convert data to percent of max binding well (% B/Bo)

OK Cancel

1. Click on the Info icon on the Main Toolbar.
2. Enter name of assay and any experimental info.
3. Enter concentration unit ng/ml in the Info window. This unit will appear in the header of the Report Tables.

Dilution Data Assay Tutorial

1. Defining the Template
2. Setting up the Dilutions
3. Selecting Curve Fit and Customizing Curve Fit Plot
4. Entering Experimental Info
5. Setting up the Report

An example of how to set up an ELISA Assay including a dilutions and a standard curve is given here. The Report is customized to show interpolated concentration, dilution factor, conc X dil factor, and mean concentration for the samples.

Open sample data file (Dilution Data.mpl) with its protocol and template

C:\Program Files\Bio-Rad\Microplate Manager 6\Sample Data

1 Defining the Template

1. Click on the *Template* icon in the Main Toolbar



2. Type 200 in well A1 and press Enter
3. Highlight area well A1-G2. (Click & hold mouse on well A1, drag mouse to well G2, and release.)
4. Click on the *Fill Series* icon in the Template Toolbar



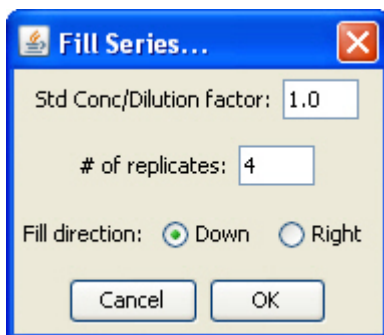
5. Enter as below and click OK



6. Type 0 in well H1 & H2 and press Enter.
7. Type S01 in well A3 and press Enter.
8. Highlight area well A3-H12. (Click & hold mouse on well A3, drag mouse to well H12, and release.)
9. Click on the *Fill Series* icon in the Template Toolbar



10. Enter as below and click OK



11. Below is the finished template

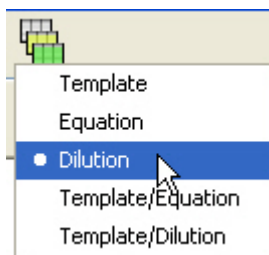
	1	2	3	4	5	6	7	8	9	10	11	12
A	200	200	S01	S03	S05	S07	S09	S11	S13	S15	S17	S19
B	100	100	S01	S03	S05	S07	S09	S11	S13	S15	S17	S19
C	50	50	S01	S03	S05	S07	S09	S11	S13	S15	S17	S19
D	25	25	S01	S03	S05	S07	S09	S11	S13	S15	S17	S19
E	12.5	12.5	S02	S04	S06	S08	S10	S12	S14	S16	S18	S20
F	6.25	6.25	S02	S04	S06	S08	S10	S12	S14	S16	S18	S20
G	3.125	3.125	S02	S04	S06	S08	S10	S12	S14	S16	S18	S20
H	0	0	S02	S04	S06	S08	S10	S12	S14	S16	S18	S20

Blank: Enter #
Standards: Enter concentration as a number
Samples: Enter identifier with alphanumeric as the first character

- Standards are defined 200, 100, 50, 25, 12.5, 6.25, 3.125, & 0.
- S01 to S20 are all of the unknown samples to be analyzed.

2 Setting up the Dilutions

1. Click *Template View* icon in the Template Toolbar
2. Select Dilution from popup



- Highlight area well A3-D3. (Click & hold mouse on well A3, drag mouse to well D3, and release.)
- Click on the *Fill Series* icon in the Template Toolbar
- Enter as below and click OK



- Dilutions are filled in automatically by the Fill Series

Dilution Factor			
	1	2	3
A	1.0	1.0	1.0
B	1.0	1.0	2.0
C	1.0	1.0	4.0
D	1.0	1.0	8.0

- Click on the *Copy* icon in the Template Toolbar



- Click on well E3

9. Click on the *Paste* icon in the Template Toolbar



10. E3 to H3 are filled

11. Highlight area well A3-H12. (Click & hold mouse on well A3, drag mouse to well H12, and release.)

12. Click on the *Fill Right* icon in the Template Toolbar



13. Below is a view of finished dilution setup

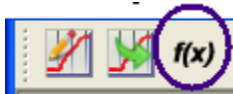
Dilution Factor												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
C	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
D	1.0	1.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
E	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
G	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
H	1.0	1.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Samples: Enter dilution factor(e.g. 1 for undiluted sample, 2 for 1:2 diluted sample) Standard, Blank: Enter 1												

3 Selecting Curve Fit and Customizing Curve Fit Plot

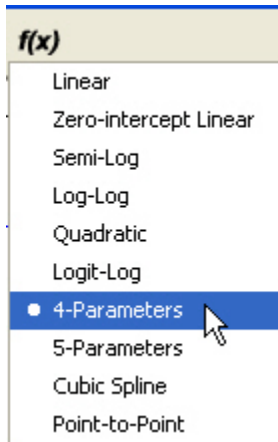
1. Click *Curve Fit Plot* icon on the Main Toolbar



2. Click *Fitting Function* icon in Curve Fit Plot Toolbar



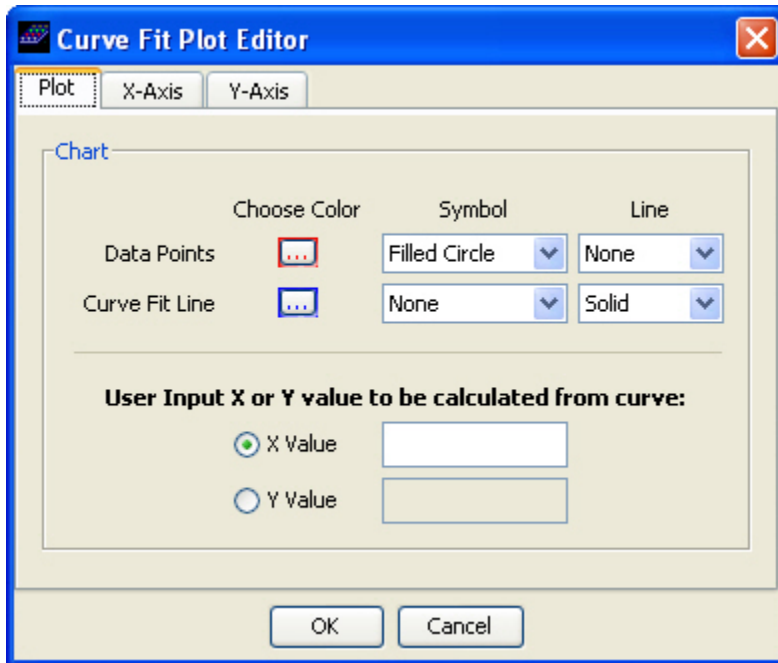
3. Select 4-Parameters from list



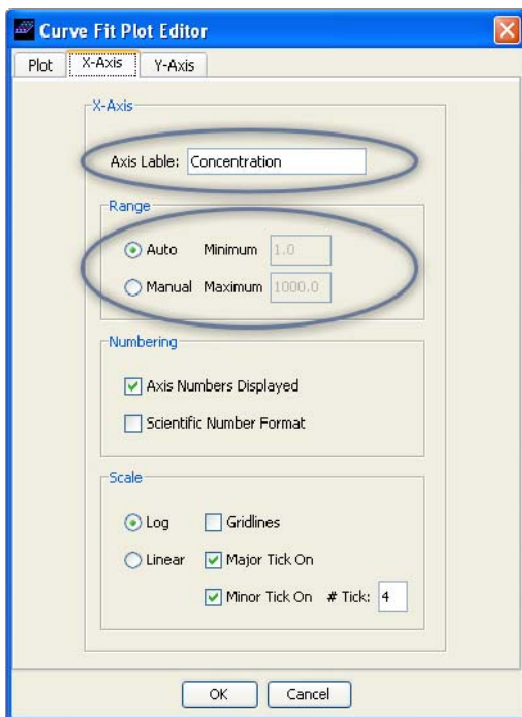
4. Click *Customize Plot* icon in Curve Fit Plot Toolbar



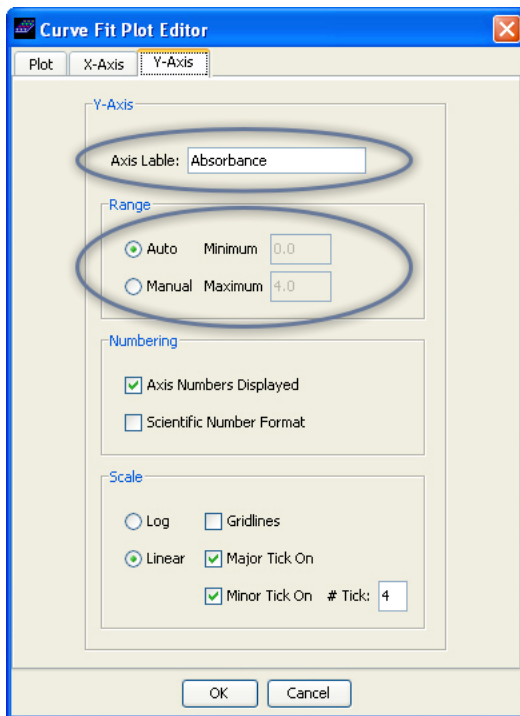
5. In the Curve Fit Plot Editor window under Plot tab, select the Color, Symbol, and Line style for the Data Points and Curve Fit Line.



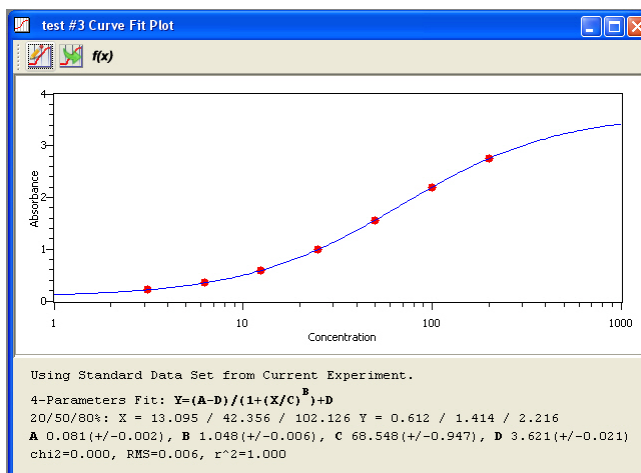
6. In the Curve Fit Plot Editor window under *X-axis* tab, enter Concentration for the axis label. Set the Range to Auto.



7. In the Curve Fit Plot Editor window under *Y-axis* tab, enter Absorbance for the axis label. Set the Range to Auto.

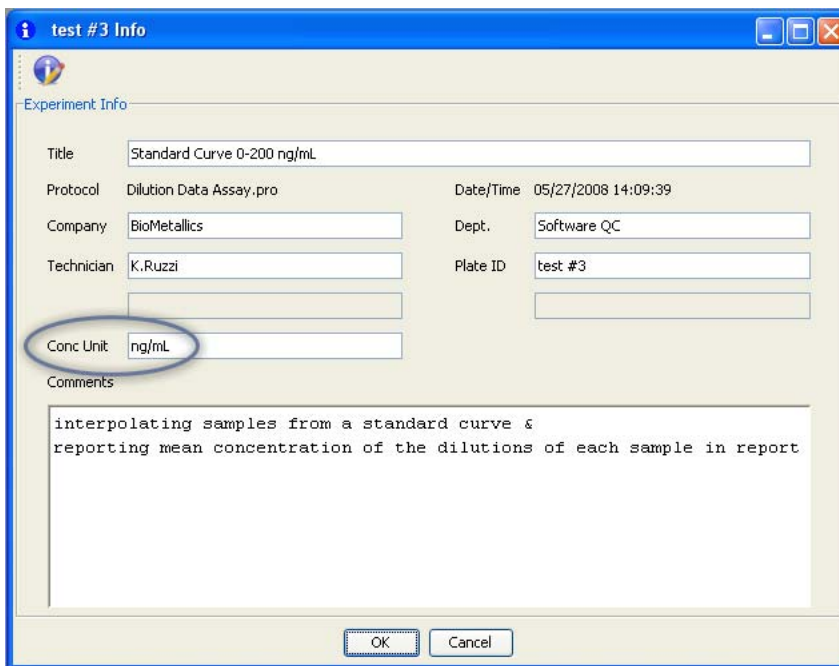


8. The customized curve fit plot shows the curve fit parameters below the curve



4 Entering Experimental Info

1. Click on the *Info* icon on the Main Toolbar



The screenshot shows a software window titled "test #3 Info". It contains several input fields for experimental data. The "Conc Unit" field, which contains the text "ng/mL", is circled in blue. Below the input fields is a large text area for "Comments" containing the text: "interpolating samples from a standard curve & reporting mean concentration of the dilutions of each sample in report". At the bottom of the window are "OK" and "Cancel" buttons.

Title	Standard Curve 0-200 ng/mL		
Protocol	Dilution Data Assay.pro	Date/Time	05/27/2008 14:09:39
Company	BioMetallics	Dept.	Software QC
Technician	K.Ruzzi	Plate ID	test #3
Conc Unit	ng/mL		
Comments	interpolating samples from a standard curve & reporting mean concentration of the dilutions of each sample in report		

2. Enter Title of assay and any experimental Comments.
3. Enter concentration unit (ng/mL) in the Info window. (This unit will appear in the Conc column header of the Report Tables.)

5 Setting up the Report

1. Click *Report* icon in Main Toolbar



2. Click *Select Report Contents* icon in the Report Toolbar



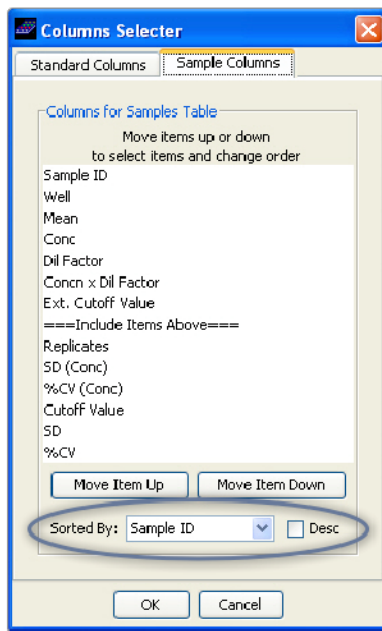
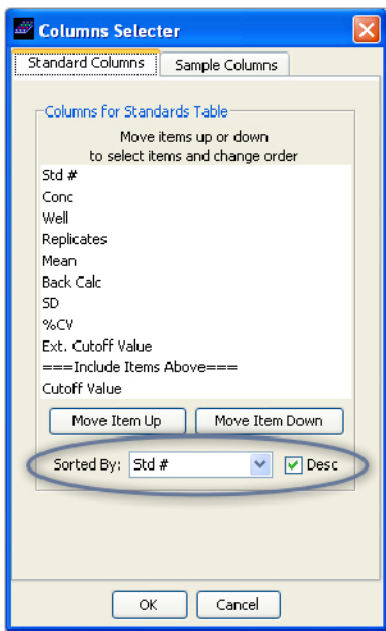
3. Move the items up or down to select which items to include in the Report and change their order. Select Dilution Factor to list the template dilutions in the Report. Select Fitting Info to list the fitting parameters in the Report.



4. Click the *Select Table Columns* icon in the Report Toolbar



5. Select Columns to be included for Standard and Sample Tables in Report window. Move the items up or down to select which items to include in the Report and change their order. Select Sorted By Std # and Descending order for Standards. Select Sorted By Sample ID and Ascending order for Samples.



Appendix

A section of the Data Analysis Report for Standards and Samples Tables with Average Concentration is shown here.

Standards Report:								
Std #	Conc (ng/mL)	Well	Replicates	Mean	Back Calc	SD	%CV	Ext. Cutoff Value
8	200	A1	2.750	2.750	199.546	0.000	0.000	POS
		A2	2.750		199.546			
7	100	B1	2.220	2.200	102.645	0.028	1.286	7
		B2	2.180		98.141			
6	50	C1	1.580	1.560	51.060	0.028	1.813	5
		C2	1.540		48.846			

Data Analysis Report:							
Sample ID	Well	Mean	Conc (ng/mL)	Dil Factor	Concn x Dil Factor	Ext. Cutoff Value	
S01	A3	2.130	92.838	1.0	92.838	7	
	B3	1.510	47.238	2.0	94.476	5	
	C3	0.937	23.036	4.0	92.142	3	
	D3	0.561	11.703	8.0	93.625	1	
					Avg: 93.270		
S02	E3	0.145	1.512	1.0	1.512	0	
	F3	0.110	0.701	2.0	1.403	0	
	G3	0.090	0.225	4.0	0.902	0	
	H3	0.095	0.347	8.0	2.772	0	
					Avg: 1.647		
S03	A4	2.430	131.052	1.0	131.052	8	
	B4	1.850	68.473	2.0	136.945	6	
	C4	1.250	34.909	4.0	139.635	4	
	D4	0.761	17.405	8.0	139.242	2	
					Avg: 136.718		

6. Click the *Print Preview* icon in the Report



Print preview:

Navigation icons: back, forward, search, print, etc.

Dilution Data.mpl **Dilution Data Assay.pro** 05/19/2008 10:45 AM

Title: Standard Curve 0-2000 ng/mL
Protocol: Dilution Data Assay.pro
Date/Time: 05/27/2008 14:09:39
Company: BioAssistatics
Dept: Software QC
Technician: K. Russell
Plate ID: test #3
Unit: ng/mL
Comments: interpolating samples from a standard curve & reporting mean concentration of the dilutions of each sample in report

Raw Data:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.750	2.750	2.130	2.430	2.490	1.780	0.539	1.880	0.492	0.149	0.308	0.201
B	2.220	2.180	1.510	1.850	1.870	1.170	0.305	1.210	0.277	0.100	0.183	0.120
C	1.580	1.540	0.937	1.250	1.240	0.672	0.189	0.707	0.182	0.097	0.132	0.106
D	1.010	0.979	0.561	0.761	0.757	0.401	0.137	0.425	0.145	0.098	0.112	0.093
E	0.591	0.572	0.145	3.060	3.080	1.840	3.110	3.080	1.620	2.750	0.955	2.830
F	0.364	0.350	0.110	3.100	2.940	1.270	3.150	2.900	1.030	2.230	0.569	2.430
G	0.218	0.215	0.090	3.110	2.540	0.772	3.160	2.560	0.607	1.610	0.334	1.780
H	0.075	0.080	0.095	3.180	2.000	0.459	3.170	2.020	0.375	1.020	0.202	1.180

Dilution Factors:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
C	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
D	1.0	1.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
E	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
G	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
H	1.0	1.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

QPTX 6 build 2.4.8 test #3 Page 1

7. Click the *Print* icon in the Print Preview Toolbar to Print the Report



Percent Control Assay Tutorial

- 1 Defining the Template
- 2 Setting up the Equations
- 3 Setting up the Screening Report
- 4 Entering Experimental Info
- 5 Setting up the Report

An example of how to set up an ELISA Assay that uses a control and equations to transform the absorbance data into percentage values of the control well (CNTRL) is given here. This assay has no standards and no standard curve.

Open sample data file (Percent Control.mpl) with its protocol and template.

C:\Program Files\Bio-Rad\Microplate Manager 6\Sample Data

1 Defining the Template

1. Click on the *Template* icon in the Main Toolbar



Template			
	1	2	3
A	#	#	#
B	BLK	BLK	BLK
C	CNTRL	CNTRL	CNTRL
D	U01	U01	U01
E	U02	U02	U02

- Blanks are defined as #. The average of these # wells will be subtracted from all the wells
- BLK are a second reagent blank. The average of these BLK wells will be subtracted from all the wells.

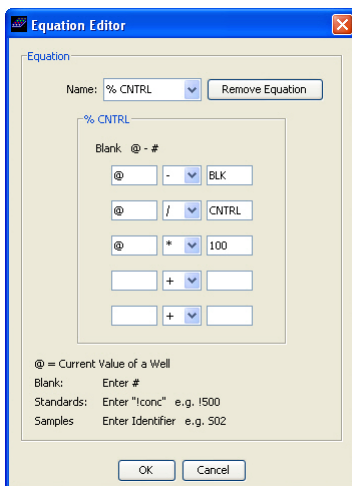
- CNTRL is for the control wells.
- U01 to U29 are all of the unknown samples to be analyzed.

2 Setting up the Equations

1. Click *Equation Editor* icon in the Template Toolbar



2. Choose % CNTRL from Name popup to view equations to be applied sequentially to data



@ - # — Subtracts mean of blank (#) wells

@ - BLK — Subtracts mean of second reagent blank (BLK) wells

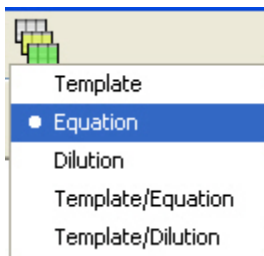
@ / CNTRL — Divides by mean of control (CNTRL) wells

@ * 100 — Multiplies by 100 to convert to % CNTRL (percent control)

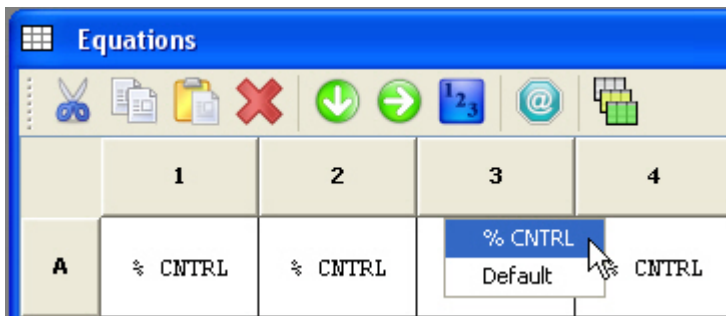
3. Click *Template View* icon in the Template Toolbar.



4. Select Equation from popup.

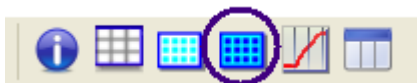


5. To apply this equation to the data, right click the mouse and select the % CNTRL equation from the popup.

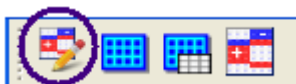


3 Setting up the Screening Report

1. Click on the *Result Data* icon in the Main Toolbar



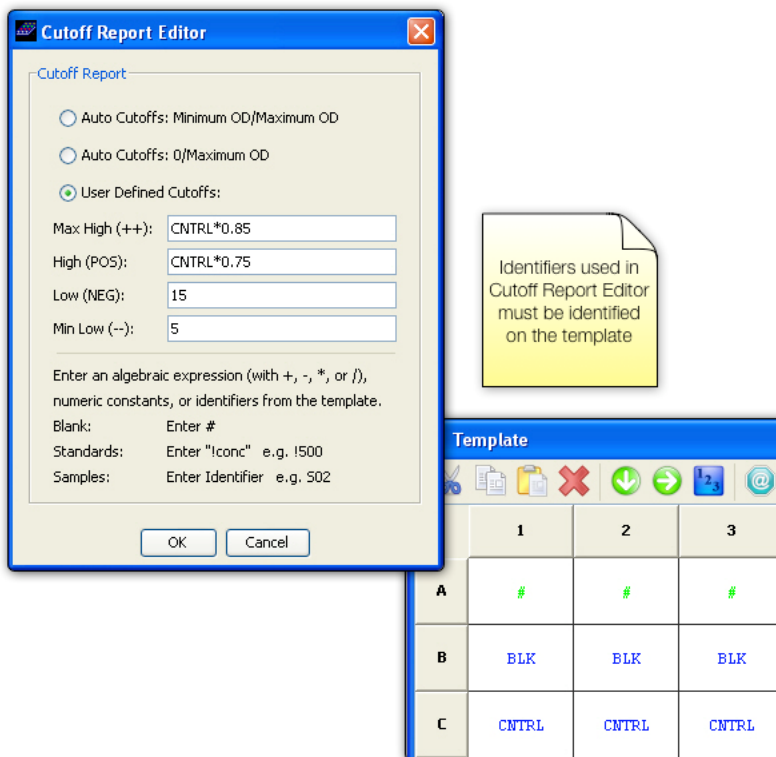
2. Click the *Cutoffs Report Editor* icon in the Result Toolbar



- Define the desired cutoffs in the Cutoffs Report Editor window. Well identifiers used in the Cutoff Report Editor must be first defined on the template.

User defined cutoffs are used in this data file.

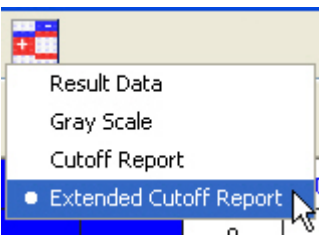
- The Max High (++) is defined as $\geq 85\%$ of the control (CNTRL) for all samples.
- The High (POS) is defined as $\geq 75\%$ of the control (CNTRL) for all samples.
- The Low (NEG) is defined as $\leq 15\%$ result value for all samples.
- The Max Low (--) is defined as $\leq 5\%$ result value for all samples.



4. Click the Report View icon in the Result Toolbar



5. Select Extended Cutoff Report from popup

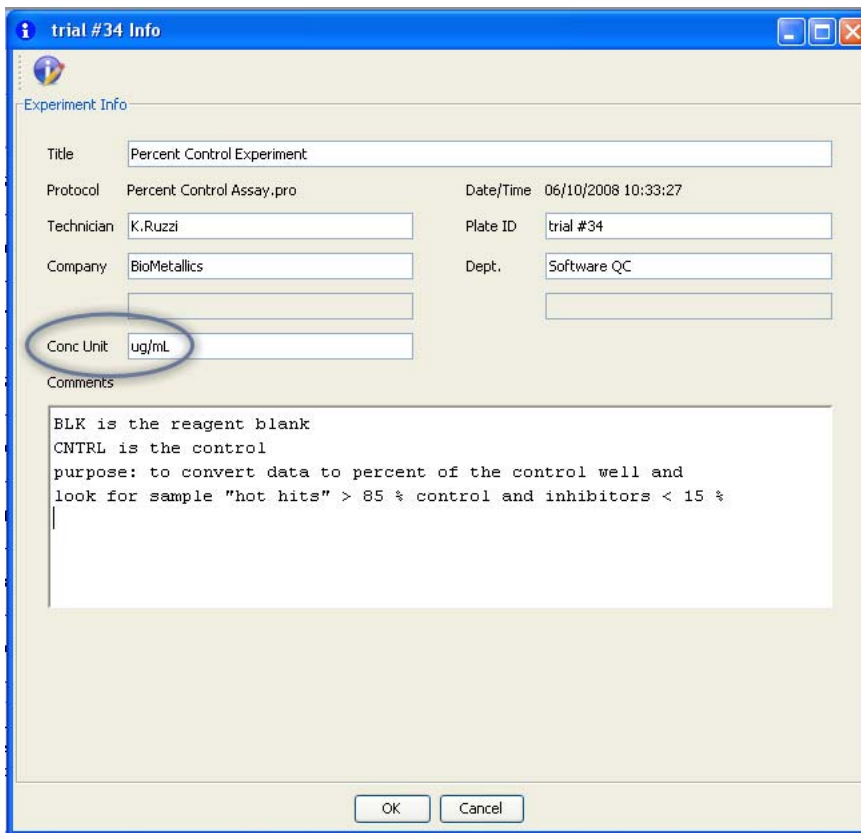


6. The resulting Extended Cutoff Report is shown here with the high values shown in red, the low values in blue, and the intermediate values in white:

Extended Cutoff Report												
	1	2	3	4	5	6	7	8	9	10	11	12
A	#	#	#	U06	U06	U06	U14	U14	U14	U22	U22	U22
	--	--	--	9	9	9	8	8	8	3	3	3
B	BLK	BLK	BLK	U07	U07	U07	U15	U15	U15	U23	U23	U23
	--	--	--	POS	POS	POS	4	4	4	NEG	NEG	NEG
C	CNTRL	CNTRL	CNTRL	U08	U08	U08	U16	U16	U16	U24	U24	U24
	++	++	++	6	6	6	NEG	NEG	NEG	++	++	++
D	U01	U01	U01	U09	U09	U09	U17	U17	U17	U25	U25	U25
	4	4	4	0	0	0	++	++	++	6	6	6
*To exclude an outlier well from analysis, double click on well. To re-include we												
*A Template must be defined in order to see Results View												
Blank=0.002 Max High Cutoff (++) : 85.000 High Cutoff (POS) : 75.000 Low Cut												
0	NEG	0	1	2	3	4						
<=5.000	<=15.000	<21.000	<27.000	<33.000	<39.000	<45.000						
5	6	7	8	9	POS	++						
<51.000	<57.000	<63.000	<69.000	<75.000	>=75.000	>=85.000						

4 Entering Experimental info

1. Click on the *Info* icon on the Main Toolbar



trial #34 Info

Experiment Info

Title	Percent Control Experiment		
Protocol	Percent Control Assay.pro	Date/Time	06/10/2008 10:33:27
Technician	K.Ruzzi	Plate ID	trial #34
Company	BioMetallics	Dept.	Software QC
Conc Unit	ug/mL		

Comments

BLK is the reagent blank
CNTRL is the control
purpose: to convert data to percent of the control well and
look for sample "hot hits" > 85 % control and inhibitors < 15 %

OK Cancel

2. Enter Title of assay and any experimental Comments.
3. Enter concentration unit (ug/mL) in the Info window. (This unit will appear in the conc column header of the Report Tables.)

5 Setting up Report

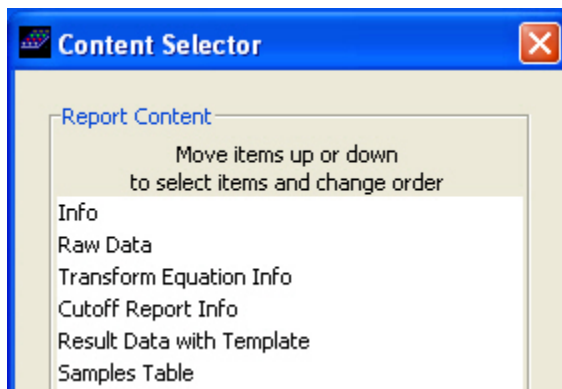
1. Click *Report* icon in Main Toolbar



2. Click *Select Report Contents* icon in the Report Toolbar.



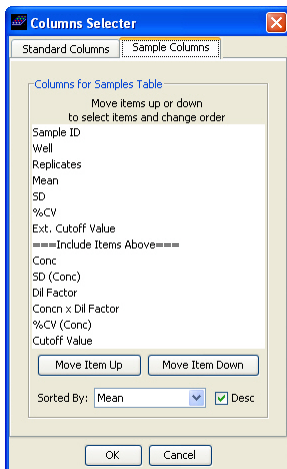
3. Move the items up or down to select which items to include in the Report and change their order.



4. Click the Select Table Columns icon in the Report Toolbar.



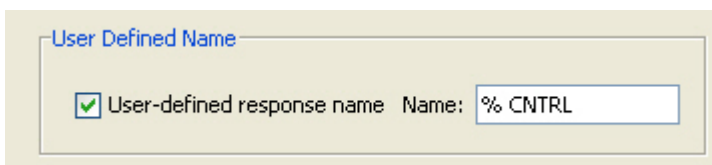
5. Select Columns to be included for Sample Table in Report window. Move the items up or down to select which items to include in the Report and change their order. Select Sorted By Mean and Descending order.



6. Click the *Format Report* icon in the Report Toolbar.



7. For User-defined response name, Check *ON* and enter % CNTRL in Name Edit field

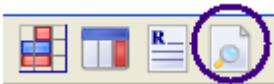


A section of the Data Analysis Report for Samples with user-defined name % CNTRL as a column header is shown here.

Data Analysis Report:

Sample ID	Well	Replicates	Mean % CNTRL	SD	%CV	Ext. Cutoff Value
CNTRL	C1	99.629	100.000	0.371	0.371	++
	C2	100.371				
	C3	100.000				
U19	F7	97.032	97.093	0.283	0.292	++
	F8	97.403				
	F9	96.846				
U05	H1	93.692	94.310	0.652	0.691	++
	H2	94.991				
	H3	94.249				

8. Click the *Print Preview* icon in the Report Toolbar



Print preview:

Percent Control.mpl Percent Control Assay.pro 09/16/2008 15:15:PM

Title: Percent Control Experiment
Protocol: Percent Control Assay.pro
Date/Time: 09/16/2008 10:33:27
Technician: K.Rajal
Plate ID: Trial #54
Company: BioMaddux
Dept: Software QC
Unit: ug/mL
Comments: BLK is the reagent blank
CNTRL is the control
Duplicate to correct data to percent of the control well and
look for sample "threshold" = 85% control and inhibitors = 15%

Raw Data:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.002	0.002	0.398	0.402	0.399	0.368	0.389	0.370	0.201	0.302	0.201
B	0.018	0.018	0.018	0.425	0.426	0.424	0.283	0.247	0.245	0.045	0.046	0.047
C	0.035	0.035	0.037	0.301	0.304	0.303	0.262	0.079	0.091	0.494	0.488	0.495
D	0.236	0.249	0.251	0.120	0.121	0.119	0.050	0.008	0.007	0.315	0.314	0.316
E	0.198	0.190	0.164	0.486	0.488	0.459	0.446	0.443	0.445	0.221	0.224	0.222
F	0.342	0.340	0.344	0.287	0.289	0.290	0.541	0.543	0.540	0.357	0.358	0.356
G	0.476	0.482	0.477	0.213	0.214	0.212	0.387	0.384	0.386	0.287	0.289	0.288
H	0.523	0.530	0.526	0.186	0.188	0.185	0.261	0.263	0.262	0.164	0.165	0.163

Single Equation Transformation applied:
% CNTRL BLK=K (BLK/0.002)
1. @ BLK (BLK/0.016)
2. @CNTRL (CNTRL/0.009)
3. @F100

User Defined Cutoffs Applied:
Blank=0.002
Max High(+) =>=85.000 CNTRL/0.85 Mean CNTRL=100.000
High Cutoff(POS) =>=75.000 CNTRL/0.75 Mean CNTRL=100.000
Low Cutoff(NEG) =<15.000 15
Max Low(-) =<5.000 5

MMV 6/26/08 2:14 PM Trial #54 Page 1

9. Click the *Print* icon in the Print Preview Toolbar to Print the Report



Percent Max Binding Assay Tutorial

- 1 Defining the Template
- 2 Setting up the Equations
- 3 Selecting Curve Fit and Customizing Curve Fit Plot
- 4 Entering Experimental Info
- 5 Setting up the Report

An example of how to set up an ELISA Assay including a standard curve and equations to transform the absorbance data into percentage values of the maximum bound well is given here.

Open sample data file (Percent Max Binding.mpl) with its protocol and template
C:\Program Files\Bio-Rad\Microplate Manager 6\Sample Data

1 Defining the Template

- 1. Click on the *Template* icon in the Main Toolbar



run #23 Template								
	1	2	3	4	5	6	7	8
A	#	500	31.3	S02	S06	S10	S14	S18
B	#	500	31.3	S02	S06	S10	S14	S18
C	MAX	250	15.6	S03	S07	S11	S15	S19
D	MAX	250	15.6	S03	S07	S11	S15	S19
E	NSB	125	7.8	S04	S08	S12	S16	S20
F	NSB	125	7.8	S04	S08	S12	S16	S20
G	1000	62.5	S01	S05	S09	S13	S17	S21
H	1000	62.5	S01	S05	S09	S13	S17	S21

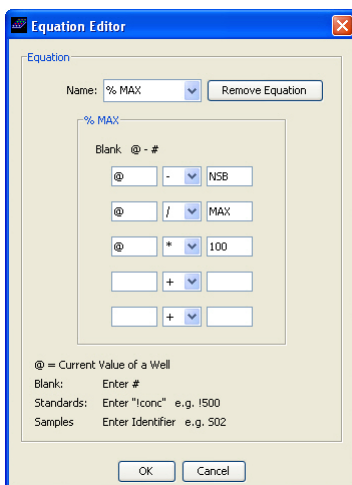
- Blanks are defined as #. The average of these # wells will be subtracted from all the wells.
- NSB are non-specific binding wells.
- MAX is for the maximum binding well (i.e. the Bo well).
- S01 to S21 are all of the unknown samples to be analyzed.

2 Setting up the Equations

1. Click *Equation Editor* icon in the Template Toolbar.



2. Choose % MAX from Name popup to view equations to be applied sequentially to data

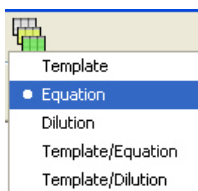


- @ - # — Subtracts mean of blank (#) wells
- @ - NSB — Subtracts mean of non specific binding (NSB) wells
- @ / MAX — Divides by mean of maximum binding (MAX) wells
- @ * 100 — Multiplies by 100 to convert to % bound

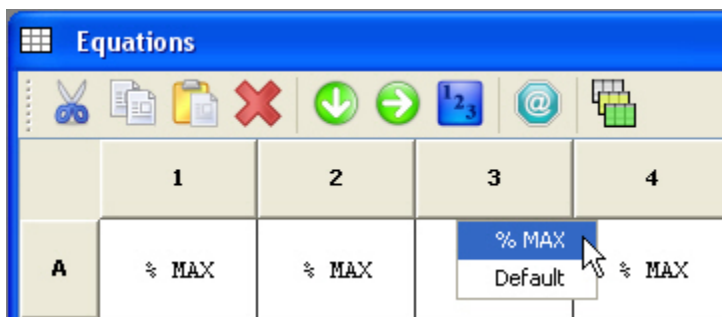
- Click *Template View* icon in the Template Toolbar



- Select *Equation* from popup



- To apply this equation to the data, right click the mouse and select the % MAX equation from the popup.



3 Selecting Curve Fit and Customizing Curve Fit Plot

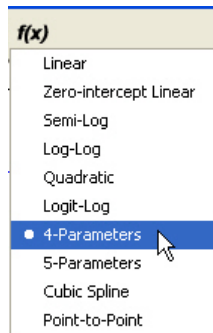
- Click *Curve Fit Plot* icon on the Main Toolbar



- Click *Fitting Function* icon in Curve Fit Plot Toolbar



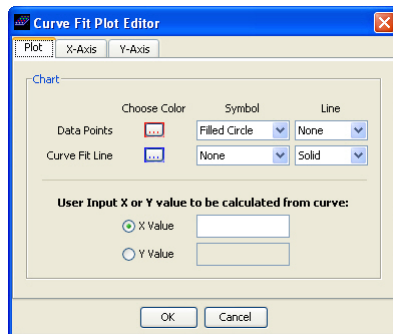
3. Select 4-Parameters from list



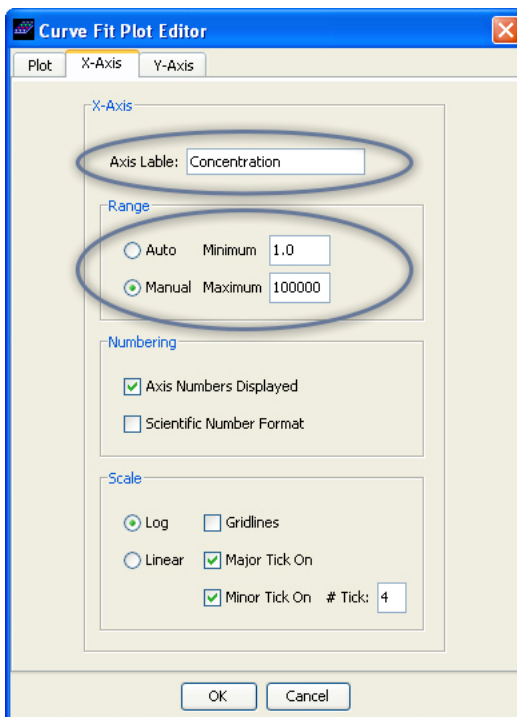
4. Click *Customize Plot* icon in Curve Fit Plot Toolbar



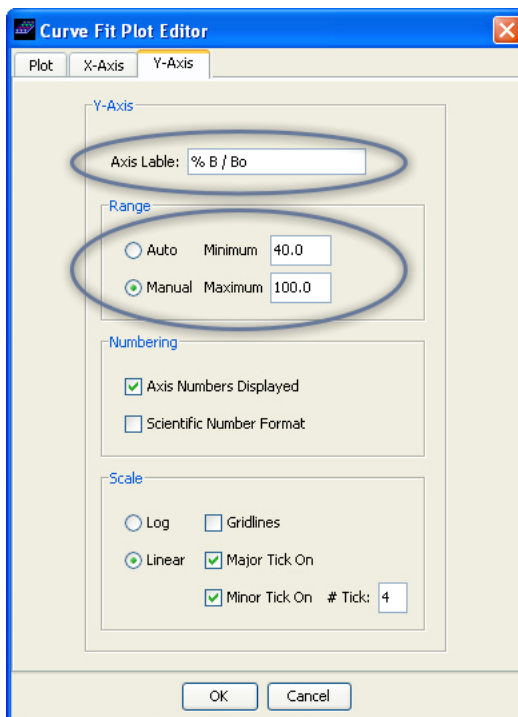
5. In the Curve Fit Plot Editor window under *Plot* tab, select the Color, Symbol, and Line style for the Data Points and Curve Fit Line.



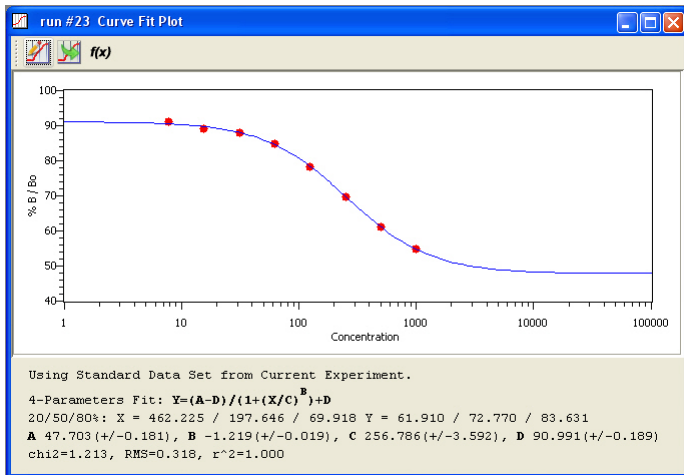
6. In the Curve Fit Plot Editor window under *X-axis* tab, enter Concentration for the axis label. Set the Range to Manual with the minimum 1.0 and the maximum 100000.



7. In the Curve Fit Plot Editor window under *Y-axis* tab, enter % B / Bo for the axis label. Set the range to Manual with the minimum 40.0 and the maximum 100.00.

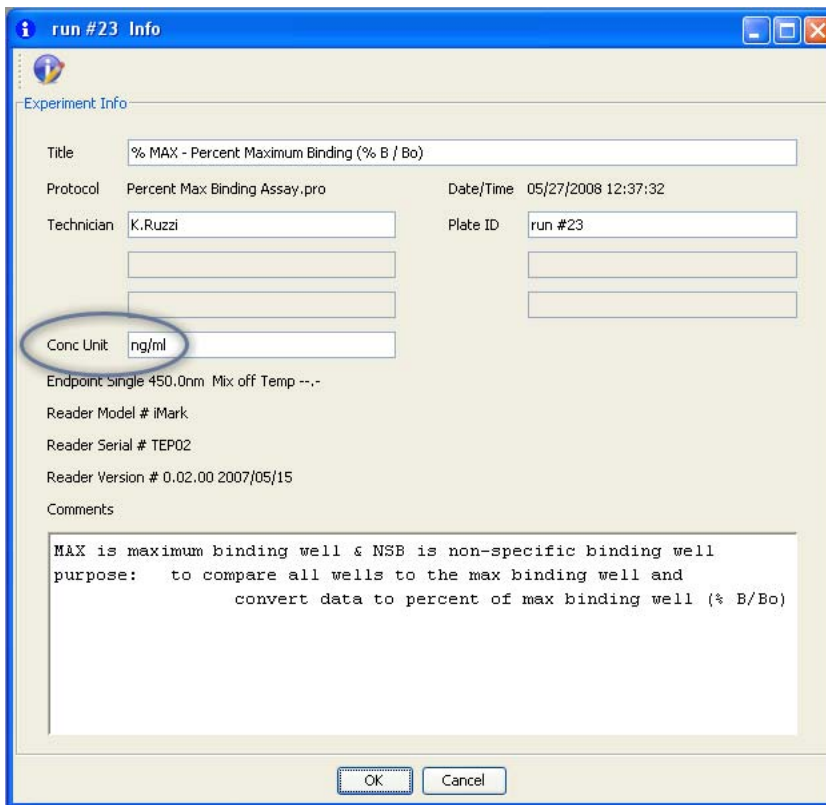


8. The customized curve fit plot shows the curve fit parameters below the curve.



4 Entering Experimental Info

1. Click on the *Info* icon on the Main Toolbar



The screenshot shows a software window titled "run #23 Info". It contains the following fields and text:

- Title: % MAX - Percent Maximum Binding (% B / Bo)
- Protocol: Percent Max Binding Assay.pro
- Date/Time: 05/27/2008 12:37:32
- Technician: K.Ruzzi
- Plate ID: run #23
- Conc Unit: ng/ml (highlighted with a red circle)
- Endpoint Single: 450.0nm
- Mix off Temp: --.-
- Reader Model #: iMark
- Reader Serial #: TEP02
- Reader Version #: 0.02.00 2007/05/15
- Comments: MAX is maximum binding well & NSB is non-specific binding well purpose: to compare all wells to the max binding well and convert data to percent of max binding well (% B/Bo)

At the bottom are "OK" and "Cancel" buttons.

2. Enter Title of assay and any experimental Comments.
3. Enter concentration unit (ng/ml) in the Info window. (This unit will appear in the conc column header of the Report Tables.)

5 Setting up the Report

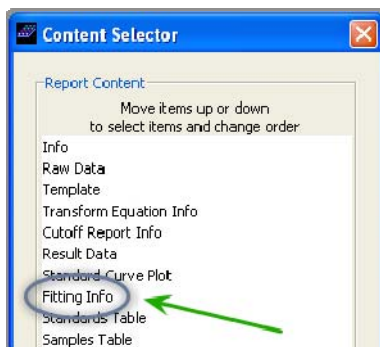
1. Click *Report* icon in Main Toolbar



2. Click *Select Report Contents* icon in the Report Toolbar.



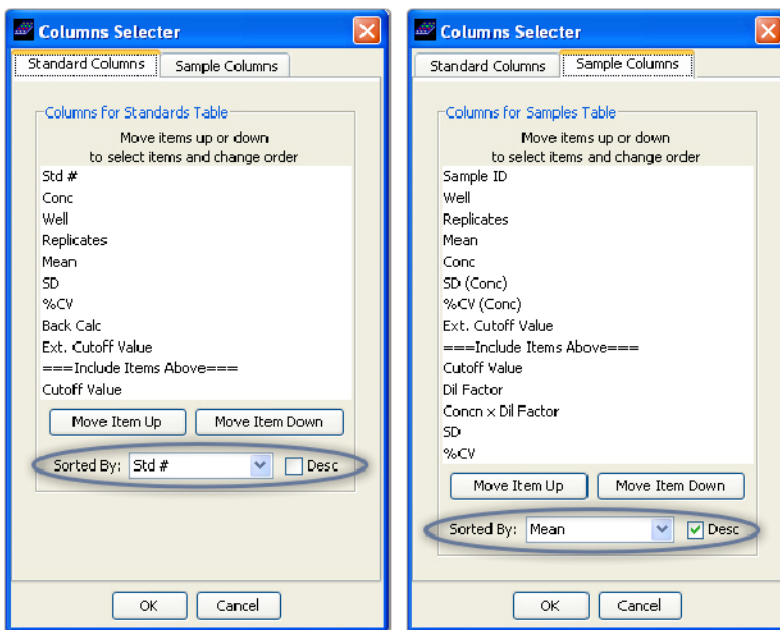
3. Move the items up or down to select which items to include in the Report and change their order. Select Fitting Info to list the fitting parameters in the Report.



4. Click the *Select Table Columns* icon in the Report Toolbar



5. Select Columns to be included for Standard and Sample Tables in Report window. Move the items up or down to select which items to include in the Report and change their order. Select Sorted By Std # and Ascending order for Standards. Select Sorted By Mean and Descending order for Samples.



6. Click the *Format Report* icon in the Report Toolbar



7. For User-defined response name, Check *ON* and enter % B/B₀ in Name Edit field



Appendix

A section of the Data Analysis Report for Standards and Samples Tables with user-defined name % B/Bo as a column header is shown here.

Standards Report:						
Std #	Conc (ng/ml)	Well	Replicates	Mean % B/Bo	SD	%CV
1	7.8	E3	90.766	90.871	0.148	0.163
		F3	90.976			
2	15.6	C3	88.877	89.087	0.297	0.333
		D3	89.297			
Data Analysis Report:						
Sample ID	Well	Replicates	Mean % B/Bo	Conc (ng/ml)	SD (Conc)	%CV (Conc)
MAX	C1	100.210	100.000	{ + }	{ * }	{ * }
	D1	99.790				
S10	C8	89.717	89.507	16.506	2.822	17.018
	D8	89.297				
S11	C6	88.248	88.458	26.279	2.684	10.214
	D6	88.667				

8. Click the *Print Preview* icon in the Report Toolbar



Print preview:

Percent Max Binding.mpl Percent Max Binding Assay.pro 06/17/2008 14:09 PM

Title %MAX - Percent Maximum Binding (% B/B₀)

Protocol Percent Max Binding Assay.pro

Date/Time 06/27/2008 12:37:32

Technician K.Ruzal

Plate ID run#23

Unit ng/ml

Reader Setup Endpoint Single 450.0nm Mix off Temp----

Reader Model # MMark

Reader Serial # TER02

Reader Version # 0.02.00.2007/05/15

Comments MAX is maximum binding well & NSB is non-specific binding well
purpose: to compare all wells to the max binding well and
convert data to percent of max binding well (% B/B₀)

Raw Data(Wavelength:450.0):

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.243	0.540	0.666	0.596	0.652	0.601	0.583	0.536				
B	0.246	0.538	0.667	0.601	0.657	0.604	0.587	0.537				
C	0.726	0.579	0.672	0.574	0.541	0.669	0.565	0.676				
D	0.724	0.582	0.674	0.578	0.543	0.671	0.551	0.674				
E	0.248	0.619	0.681	0.624	0.635	0.643	0.491	0.612				
F	0.249	0.624	0.682	0.625	0.639	0.648	0.497	0.615				
G	0.510	0.651	0.523	0.649	0.563	0.500	0.512	0.686				
H	0.506	0.653	0.526	0.641	0.567	0.506	0.518	0.688				

Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A	#	500	313	502	506	S10	S14	S18				
B	#	500	313	502	506	S10	S14	S18				
C	MAX	250	15.6	503	507	S11	S15	S19				
D	MAX	250	15.6	503	507	S11	S15	S19				
E	NSB	125	7.8	504	508	S12	S16	S20				
F	NSB	125	7.8	504	508	S12	S16	S20				
G	1000	62.5	501	505	509	S13	S17	S21				
H	1000	62.5	501	505	509	S13	S17	S21				

MMark Build 2.1.8 run#23 Page 1

9. Click the *Print* icon in the Print Preview Toolbar to Print the Report





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