

Microplate Manager® 5.0 PC User Guide

BIO-RAD

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1. Introduction

This manual assumes that you are familiar with your computer and standard Windows commands and functions, such as opening, closing, and saving files, and moving and clicking your mouse.

Some of the features and functions in Microplate Manager may be slightly different depending on whether you are using the Ultramark, Model 550, or Benchmark microplate reader. These differences are noted in the text.

1.1 Overview of Microplate Manager

Microplate Manager is designed to collect, analyze, and output data from Bio-Rad's Ultramark, Model 550, and Benchmark microplate readers. It runs as a Windows 95, Windows 98, or Windows NT 4.0 application on a computer that is directly connected to the reader. It features a standard Windows[®] interface, with pulldown menus, toolbars, and keyboard shortcuts.

Using Microplate Manager, you first select the type of reading you want to perform and specify the layout of your microplate. Then you capture your data using the microplate reader. Finally, you display your reports and print and/or export your data.

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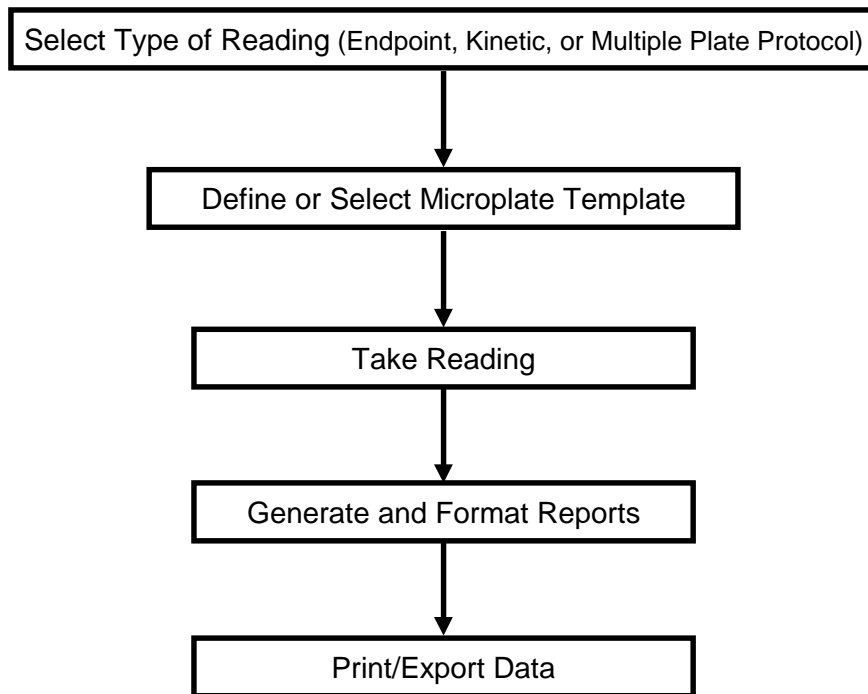


Figure 1. Workflow of Microplate Manager

Microplate Manager can perform three general types of microplate readings. These are called “protocols.”

- **Endpoint protocols** are used to acquire a single absorbance reading from each well.
- **Kinetic protocols** are used to acquire a series of absorbance readings from each well over a user-defined interval. These protocols are used to calculate reaction velocities.
- **Multiple plate protocols** are used with a stack loader to collect endpoint data from a series of plates. Multiple plate protocols save each endpoint data set in a separate file.

First you select the type of protocol you want to create. Then you specify the settings for your particular protocol and define or select a template for your microplate.

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Finally you take the reading. If you want to use the particular protocol you've created again, you can save it as a file.

Once absorbances have been read, you can save them as a data file and display them in a selection of reports. Your data can be viewed, printed, or exported to other applications.

1.2 Types of Files

There are two types of files used by Microplate Manager. Protocol files contain the settings for reading a microplate. Data files contain the data from the reading and any formatting associated with that data.

Protocol Files

A protocol file contains the parameters of a reading. It includes such information as mixing times and measurement wavelengths. Each of the three protocol types (**endpoint**, **kinetic**, and **multiple plate**) is slightly different, to accommodate the different parameters used in each type. File names for protocols have the extensions .epr (endpoint reading), .mpr (multiple plate reading), or .kpr (kinetic plate reading).

The protocol file also includes a **plate template**. The template specified in the protocol file is copied into the data file at the completion of a plate reading. If there is no template specified in the protocol file, then the default template is copied into the data file. Defining a template in the protocol file before a reading allows you to create data files that contain all the information necessary to evaluate the absorbance data and automatically generate the desired reports.

(You can edit or replace the template in the data file after a reading. However, changes made to the protocol file template after a reading are not updated in the data file.)

You may specify labels for reports; these are stored in the protocol file.

A list of reports to be printed and/or displayed at the end of a run are saved in the protocol file.

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Data Files

The data file contains the raw data from the microplate reader. It has the extension .mpm.

For an **endpoint reading**, this data consists of the individual absorbance readings from the wells on the microplate. For a **kinetic reading**, this data is the time course series of the individual absorbance readings. A **multiple-plate reading** creates a new data file for each plate; each file contains endpoint absorbance measurements.

The data file also contains a **plate template**, which contains all the information necessary to analyze the absorbance data, including a description of the assay, well types, concentrations, dilutions, and the regression method used for computing unknowns from standards. When the data file is first created after the absorbance data is collected, the template associated with the protocol file is copied into the data file. You can later edit the template or completely replace it with a template from another data file.

Finally, the data file includes some information on how the plate was read (e.g., measurement wavelength, date/time of reading).

1.3 New Features in Version 5.0

This version of Microplate Manager features an enhanced user interface with image display and grid overlay features. Some of the new features of the software include:

- Flexible plate definition to handle images reads of plates from 1 to 1,536 wells, including drag-and-drop well layout and multilevel undo when formatting images.
- Improvements in template formatting, including automatic scrolling when formatting large plates, noncontiguous well selection for changing assay and replicate set association, better handling of renumbering standards and unknowns on densely formatted plates, and autofilling for rapidly assigning standard and unknown replicate sets.
- Automated setup. The software automatically identifies the microplate reader and establishes the connection to the reader.

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- Major improvements in associating protocols and templates.
- More flexibility in tagging outliers in replicate sets.
- Up to 12 different assays on one plate, each with its own blanks, controls, and standards.
- More status bar and cursor feedback in templates and reports, including well information and cursor positions.
- External standards and standard curve checking. A quantitative method for comparing two standard curves is now available. A statistical analysis of the comparison is provided.
- Improved display of graphics reports such as standard curve and kinetic zoom.
- Incubator temperature adjustments as small as 0.2° C.
- Improved handling of multipage printouts for larger plates.
- Permanent tagging of user-edited or imported data on screen and in printouts.
- Fully integrated simulated scanner for demos and practice runs, capable of producing simulated data.
- Complete reading protocols. Reading parameters, plate template, analysis options, and print settings may be saved for repeated applications.
- Auto-scaling of kinetic data in Kinetic Zoom Plot windows.

2. Installation and Setup

2.1 Instrument Specifications

2.1.1 Host Computer and Operating System

Microplate Manager will run under Windows 95, Windows 98, or Windows NT 4.0.

The host machine must be based on a Pentium Intel class CPU or higher. It should have a hard disk of at least 200 MB, and a minimum of 16 MB RAM. It must have a monitor and video card capable of displaying 256 colors at 1024 by 768 resolution. There must be a SCSI-2 port available for connecting to the reader. A mouse is required to use the software. A parallel port is required in order to print reports directly from the PC.

The Ultramark communicates with the host computer via a SCSI-2 connection. You can use the SCSI-2 cable and Adaptec card provided by Bio-Rad (cat. no. 170-9521) or purchase them from any computer store.

The Model 550 and Benchmark microplate readers communicate with the host computer via a standard serial port and cable.

2.1.2 Microplate Readers

Microplate Manager 5.0 supports Bio-Rad's Ultramark, Model 550, and Benchmark microplate readers.

2.2 Connecting the Microplate Readers to the Host Computer

A SCSI-2 cable and card (cat. no. 170-9521) are required to connect the Ultramark to the host PC. Make sure all devices are turned off before making or changing cable connections. See the hardware manual for details.

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A serial cable is included with the Model 550 or Benchmark and is used to connect the instrument to the host PC. Make sure all devices are turned off before making or changing cable connections. See the hardware manual for details.

2.3 Attaching the Hardware Protection Key

A hardware protection key (HPK) is included with this version of Microplate Manager. You must attach the HPK to your computer before you can run Microplate Manager.

Before attaching the HPK, turn off your computer. If you have a printer attached to your computer's parallel port, turn that off as well.

The HPK attaches to the parallel port on the back of your PC. If a printer cable is attached to this port, disconnect it. After you have attached the HPK, you can attach the printer cable to the key itself and restart your computer and printer.

The HPK has a driver that is automatically installed when you install Microplate Manager.

2.4 Installing Microplate Manager

Insert the Setup disk (Disk 1) into the floppy disk drive on your computer. On your Windows taskbar, click the *Start* button, then select *Run*. Type **a:\setup** in the field, and click on *OK*.

The installer will prompt you to insert the remaining disks. The default program directory is C:\Program Files\Bio-Rad\Microplate Manager. You can select a different directory when prompted to do so by the installer.

The installer will place a startup icon on your desktop and create a Microplate Manager directory on your Windows Start menu. When the installer is finished, click on the *Finish* button.

2.5 Starting Microplate Manager

To start Microplate Manager, click on the application icon on your desktop or select Microplate Manager from the Programs directory on your Start menu.



Figure 2. Microplate Manager desktop icon.

The software will open, displaying the menu bar, main toolbar, main application window, and status bar.

2.6 Selecting a Serial Port

The Ultramark connects to the computer via the SCSI port. If you are connecting to a microplate reader other than the Ultramark, you can specify the serial port connection by selecting *Communications Setup* from the **OPTIONS** menu in Microplate Manager. This opens a dialog box in which you can select one of the available ports on your computer.

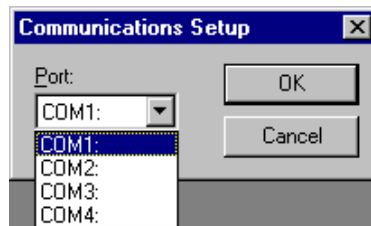


Figure 3. Communications Setup dialog box.

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When you open a protocol, Microplate Manager will look first for a reader at the serial port you select. If a reader is not found at that port, the software will scan the remaining ports as well as the SCSI bus for a reader.

If a reader is found at a particular port, that port will be selected. If no reader is found at any serial port or the SCSI port, you will receive an error message.

2.7 Setting Filters

If you change the filters in the Ultramark or Model 550 microplate readers, you can specify the wavelengths of the new filters from within Microplate Manager. (Benchmark users should refer to the hardware manual for instructions on changing filter settings.)

2.7.1 Ultramark Setup

Select *Ultramark Setup* from the **OPTIONS** menu

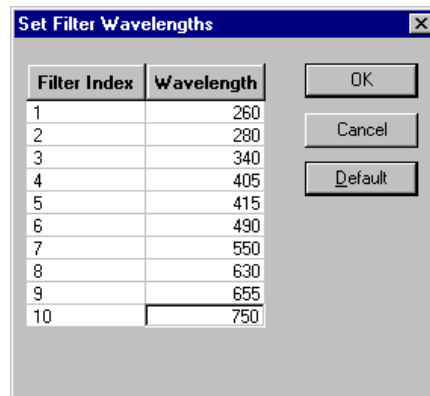


Figure 4. Ultramark Setup dialog box.

For each filter, enter the appropriate wavelength. The wavelengths of the filters that are shipped with the Ultramark will be included with your Ultramark documentation. If you change any of the filters, you will need to change these settings.

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Click on *OK* to implement your changes.

2.7.2 Model 550 Setup

Select *Model 550 Setup* from the **OPTIONS** menu

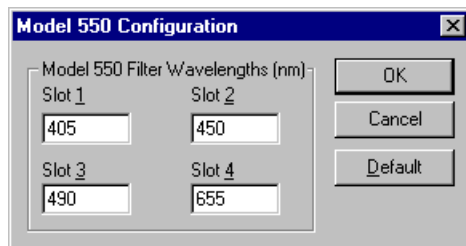


Figure 5. Model 550 Setup dialog box.

For each filter, enter the appropriate wavelength. The wavelengths of the filters that are shipped with the Model 550 will be included with your reader documentation. If you change any of the filters, you will need to change these settings.

Click on *OK* to implement your changes.

2.8 Preferences

To set the user-defined preferences for Microplate Manager, select *Preferences* from the **FILE** menu.

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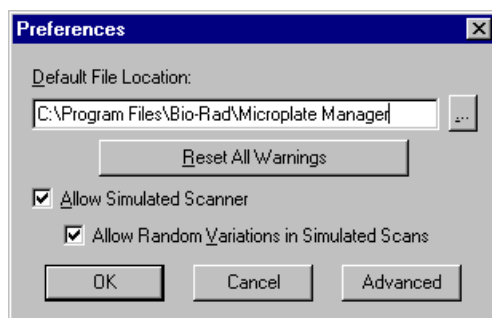


Figure 6. Preferences dialog box.

In the **Preferences** dialog box, you can enter or select the *Default File Location* for opening and saving files. To select the directory, click on the “...” button next to the text field and scroll through the directory tree. You can also type the full directory path and name directly in the field. If an invalid path is entered, it will be ignored, and the working directory will be used as the home directory.

Microplate Manager has a number of “warning” messages that pop up if you try to perform certain operations. Those messages include a checkbox to disable the warning (“Don’t show this warning again”). If you select that checkbox, and then decide that you want to display the warning again, click on the *Reset All Warnings* button in the **Preferences** dialog box.

If you are not connected to a microplate reader, the *Allow Simulated Scanner* checkbox allows you to operate the software as if you were connected to an Ultramark. In this mode, all the controls will appear active, but instead of real readings, you will create “dummy” reports containing manufactured data. This is useful for practice and demonstration purposes.

To enter simulated mode, the checkbox must be checked and, if you are connected to a reader, the reader must be turned off.

When the simulated scanner function is enabled, you can also select *Allow Random Variations*. This will generate random numbers in your simulated scans.

- The *Advanced* button accesses additional preferences that should not be changed except with the assistance of Bio-Rad support personnel.

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When you are finished making your selections, click on the *OK* button.

2.9 Sample Data Files

Six sample data files are installed with Microplate Manager, in a directory called **Examples** in your Microplate Manager directory on your hard drive.

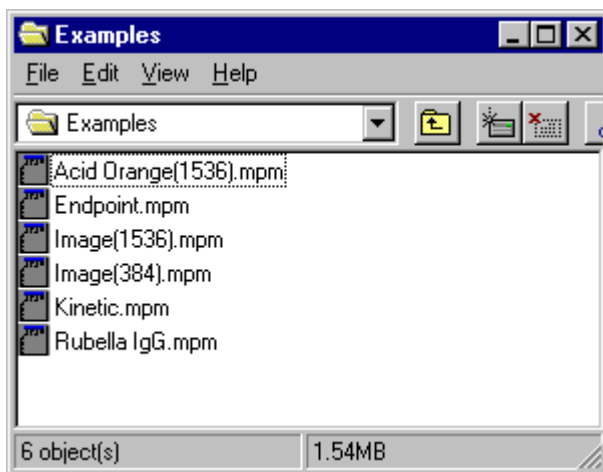


Figure 7. Examples directory.

Clicking on any one of these data files will open Microplate Manager and display the template for that file. Then you can view the sample data using the commands described in Chapter 6.

3. Getting Started

3.1 Menus, Toolbars, and Other Features

Menu Bar

Microplate Manager has a standard **menu bar** with pulldown menus that include all the commands and functions in the software. The available menus and menu items are different depending on the specific actions you are performing.

For example, when the software first opens, the menus will include basic file opening and set-up features. When you are formatting a template, the menus will change to include functions for formatting a template. When you are creating a protocol, the menus will change to include related functions. After you have read a microplate and the data is displayed, the menus will change again to include functions for creating different reports and exporting your data.

Toolbars

Microplate Manager has a **main toolbar** that contains standard file and formatting tools. It is displayed below the menu bar. Display of the main toolbar can be toggled on and off from the **VIEW** menu.

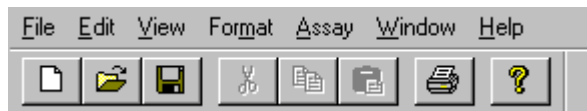


Figure 8. Default menu bar and main toolbar.

Other tools are associated with particular features of the software, and only appear when you are using those features. For example, the **template toolbar** is only displayed when you are formatting a template. It is located at the top of the template form.

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Right-click Commands

You can access many of the commands associated with the action you are currently performing by using the **right mouse button**. For example, if you are in the process of defining an assay on your template, clicking the right mouse button will display many of the commands associated with that action. The commands are displayed in a drop-down menu next to the cursor.

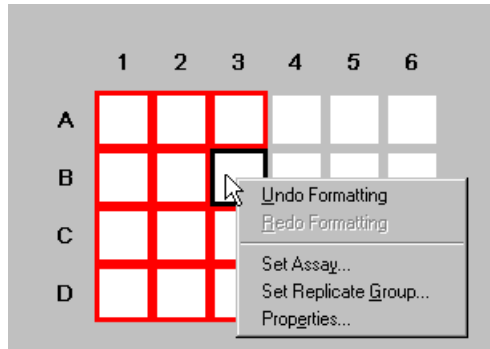


Figure 9. Example of commands available by clicking right mouse button.

Status Bar

The **status bar** appears at the bottom of the Microplate Manager window. If your cursor is positioned over a button or menu command, a brief description of that function will appear in the status bar.

In some reports (**Raw Image, Standard Curve**), the status bar indicates the position of the cursor. It also indicates the position of the cursor when you are working with **templates**.

Display of the status bar can be toggled on and off from the **VIEW** menu.

3.2 Opening and Saving Files

Microplate Manager has two basic file types: Protocol files and data files. Protocol files have a .kpr, .epr., or .mpr extension. Data files have a .mpm extension.

To open an existing protocol file or data file, select *Open...* from the **FILE** menu, select the name of the file you want to open, and click on the *Open* button.

To save a protocol file, select *Save Protocol* or *Save Protocol As...* from the **FILE** menu. To save a data file, select *Save Data/Template* or *Save Data/Template 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.

See the individual chapters on templates (Chapter 4), protocols (Chapter 5), and data and reports (Chapter 6) for more information.

3.3 Reading Data from Other Applications

The software will read files from the previous version of the Microplate Manager/PC software (version 4.0.x). It also imports data in several ASCII formats, including tab delimited and comma delimited (CSV). Kinetic data is imported from one of three formats defined in Kinetic Collector PC: COL, ROW, MTX. The software will not read native files from the Macintosh Microplate Manager software, but it will read tab delimited files generated by the Macintosh Microplate Manager software.

Data from all reports can be copied onto the clipboard, as well as exported to a file in either tab delimited or comma delimited formats. Both tab delimited and comma delimited formats may be exported into Excel®.

3.4 Quick Start

To read a plate immediately, perform the following steps:

1. Turn on the plate reader and let it warm up. Prepare a microplate and place it in the plate reader.

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2. Open Microplate Manager.
3. Under the **FILE** menu, select *New Endpoint Protocol*, *New Kinetic Protocol*, or *New Multiple Plate Protocol*, depending on the type of analysis you want to perform. The protocol dialog box will open.
4. In the dialog box, select the size of your microplate from the *Plate Type:* pulldown list and click on the *Run* button. The plate will be read and your raw absorbance or velocity data will be displayed.

At this point, you can format the template, save and analyze the data, and generate, display, and print any additional reports. (See the following chapters for information about to perform these functions.)

3.5 Typical Scenarios

The following scenarios describe the workflow for typical kinds of analysis. Refer to the following chapters for details on how to perform the particular functions.

Scenario 1. Endpoint Assay with Previously Formatted Template

You want to perform an endpoint assay using a template layout that you have already defined. From the **FILE** menu, you select *New Endpoint Protocol*. This opens the **Endpoint Protocol** dialog box. You enter *Mix Time*, *Initial Wait*, and *Incubator* settings for the reader.

Chapter 3. Getting Started

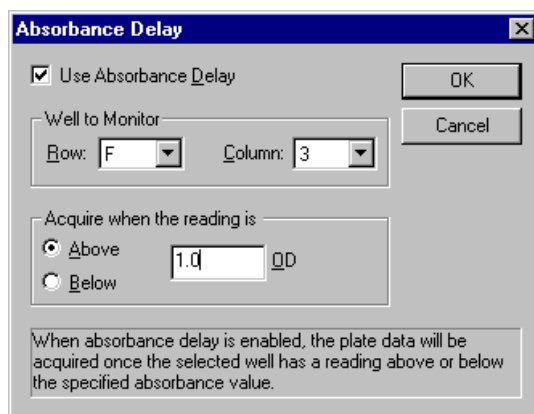


Figure 10. Performing an endpoint reading.

You have a data file saved from a previous run with the template you want to use for this plate, so you click on *Pick Template...* and choose that data file. Now that everything is set, you click on the *Run* button.

After the reading is complete, the raw data values pop up. The plate template is also displayed. Since the template already contains the concentrations of the standards, you go straight to the **Unknown Concentration Report** (select *Unknown Concentration Report* from the **VIEW** menu). You select *Print...* from the **FILE** menu to print this report.

You decide that you will be repeating this operation frequently, so you go back to the **Endpoint Protocol** dialog box. The *Reports...* button opens a dialog box in which you specify the reports to view and/or print after each reading. Select the **Unknown Concentration Report** for printing, and click on *OK*. You select *Save Protocol* from the **FILE** menu and enter a file name to store this protocol on disk.

The next day, you want to repeat the protocol. Select the protocol file name from the **Open** dialog box, place your plate in the reader, click on the *Run* button, and wait at the printer for a hard copy of the new **Unknown Concentration Report**.

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Scenario 2. Kinetic Assay

You want to perform a new kinetic assay. From the **FILE** menu, you select *New Kinetic Protocol*. The **Kinetic Protocol** dialog box opens.

For this assay, you would like to make five readings at 21-second intervals followed by 20 readings at one-minute intervals. You want to shake the plate for one second before each of the first five readings.

To accomplish this, you define two reading sets. The first set has 5 repetitions, a 1-second mix time, and a 22-second interval (the interval you want plus the mix time). The second set has 20 repetitions, 0 seconds mix time, and a 60-second interval.

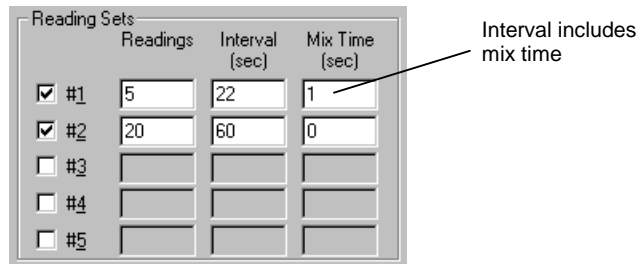


Figure 11. Defining the Reading Sets.

After inserting your 12 x 8 plate into the reader, you click on *Run* and the reader begins collecting data. At this point, both the template and the **Kinetic Plots Report** automatically pop up.

The **Kinetic Plots Report** shows a graph of absorbance versus time for each well. As each reading is completed, the graphs are updated. After all of the data has been read, you can display any relevant report (**Limit**, **Control**, etc.) by selecting it from the **VIEW** menu. To change the velocity calculation, you go to *Analysis Options...* under the **OPTIONS** menu and select one of the two calculation methods under *Kinetic Mode*. When you click on *OK*, the velocities are automatically recalculated.

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Scenario 3. Using External Standards and Curve Comparisons

You are running the same assay on many samples. To increase throughput, you would like to place a set of standards on the first plate and then use those to determine unknown concentrations on all subsequent plates. Near the end of the day, you want to check the consistency of the readings by comparing the external standards (from the first plate) to a fresh set of standards.

First, you measure the absorbances of the standards on Plate 1 and save the resulting data as Data File 1. Next, while creating a protocol file for all subsequent data collection, open the **Standard Curve Report** or **Unknown Concentration Report** and select *External Standards...* from the **OPTIONS** menu. Choose Data File 1 as the source file for the external standards. Now the unknown concentrations on all plates read using this new protocol file will automatically be calculated using the standards from Plate 1.

To compare a new standard curve to the external standard curve (from Plate 1), define standards on the new plate (internal standards) and run the plate. When the data is displayed, open the **Standard Curve Report** and select *External Standards...* from the **OPTIONS** menu. Choose Data File 1 as the source file for the external standards. Both the internal and external standard curves will be displayed. Now select *Analysis of Variance...* from the **OPTIONS** menu and a statistical summary of the comparison of the two curves will be displayed.

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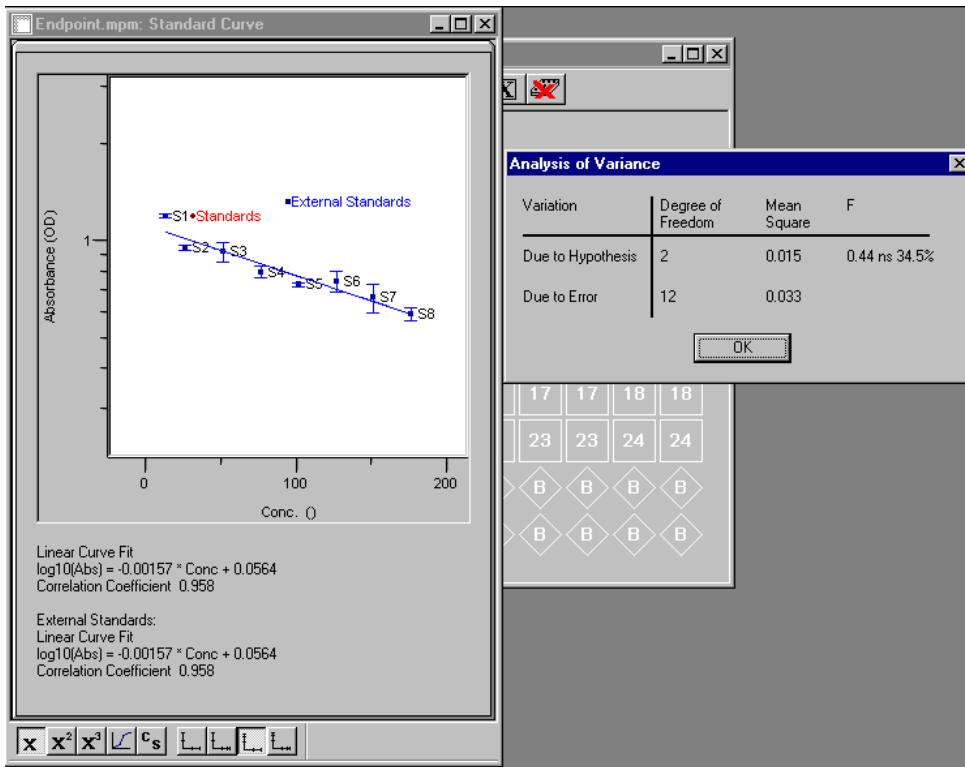


Figure 12. Comparing external and internal standards.

4. Templates

Templates allow you to specify the number of wells (Ultramark only) and the layout of the standards, unknowns, controls, etc. on your microplates. You use this layout information both when reading the microplate and when analyzing your data and organizing it in your final report(s).

- Ultramark only: This version of Microplate Manager allows you to capture and display an image of your microplate using the *Custom Template* feature. If you create or select a custom template, you can view the resulting image using the **Raw Image Report**. This feature also allows you to specify any plate dimensions up to a total of 1,536 wells.

You can define your template at several stages in the reading process.

- You can define the template first, by selecting an existing or new template directly from the **FILE** menu, formatting it, then saving it as a file. You can then select this template file from within a protocol, and it will be automatically read into the final data file.
 - You can open and format a template from within the protocol dialog box. It will then be automatically read into the final data file.
 - You can format the template in the final data file, or replace the template in the data file with a different template. (However, your changes will not be saved with the protocol.)
- It is useful to format your template *before* reading the microplate so that this information can be included in the protocol file. This allows reports to be automatically generated, displayed, and printed immediately after plate reading.

4.1 Opening a Template

4.1.1 Opening an Existing Template

To open an existing template, select *Open* from the **FILE** menu or click on the *Pick Template* button from within a protocol dialog box. This will open the **File Open**

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dialog box. Select the appropriate directory and double-click on the template file to open it.

Template files have a .mpm extension—that is, they are saved as data files, even if no data has been read into the template. Templates with no data have “0.000” entered into the data fields.

4.1.2 Opening a New Template

To open a new template, select *New Template* from the **FILE** menu. (See Chapter 5 for information on selecting a new template from within a protocol dialog box.)

Templates are listed in terms of numbers of columns and rows. For example, a 96-well microplate will have the dimensions of 12 columns x 8 rows. A 1536-well microplate will have the dimensions of 48 x 32.

Ultramark users can select from one of three standard template sizes: 12x8, 24x16, or 48x32. Model 550 and Benchmark users can only select the standard 12x8 template.

If you are using the Ultramark, you can also define your own template size by selecting *Custom*.

- **If you select a custom template, the Ultramark will capture an image of the microplate at 500 micron resolution; standard templates will simply capture well data.** There is no difference in the resulting data and analysis; however, if you use a custom template, you can view the resulting microplate image in the **Raw Image Report**. This report is not available if you use a standard template. See section 6.6.5 for more details.

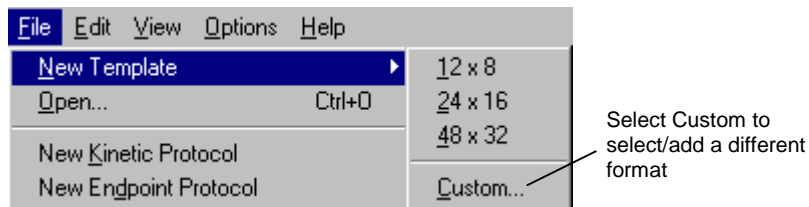


Figure 13. Selecting a template.

Creating a Custom Template Format

- This feature is only available to users of the Ultramark.

If the layout of your microplate is not listed, select *Custom*. A dialog box will open, allowing you to create a new template format.

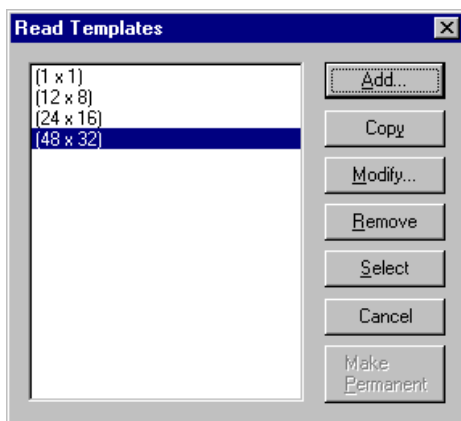


Figure 14. Creating a custom template.

In this dialog box, if the template format you want is listed, simply double-click on it to select it (or click on it once to highlight it, then click on the *Select* button to select it).

- To copy a format, click on the format name, then click on *Copy*. The copy will have the original format name plus a “:1” extension. You can then modify the copy (see below). This is useful if two microplates have the same number of wells, but the physical dimensions of the plates are different.
- To delete a format, click on the format name, then click on *Remove*. You will be asked to confirm the deletion.
- To close the dialog box without selecting a template, click on the *Cancel* button.
- To add a format, click on the *Add* button. To modify or rename an existing format, click on it, then click on *Modify*. Both of these buttons will open the **Define Plate Format** dialog box.

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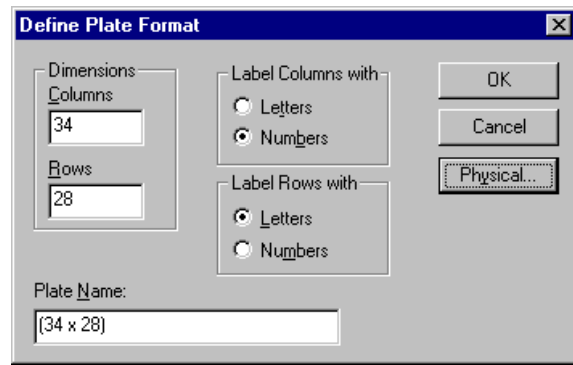


Figure 15. Defining the format of a plate.

In the **Define Plate Format** dialog box, you can enter the numbers of *Columns* and *Rows* in your microplate in the appropriate fields.

You can also choose how your columns and rows should be labeled—with *Letters* or *Numbers*. The default labeling is numbers for columns and letters for rows; however, if the number of rows exceeds 26, numbers will be substituted for letters.

You can enter a different name for your plate in the *Plate Name* field.

When you are finished making your selections, click on the *OK* button.

Defining the Physical Dimensions of the Plate

- This feature is only available to users of the Ultramark.

If you need to change the physical dimensions of your plate (the distance from the edge of the plate to the center of a well, the shape of the wells, etc.), click on the *Physical* button. This will open the **Physical Dimensions** dialog box.

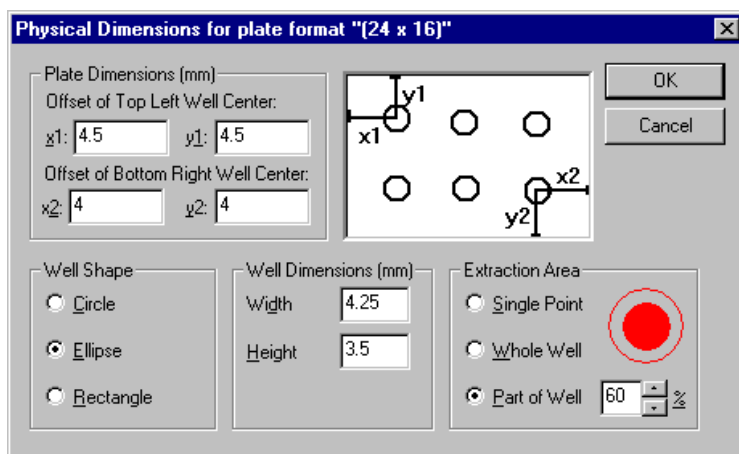


Figure 16. Entering the physical dimensions of your plate.

In the **Physical Dimensions** dialog box, you can enter the distance in millimeters from the edges of the plate to the centers of the corner wells. Entering the offset distances for the top left well ($x1$, $y1$) and bottom right well ($x2$, $y2$) will provide the dimensions for the entire plate. Refer to the diagram in the dialog box for guidance on which distance to enter into which field.

- All dimensions are in millimeters and are from the edge of the plate to the *center* of the well.

You can also select the shape of your wells—*Circle*, *Ellipse*, or *Rectangle*—by clicking on the appropriate option button.

If your well shape is a circle, you can enter the *Diameter* (in millimeters) of each well. If your well shape is an ellipse or a rectangle, you can enter the *Width* and *Height*.

Extraction Area is the area of each well that is measured for data. You can obtain data from a *Single Point* at the center of each well, the *Whole Well*, or part of a well. If you select *Part of Well*, specify the percentage of the well you want to measure. The red circle gives you a visual representation of part of the well you are selecting.

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When you have specified the physical dimensions of your plate, click on the *OK* button.

4.2 Viewing the Template

You can display a template in three sizes. Under the **VIEW** menu, select *Show Full Size*, *Show Compact*, or *Show Tiny*. The smaller sizes are useful for viewing larger templates.

If a template is too large to fit in the display window, it will be displayed with scroll bars along the right side and bottom of the template window.

Cursor Feedback

When you move your cursor over a template, the well number is displayed next to the cursor and in the status bar at the bottom of the Microplate Manager window. In addition, if applicable, the assay number, well formatting, and current value of the well are displayed in the status bar.

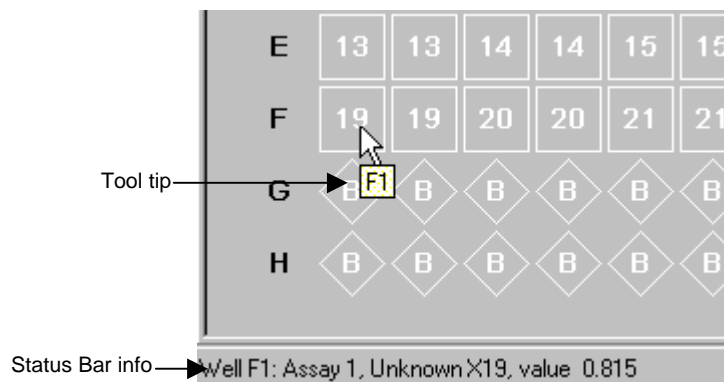


Figure 17. Example of cursor feedback in a template.

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Information about Individual Wells

Another source of information about your template is the **Well Info** dialog box. To open this dialog, click on a well and select *Properties* from the **EDIT** menu, or right-click and select *Properties* from the drop-down list. This will open the info box.

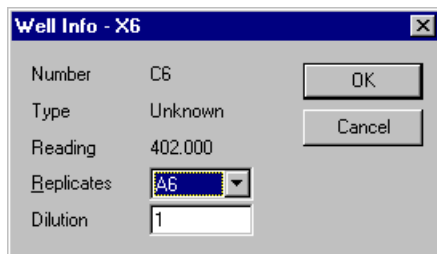


Figure 18. Well Info dialog box.

The info box shows the number, type, and reading of the well you selected. It also lists all the wells in its replicate group, and shows the dilution for the replicate group. You can enter a new dilution here, and the change will be reflected throughout the application.

You can only display the properties for one well at a time.

4.3 Features of a Template

When you select a new template from the **FILE** menu (or from within one of the protocol dialog boxes), a **blank template** will open, displaying the layout grid you selected and the template toolbar.

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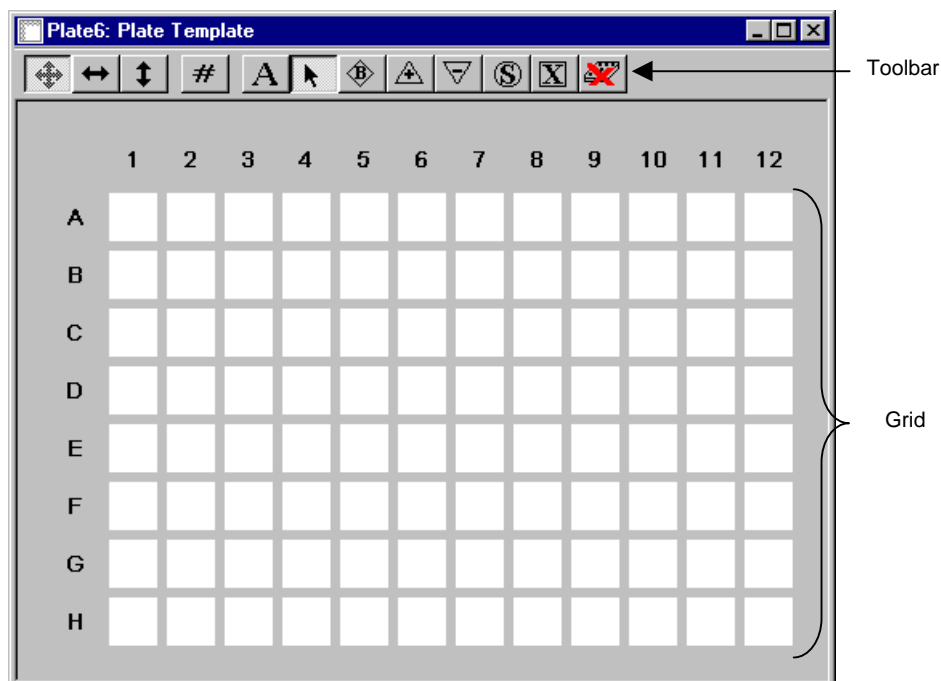


Figure 19. Blank 12 x 8 template.

Using the **template toolbar**, you define the wells that contain your standards, unknowns, blanks, and controls. You can also identify replicate groups within your unknowns and standards. Wells may be grouped by assay within the template, and you can enter concentrations of standards and dilution factors for unknowns.

The following is a summary of the information that can be stored in a template:

- Number and location of assays on the plate.
- Well type (i.e., standard, unknown, positive or negative control, blank, or undefined).
- Replicate groups (if any) for standards and unknowns within each assay.
- Concentrations of standards for each assay.
- Dilutions of unknowns for each assay.

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- Customized name for each assay.
- Notes about each assay to be printed on reports.
- Type of regression for each assay.
- Type of axes for each regression.
- External standards (if any) to be applied to each assay

Types of Wells

A template allows you to define six different types of wells:

- **Standard wells** contain your standards. They are indicated by a circle with a number at the center that indicates the replicate group.
- **Unknown wells** contain your unknowns. They are indicated by a square with a number at the center that indicates the replicate group.
- **Blank wells** are used to measure background noise for accurate absorbance calculation. They are indicated by a diamond with a letter B at the center.
- **Positive control wells** contain samples that define the upper limit of your data range. They are indicated by a triangle with a “+” at the center.
- **Negative control wells** contain samples that define the lower limit of your data range. They are indicated by an inverted triangle with a “-” at the center.
- **Undefined wells** are undefined in the template. They are indicated by an empty white square.

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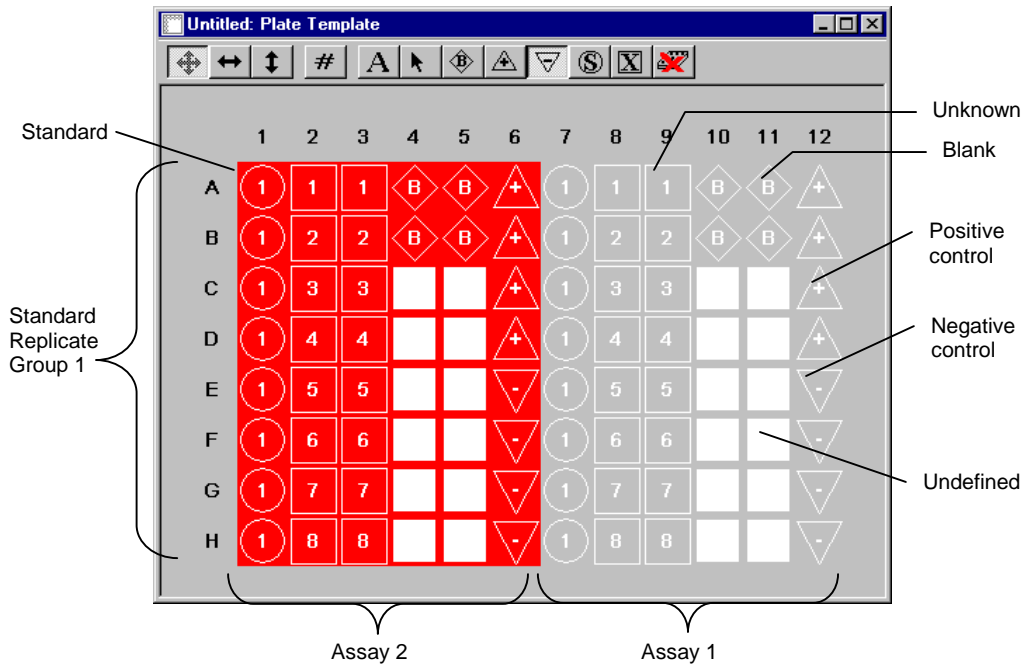


Figure 20. Example of a plate template with two different assays defined.

Replicate Groups

A replicate group is a group of wells with the same standard or unknown. For each replicate group, a mean absorbance value (in the case of endpoint or multiple plate protocols) or velocity (in the case of kinetic protocols) is calculated, along with the standard deviation and coefficient of variation for the replicate group. If more than one blank, positive, or negative control is defined for a template, they are treated like a replicate group, and the same statistical analyses are performed (mean, standard deviation, and coefficient of variation).

Assays

Up to 12 assays may be defined on a single plate. These are differentiated by color. The first assay always appears as the standard background gray. If you define subsequent assays, they appear as different colors. Independent statistical analysis and data reduction are performed for each assay.

Concentrations and Dilution Factors

Concentrations of standards and dilution factors for unknowns may also be entered using separate dialog boxes. If the concentration or dilution values are in a geometric series, you only need to enter the initial value and the ratio; Microplate Manager will calculate the remaining values.

4.4 Formatting a Template

After you have opened a template (see section 4.1.2), you are ready to format it.

- If you have reports that are associated with your displayed template, you should close them before formatting or editing the template. Reports are updated every time the template changes, and the recalculation time may make the program much less responsive.

The commands for formatting a template are located on the template toolbar and on the **FORMAT** menu.

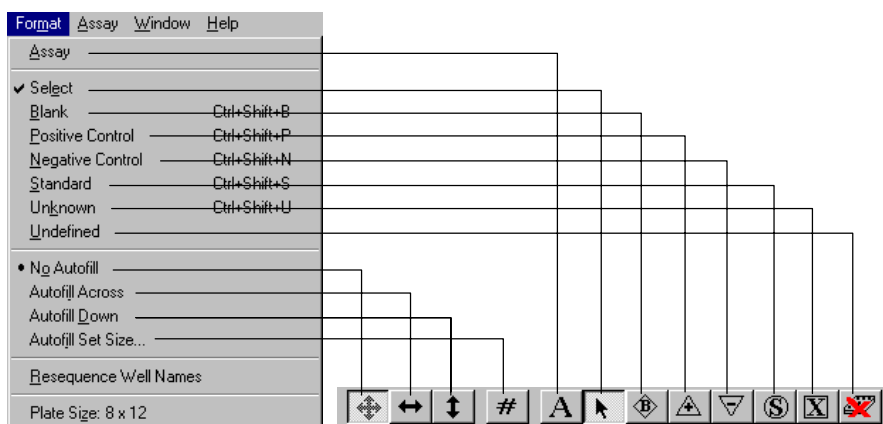


Figure 21. Format menu and template toolbar.

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Some additional assay-related commands are located on the **EDIT** and **ASSAY** menus.

Undo and Redo Formatting Commands

If you make a mistake while formatting, select *Undo Formatting* from the **EDIT** menu. This will undo the last formatting change you made to the plate template. Once undone, the formatting can be redone by choosing *Redo Formatting* from the **EDIT** menu.

The *Undo* and *Redo* commands can also be accessed by clicking the right mouse button when the pointer is positioned over the template.

4.4.1 Step 1. Define the Assays

If there is more than one assay on a plate, you should define the assays first, then define the well types. If you accidentally define a new assay over a defined well in another assay, well numbering will be affected.

To define an assay, click on the *Assay* button on the **template toolbar** (or select *Assay* from the **FORMAT** menu). The cursor will change into the *Assay* tool. Then drag the mouse to select all wells in the assay. As you drag, a box will appear on the template marking the selected wells.

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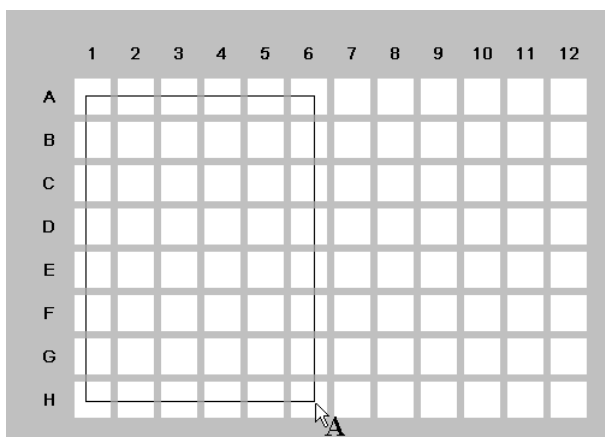


Figure 22. Defining an assay with the Assay tool.

When the mouse is released, a new assay will be created, and all the wells in the assay will have the same color.

You can define up to 12 assays on a single template. When you are done defining your assay(s), click on the *Select* button on the toolbar to change your cursor back to normal functionality.

- When a new template is first displayed, all wells are considered to be part of Assay 1. The first assay defined with the *Assay* tool is named Assay 2, the second assay defined with the *Assay* tool is named Assay 3 and so forth, and all remaining wells are considered part of Assay 1. If all wells are eventually reassigned from Assay 1 to another assay, the assay numbers are not resequenced and there will not be an Assay 1 on the plate or in the reports.

Assay Info

To rename your assays, annotate them, or change their colors, select *Assay Info...* from the **ASSAY** menu. This will open the **Assay Information** dialog box.

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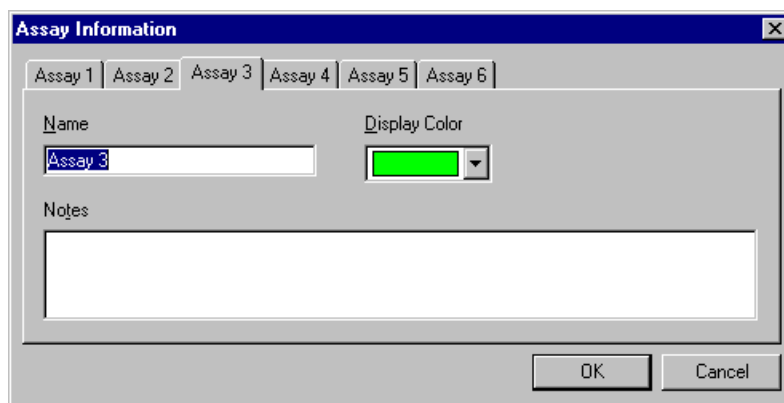


Figure 23. Assay Information dialog box.

Each assay will have its own tab. Click on the tab of the assay you want to change.

Enter a new assay name and notes about the assay in the appropriate fields. Assays may have any name up to 30 characters in length. Notes are printed with the reports.

To change the display color, use the pulldown menu under *Display Color* to select from the available colors.

Changing a Well to a Different Assay

A well may be reassigned to a different assay by clicking on the well with the *Select* tool assigned and selecting *Set Assay...* under the **EDIT** menu (you can also right-click with your cursor over the well and select *Set Assay...* from the drop-down menu). This will open the **Change Assay** dialog box. Choose the assay to move the selected well into from the drop down list of assays.

4.4.2 Step 2. Define the Well Types

The next step is to identify the standard wells and unknown wells on your template. Click on either the *Standard* button or the *Unknown* button on the **template toolbar** (or select these commands from the **FORMAT** menu). If you are defining standards, your pointer will have an S attached to it. If you are defining unknowns, your pointer will have an X attached to it.

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You can then click and/or drag on your template to define your well types.

Clicking on a particular well will mark that well as a single standard or unknown. Clicking on several wells in succession will number them sequentially.

You can also number your wells sequentially by using the *Autofill* buttons. Click on *Autofill Left to Right* and drag across a row of wells with your *Standard* or *Unknown* pointer. The wells will be numbered sequentially from left to right. Click on *Autofill Top to Bottom* and drag down a column of wells, and the wells will be numbered sequentially from top to bottom.

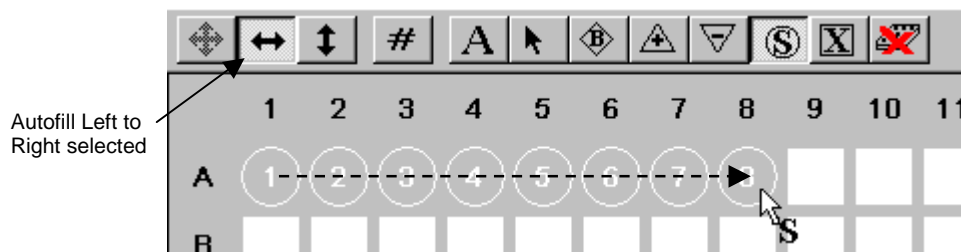


Figure 24. Autofilling a row of standards.

If you drag across multiple rows or columns, the wells will be numbered sequentially from top to bottom, left to right, across the rows and columns.

You can define as many different standards and unknowns as you have wells on your template.

Replicate Groups

You can also define replicate groups by dragging on the template.

With your *Standard* or *Unknown* pointer, click on the *Turn Off Autofill* button, then drag across a group of wells. All the wells you select will have the same number, indicating that they are in the same replicate group.

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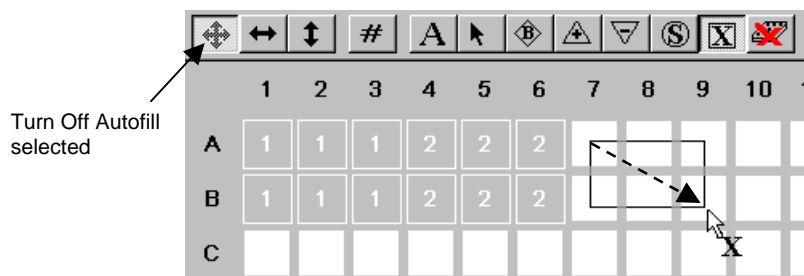


Figure 25. Defining a replicate groups of unknowns.

Dragging across another group of wells will assign those to a second replicate group, and so on.

Suppose you have several replicate groups aligned in single rows or columns. If there are the same number of replicates in each group, you can use the *Autofill* buttons and the # button to define these groups quickly and easily.

With your *Standard* or *Unknown* pointer, click on the # button, and specify the number of replicates in each group. Then click on the *Autofill Left to Right* or *Autofill Top to Bottom* button, depending on whether your replicates are in rows or columns. Drag across a row or down a column. When you finish dragging, your replicate groups will appear, numbered sequentially.

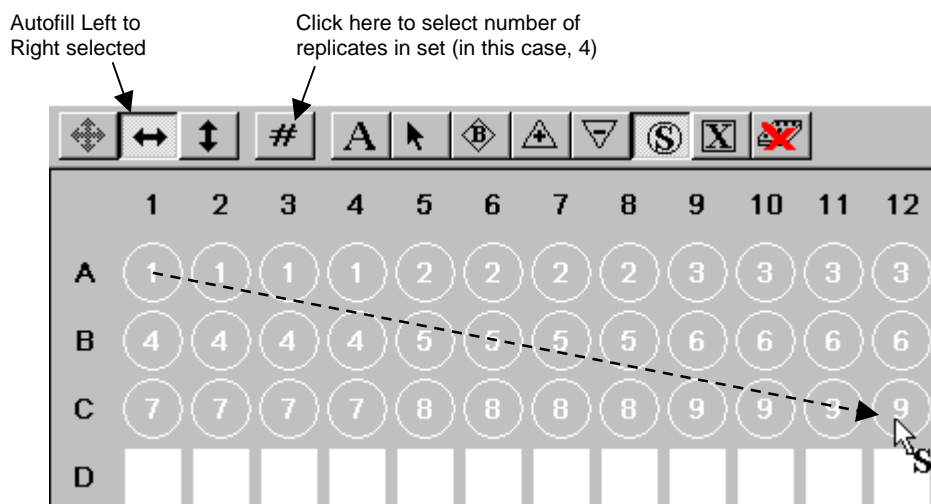


Figure 26. Defining multiple standard replicate groups in a row.

If you drag across multiple rows or columns, the replicate groups will be numbered sequentially, top to bottom and left to right.

Standards and unknowns (and replicate groups) are numbered sequentially within each assay. If you have defined more than one assay on your template, each will have its own Unknown 1, 2, 3, etc. You cannot define well types across assays.

- To change the type of a well or group of wells, simply overwrite them as if you were defining for the first time.
- To change the replicate group of a well or group of wells, first select the well(s) with the *Select* tool, then choose *Set Replicate Group...* from the **EDIT** menu or the **right-click** list of available commands. This will open a small dialog box. Select the desired replicate group from the pulldown list of existing replicate groups within the dialog box.
- If your well numbering is out of sequence and you want to correct it, select *Resequence Well Names* from the **FORMAT** menu. The wells will be renumbered in the correct order. This is a cosmetic change, and will not affect the analysis in any way.

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- To undefine wells or replicate groups, click on the *Format as Undefined* button (or select *Undefine* from the **FORMAT** menu) and click or drag on the wells to be undefined. All formatting will be erased from these wells.

4.4.3 Step 3. Enter the Concentrations of the Standards

If you have identified standard wells on your template, you can enter the concentrations for those standards.

Select *Edit Standard Conc...* from the **EDIT** menu. This will open the **Edit Standards** dialog box.

Standard	Conc.
S1	12.000
S2	6.000
S3	3.000
S4	1.500
S5	0.750
S6	0.375
S7	0.188

Figure 27. Edit Standards dialog box.

Each defined assay with standards will have a tab that is labeled with the name of the assay (default names are Assay 1, Assay 2, etc.). Concentrations for each assay are entered separately on a form under each tab. To enter concentrations for Assay 2, click on the Assay 2 tab.

Each standard is listed in the column at the left. The default concentration is 1.

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Click on the *Enter Manually* button to directly input the concentrations of your standards. Then type in the concentrations in the appropriate fields in the *Conc.* column.

If a dilution series was created for the standards, the software can calculate the concentrations of each standard. Click on the *Enter Automatically* button, then click on the button that identifies the standard with the highest concentration and enter that concentration and the dilution factor in the appropriate fields.

- Calculations are made by dividing by the dilution factor, so do not input fractional values for the dilution factor. If each standard is half the concentration of the previous standard, enter 2 for the dilution factor. If each standard concentration is one-fourth the previous, enter 4, and so on.

Click on *Calculate* when all the information is entered. The concentrations in the *Conc.* column will be automatically updated.

To use the automatic calculation feature, the most concentrated standard must be either the first standard (S1) or the last.

You can enter the units of concentration in the appropriate field; however, they are not required. These units will appear in your reports.

Repeat this procedure for each defined assay on your plate.

To implement your changes, click on *OK*.

4.4.4 Step 4. Enter Dilutions of Unknowns

You can also enter dilution factors (if any) for your unknowns. Select *Edit Unknown Dilutions...* from the **EDIT** menu. This will open the **Edit Unknowns** dialog box.

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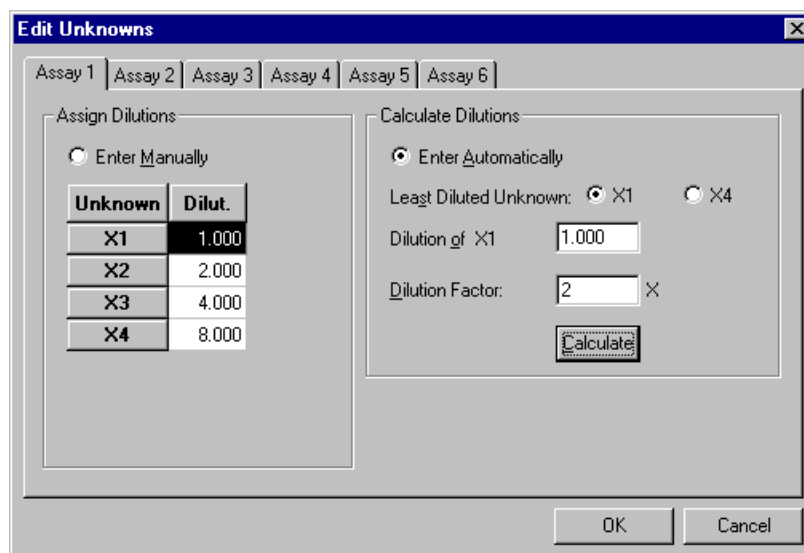


Figure 28. Edit Unknowns dialog box.

Each defined assay has a tab that is labeled with the name of the assay (default names are Assay 1, Assay 2, etc.). Dilutions for each assay are entered separately on a form under each tab. To enter dilutions for Assay 2, click on the Assay 2 tab.

Each unknown is listed in the column at the left. The default dilution is 1.

Click on the *Enter Manually* button to directly input the dilutions of your unknowns. Then type in the dilution factor in the appropriate fields in the *Dilut.* column.

- The number in the *Dilut.* column is the dilution factor, not the final concentration. For example, if the first unknown has a dilution factor of 1 (i.e., no dilution), and each unknown is twice as diluted as the previous unknown, enter 2 for the second unknown, 4 for the third unknown, 8 for the fourth unknown, etc.

The software can calculate the dilution series for you. Click on the *Enter Automatically* button, then click on the button that identifies the least diluted unknown and enter that dilution and the dilution factor in the appropriate fields.

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Click on *Calculate* when all the information is entered. The dilution factors in the *Dilut.* column will be automatically updated.

To use the automatic calculation feature, the least diluted unknown must be either the first unknown (U1) or the last.

Repeat this procedure for each defined assay on your plate.

To implement your changes, click on *OK*.

4.4.5 Step 5. Select Regression Curve

To set the type of regression method and the axes for each standard curve, choose *Standard Curve Report* from the **VIEW** menu. The **Standard Curve** dialog box will open.

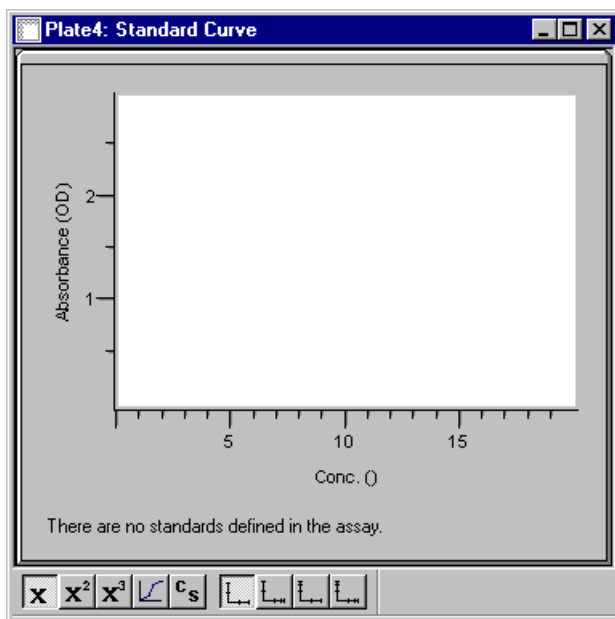


Figure 29. Standard Curve dialog box.

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Click on the tab of the assay you want to change, then choose the type of regression method from the **OPTIONS** menu or from the **standard curve toolbar** at the bottom of the dialog box.

Also select the types of axes from the **OPTIONS** menu or from the toolbar. The types of axes are linear-linear, linear-log, log-linear, and log-log. For the logistic regression method, only linear axes are available.

Repeat this procedure for each assay on your plate.

To implement your changes, close the dialog box by clicking on the *Close* box in the upper right corner.

4.4.6 Step 6. Select External Standards

If you run the same assay repeatedly, you may not want to include standards on every plate. Microplate Manager allows you to run your standards once, then include them in subsequent readings.

To do so, with your template displayed, select *Standard Curve Report* or *Unknown Concentration Report* from the **VIEW** menu and click on the tab of the desired assay.

Choose *External Standards...* from the **OPTIONS** menu to open the **External Standards** dialog box.

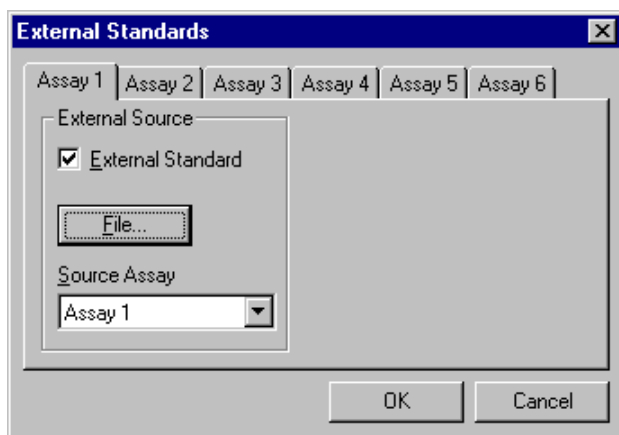


Figure 30. External Standards dialog box.

If you are already using external standards, the *External Standards* checkbox will be selected. To stop using external standards, deselect the checkbox and click on *OK*.

To begin using external standards, click on the *External Standards* checkbox. This will activate the *File* button. Click on the *File* button and a dialog box will open.

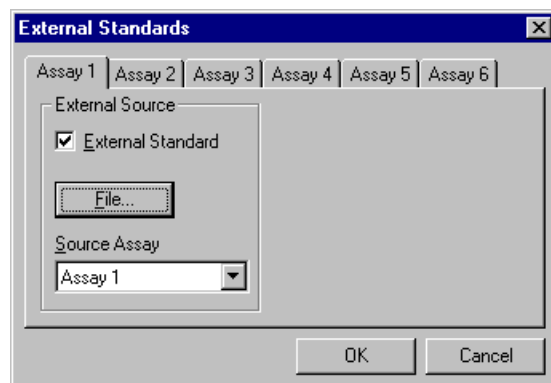


Figure 31. Selecting the source file for the external standards.

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Select the data file containing the external standards and click on *Open*. The names of all assays with defined standards within the selected data file will appear in the *Source Assay* pulldown menu.

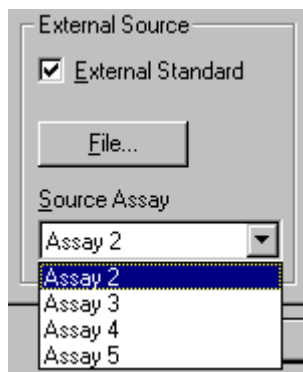


Figure 32. Selecting the source assay for your external standards.

Under the *Source Assay* pulldown menu, select the assay that contains the desired external standards. When you are finished, click on *OK* to close the dialog and implement your changes.

Notes on External Standards

When the source assay is selected for an external standard, the standards data is copied from the source file into the new data file. Any subsequent changes to the standards in the source file will not be updated in the new data file. After external standards have been copied into a data file, the imported standards in that data file can now be used as the source for subsequent files.

External standards override internal standards. If the *External Standards* checkbox is checked, then the concentrations of the unknowns are calculated using the external standards, even if internal standards are also defined on the plate. To make calculations using the internal standards, deselect the *External Standards* checkbox.

In the **Unknown Concentration** report, external standards are labeled “XS n ,” where n is an integer beginning at 1.

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4.4.7 Step 7. Saving the Template

Save the template as a data file by selecting *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu. This will open a standard **Save As** dialog box. Enter the name of the template and click on *Save*. The template will be saved with a .mpm extension.

If you try to close the file before saving, you will be prompted to save the file before closing.

5. Protocols

Protocols contain the settings for controlling the microplate reader. To read a microplate, you must first open a protocol and specify the settings.

- Some of the features and functions in the protocols are different depending on the type of microplate reader (Ultramark, Model 550, Benchmark) you are using. These differences are noted in the text. The figures depict some functions that are only available with the Ultramark.

Microplate Manager has three general types of protocols: Endpoint, kinetic, and multiple plate.

- **Endpoint protocols** are used to acquire a single absorbance reading from each well.
- **Kinetic protocols** are used to acquire a series of absorbance readings from each well over a user-defined interval. You can use these protocols to calculate reaction velocities.
- **Multiple plate protocols** are used with a stack loader to collect endpoint data from a series of plates. Multiple plate protocols save each successive endpoint data set in a separate file.

First you select the type of protocol you want to use, then you specify the settings for your particular reading, and finally you take the reading. If you want to reuse the particular protocol you've created, you can save it as a file.

Creating a New Protocol

To create a new protocol, select *New Kinetic Protocol*, *New Endpoint Protocol*, or *New Multiple Plate Protocol* from the **FILE** menu. This will open the dialog box for that particular type of protocol.

Opening an Existing Protocol

To edit an existing protocol file, choose *Open...* from the **FILE** menu, and then select the protocol name from the **Open File** dialog box. File names for protocols have the

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suffixes .epr (endpoint reading), .mpr (multiple plate reading), or .kpr (kinetic plate reading).

- Only one protocol may be open at a time.

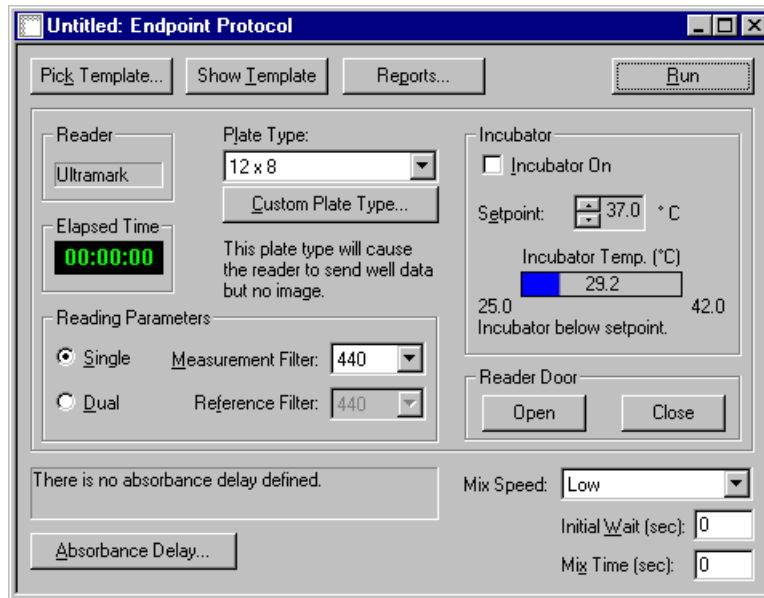


Figure 33. Example of a protocol dialog box (note that some of the functions displayed here are only available with the Ultramark microplate reader).

Each protocol dialog box has numerous settings for controlling the microplate reader. Some settings are the same for all protocols, while others are unique to the specific type of protocol. Also, some settings are only available with the Ultramark microplate reader; these settings are noted in the descriptions that follow.

The next section describes features common to all the protocols, and subsequent sections describe the features unique to each type of protocol.

5.1 Features Common to All Protocols

All three protocol types share basic reading parameters such as measurement wavelength, reader type, and delay; basic plate template and analysis parameters; reporting features; and functions for saving the protocol and running the reader.

5.1.1 Reader Identification

When you select a new or existing protocol from the **FILE** menu, the software first scans for the SCSI or serial port connection to the microplate reader. (For instructions on specifying a port, see section 2.5.)

- If no reader is found, you will receive a warning message. Make sure the reader is turned on, and check all the connections to the reader. See the microplate reader hardware manual for more details.

5.1.2 Selecting a Template

Each protocol file includes a microplate template that specifies the number of wells to be read, as well as any additional formatting information you want to include (locations of standards, unknowns, controls, etc.). This template is copied into the data file when a reading is taken.

From within the protocol dialog box, you can either create a new template (Ultramark only) or pick an existing template. See Chapter 4 for a description of templates and instructions on creating and saving them.

If no template is selected, a default template of 96 blank wells (12 x 8) is used.

Selecting an Existing Template

To select an existing template for your plate reading, click on the *Pick Template...* button. This will open a **File Open** dialog box, from which you can select the predefined template to include in the protocol file.

- Templates cannot be copied from existing protocol files. They must be copied from data files (.mpm extension).

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After you select the template, it is displayed on the screen and simultaneously copied into the protocol file.

Selecting a New Template (Ultramark only)

If you are connected to an Ultramark, you can select a new template from the pulldown list under *Plate Type*: in the center of the protocol dialog box or click on the *Custom Plate Type...* button to create a new plate type. Custom plate types are displayed inside parentheses.

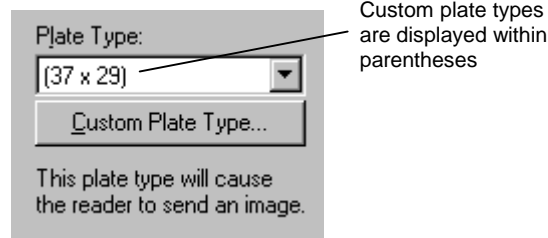


Figure 34. Selecting a new template in a protocol dialog box.

Clicking on *Custom Plate Type...* is the same as selecting *New Template > Custom...* from the **FILE** menu. See section 4.1.2 for more information about selecting a new template and creating custom templates.

Displaying and Saving Templates in Protocol Files

To display the template that is stored in the current protocol file, click on the *Show Template* button. (If you click on *Pick Template...* and select an existing template, it will be displayed automatically after selection.)

A template is not saved in the protocol file until you save the protocol. After you have selected/edited a template from within the protocol dialog, select *Save Protocol* or *Save Protocol As...* from the **FILE** menu, or click on the *Save* button on the main toolbar.

- Protocols are not automatically saved when you click on *Run*. To preserve changes to a protocol file, you must save it.

When a template is saved in a protocol file, it will remain there until you select a different template.

- If you create or edit a template in one protocol and want to copy it to another protocol, you must first save it as a data file. To do so, you have to click on *Run*. This will create a data file with a copy of the template in it. You can then save this data file and open it from within the new protocol.

5.1.3 Setting the Reading Parameters

Microplate Manager will read a plate in either a single-wavelength or a dual-wavelength mode. To select the filter wavelength (in nanometers) for measurement and reference readings, go to the *Reading Parameters* section of the protocol dialog box.

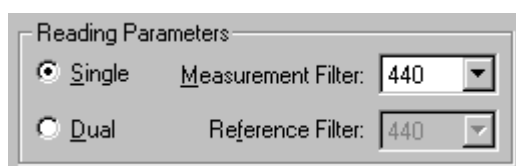


Figure 35. Reading Parameters in the protocol dialog box.

The wavelengths you have specified for your microplate reader's filters are listed in the pulldown menus. See section 2.7 for instructions on how to set the filter wavelengths for your microplate reader.

If you select the *Single* button, one measurement filter will be used for your reading.

If you select the *Dual* option, you can also select a reference filter. The reference filter will not capture any data, but will provide a baseline reading that you can subtract from your measurement reading.

5.1.4 Incubator Controls (Ultramark and Benchmark only)

To use the incubator of the Ultramark or Benchmark, click on the *Incubator On* checkbox in the protocol dialog box.

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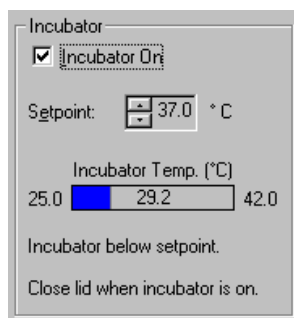


Figure 36. Incubator settings.

The *Setpoint* is the temperature that the incubator will warm up to and maintain throughout the reading. The range is 25.0–42.0°C; the default setpoint is 37°C. To change the setpoint, click on the arrows next to the temperature field.

The blue status bar displays the current temperature. If you click on the *Run* button before the incubator reaches the setpoint, a message box will warn you that the setpoint temperature has not been reached.

5.1.5 Mix Settings

Mix Time specifies the length of time that a plate is shaken before the reading is taken. Enter a mix time in seconds, then select a *Mix Speed* (Ultramark only) from the pulldown list (*Low*, *Medium*, *High*). When you click on *Run*, the reader will shake the plate for the specified mix time before taking a reading.

5.1.6 Reader Door (Ultramark only)

From within the protocol window, you can open and close the tray door of the Ultramark by clicking on the Open or Close button in the Reader Door field.



Figure 37. Ultramark reader door controls.

5.1.7 Reports

You can select certain reports to be automatically displayed and/or printed after a reading is taken. Click on the *Reports...* button to open a dialog box listing the available reports.

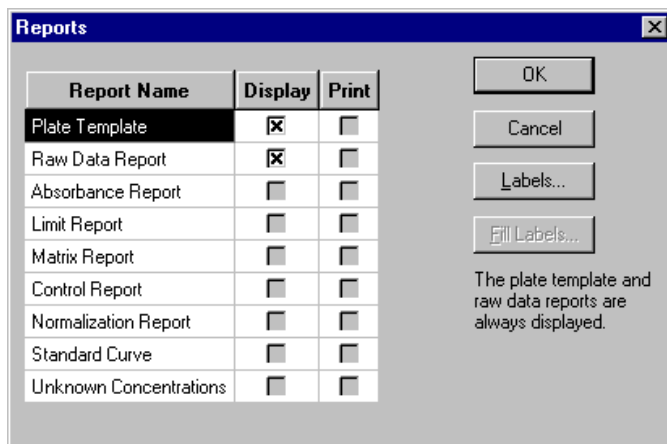


Figure 38. Reports dialog box for endpoint and multiple plate protocols.

The **plate template** is always displayed immediately after a reading. For endpoint and multiple-plate protocols, the **Raw Data Report** is also auto-displayed. For kinetic protocols, the **Kinetic Plots Report** is displayed.

To select certain options for automatic printing such as page orientation and paper source, select *Print Setup...* from the **FILE** menu. This will open a **Print Setup** dialog box.

Reports that are not selected in advance in the protocol file may be generated, displayed, and printed from the data file.

Labels

From within the **Reports** dialog box, you can create custom labels to be printed at the top of each report. If you create a label in a protocol, after the plate has been read and

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before the reports have been generated, you will be prompted for the labeling information if it has not already been entered.

For example, if the labels “Date” and “Operator” are created in a protocol file, then when that protocol is run, a dialog box will pop up after plate reading prompting you to type the date and the operator’s name. Both the label (e.g., Date) and the label contents (e.g., July 4, 1999) are printed on the report.

In the **Reports** dialog box, click on *Labels...* to open the **Define Labels** dialog box.

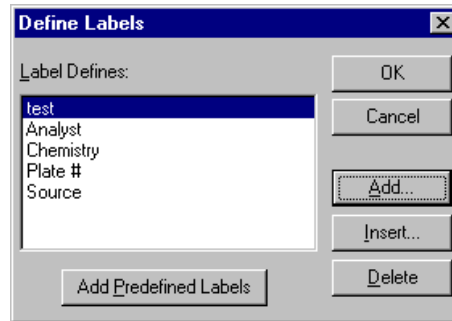


Figure 39. Define Labels dialog box.

See section 6.6.1 for information about defining labels.

After you have defined your labels, you can fill them in by clicking on the *Fill Labels...* button in the **Reports** dialog box.

See section 6.6.1 for information about filling in labels.

- You can also define and fill out labels after the protocol has been run, when the data is displayed (see section 6.6.1). Labels created after a protocol has been run cannot be saved with the protocol.

5.1.8 Running a Protocol

Once all the parameters have been set, click on the *Run* button to begin taking a reading.

While the reading is in progress, the *Run* button will change into a *Stop* button and the other controls will become inactive. A digital clock will display the elapsed time.

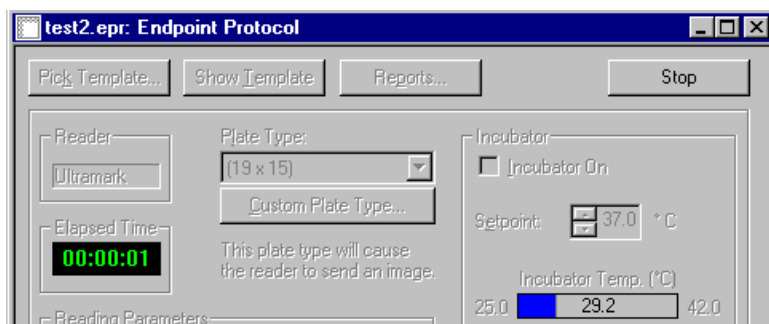


Figure 40. Example of a running protocol.

Clicking on *Stop* will stop the reading in progress.

For kinetic and multiple-plate protocols, data acquired up to the time the reading is stopped will be displayed and may be saved. For example, if a run of 100 readings is stopped during reading 51, you can save the first 50 readings. If you stop an endpoint protocol, all data will be lost.

5.1.9 Saving a Protocol

You must save your protocol to preserve any changes you have made to the protocol parameters. To save a protocol, select *Save Protocol* or *Save Protocol As...* from the **FILE** menu or click on the *Save* button on the main toolbar. Enter a name for the protocol in the dialog box. The default extensions for kinetic, endpoint, and multiple plate protocol files are .kpr, .epr, and .mpr, respectively.

- Protocols are not automatically saved when you click on *Run*. To preserve changes to a protocol file, you must save it.

5.2 Features Unique to Endpoint Protocols

Endpoint protocols collect one absorbance reading for each well on a microplate. To run a new endpoint protocol, select *New Endpoint Protocol* from the **FILE** menu.

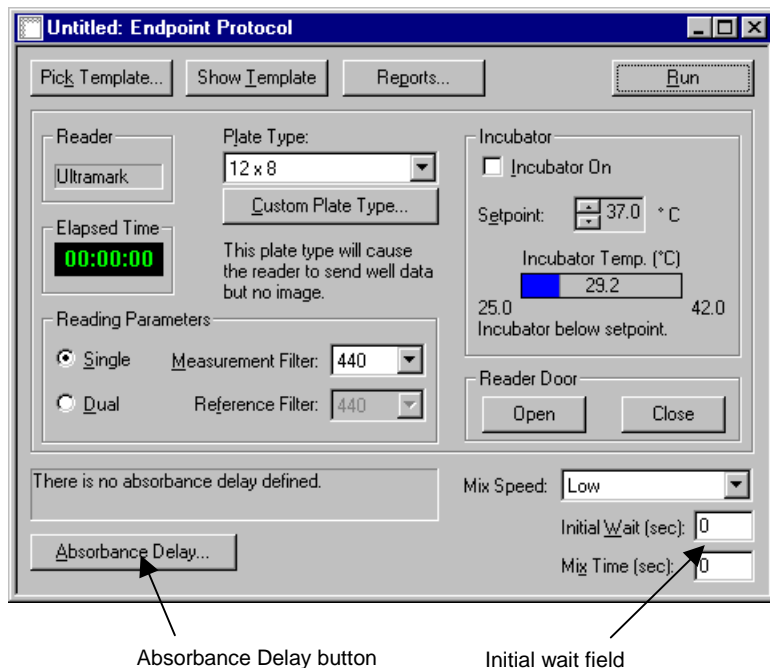


Figure 41. Features unique to an endpoint protocol dialog box.

The controls common to all protocols are described in the previous section. The controls specific to endpoint protocols are described below.

Initial Wait

The *Initial Wait* option delays the start of a reading by the specified number of seconds after you click on *Run*. This is useful for adjusting for the initial lag time associated with delayed reactions. Enter a number in the field to set an initial wait time.

Absorbance Delay

Absorbance Delay delays data collection until a well that you select reaches a specified level of absorbance. To access the controls for this feature, click on the *Absorbance Delay* button.

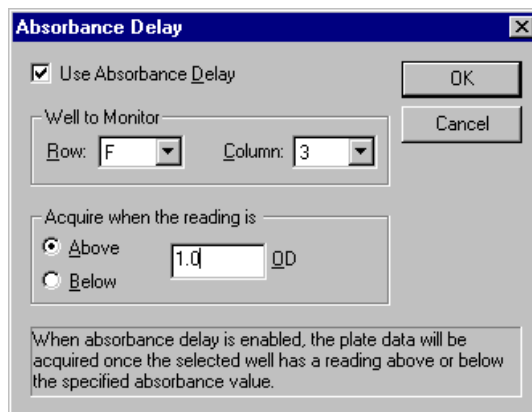


Figure 42. Absorbance Delay dialog box.

In the **Absorbance Delay** dialog box, click on the *Use Absorbance Delay* checkbox to enable this feature. Select the row and column number of the well whose absorbance you want to monitor from the pull-down lists. In the *Acquire when the reading is* section, specify an absorbance that is above or below a certain OD value. *Below* will begin data collection when the measured absorbance is below the specified OD, and *Above* will begin data collection when the absorbance is equal to or greater than the specified OD.

Click on the *OK* button to implement your changes.

5.3 Features Unique to Kinetic Protocols

Kinetic protocols are used to acquire a series of absorbance readings from each well over a time period that you specify. You can use these protocols to calculate reaction velocities.

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To run a new kinetic protocol, select *New Kinetic Protocol* from the **FILE** menu.

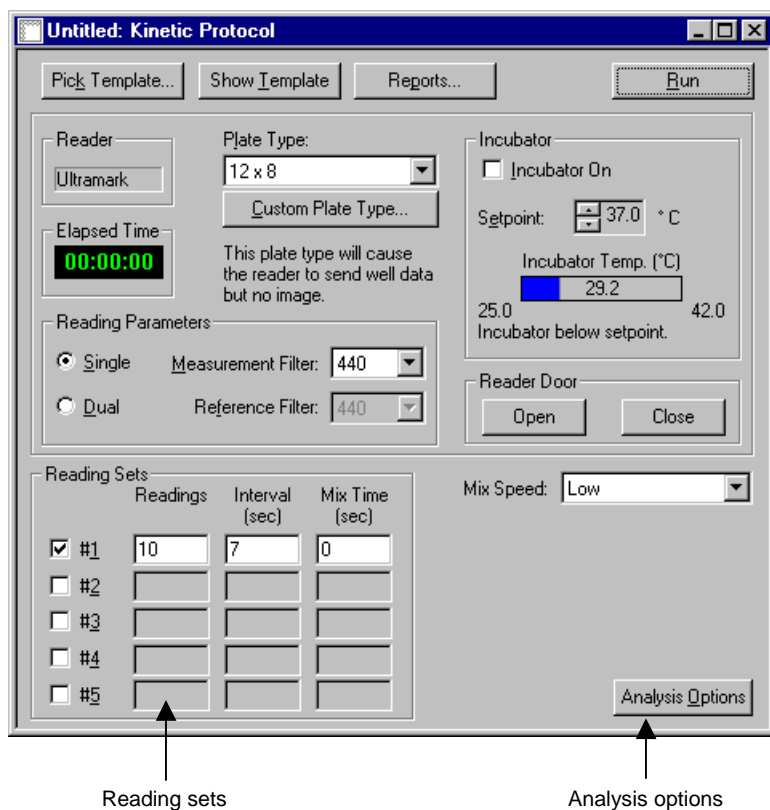


Figure 43. Features of a kinetic protocol dialog box.

The controls common to all protocols are described in section Features Common to All Protocols. The controls specific to kinetic protocols are described below.

5.3.1 Reading Sets

A reading set consists of the number of times a plate is read, the delay between each reading, and (if desired) the mixing time before each reading. You can specify up to five reading sets per protocol.

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To activate a reading set, click on the checkbox next to the set number. Only sets that are checked will be run.

Enter the number of readings you want to take in the *Readings* column.

Next enter the interval between the start of two consecutive readings in the *Interval* column. Note that this is the minimum time necessary for one complete reading plus any mixing time you specify. For a standard 12 x 8 (96-well) plate, the smallest interval you can specify is 7 seconds. For custom plate sizes, the smallest interval you can specify is 450 seconds (7.5 minutes). If the interval you enter is too short, a message box will tell you the minimum total read time.

Finally, you can specify a mixing time for your plate. If no mixing is desired, enter a 0.

- If you specify a mixing time, be sure to include this time in the interval between readings (*Interval* column).

5.3.2 Analysis Options

To access the other kinetic protocol options, click on the *Analysis Options...* button. This will open the **Analysis Options** dialog box.

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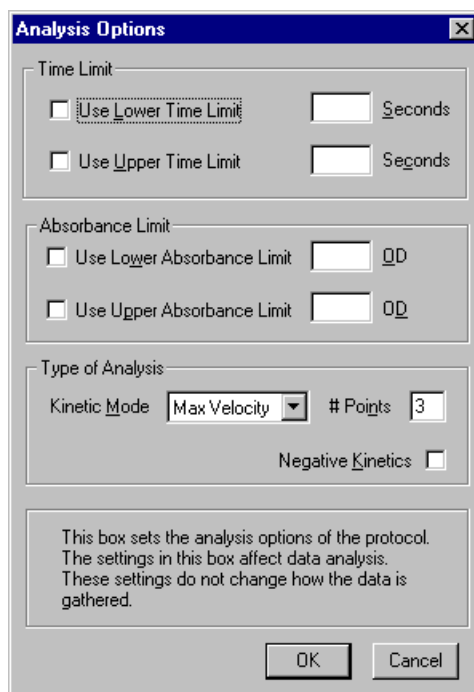


Figure 44. Analysis Options in the kinetic protocol dialog box.

The settings in this dialog box will only affect the analysis of your data. They will not change the raw data that is gathered.

Time and Absorbance Limits

In this dialog, you can specify a time range and/or an absorbance range for your data points. If time limits and/or absorbance limits are set, then only data points within these limits will be used to calculate the reaction velocity. This is useful for excluding an initial lag time in delayed reactions or for excluding the end of a reaction as it approaches equilibrium.

To set time limits, select the appropriate checkbox(es) and enter a number of seconds in the appropriate field(s).

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To set absorbance limits, select the appropriate checkbox(es) and enter ODs in the appropriate field(s).

If none of the limit checkboxes is selected, then all data points will be included in the velocity calculations.

Velocity

There are two methods for calculating kinetic velocities. The first fits a line to the entire data set and defines the reaction velocity as the slope of this line. The second fits a line to every subsegment of n points, where n is specified by the user, and identifies the greatest velocity from among these segments.

Under *Kinetic Mode*, select one of the following options:

- **Velocity.** The kinetic velocity (slope of the absorbance versus time curve) will be calculated by linear regression using all the data points. If time or absorbance limits are selected, then all the data points within the selected range are used in the velocity calculations.
- **Max. Velocity.** Kinetic velocities will be calculated for overlapping segments of the reaction, and the maximum velocity will be reported. You then select the number of data points to include in each segment of the reaction; the minimum number of points is two. The first segment begins with the first data point and extends for the number of points you have selected. The second segment begins with the second data point, the third segment with the third data point, etc.

For cases where the absorbances decrease with time, the velocities will be negative. To change the sign of the velocities, select the *Negative Kinetics* checkbox.

- If the velocities are negative, then the velocity or maximum velocity axis of the **Standard Curve Report** is constrained to linear unless the *Negative Kinetics* box is checked (logarithms cannot be taken of negative values).

5.4 Features Unique to Multiple Plate Protocols

The multiple plate protocol will perform single endpoint readings on a series of plates and store these readings in separate data files with a common root file name. This protocol may be used with or without a stack loader. With a stack loader, you program the loader to load your plates in sequence. Without a stack loader, you will be prompted to manually load and unload each plate in the sequence, up to the number you specify in the protocol.

To run a new multiple plate protocol, select *New Multiple Plate Protocol* from the **FILE** menu. This will open the **Multiple Plate Protocol** dialog box.

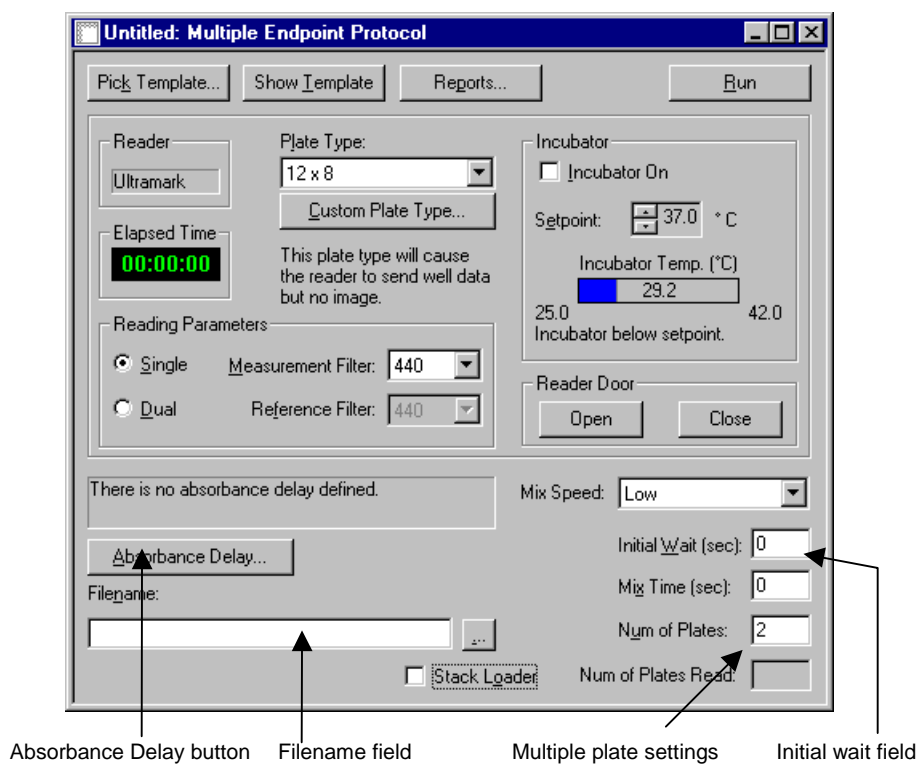


Figure 45. Features of a Multiple Plate Protocol dialog box.

The controls common to all protocols are described in section 5.1. The controls specific to multiple plate protocols are described below.

Initial Wait

The *Initial Wait* option delays the start of the protocol by the specified number of seconds after you click on *Run*. This is useful for excluding an initial lag time in delayed reactions. Enter a number in the field to set an initial wait time.

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Stack Loader Checkbox

If you are connected to Bio-Rad's Twister stack loader and have installed the associated software, the *Stack Loader* checkbox will appear checked when you first open the Multiple Plate Protocol window. (If the Twister software is not installed, this checkbox will be unavailable.) If you do not want to use the stack loader, deselect this checkbox.

With the checkbox checked, when you click on the *Run* button in the protocol window, the *Plate Handler Options* dialog box will open.

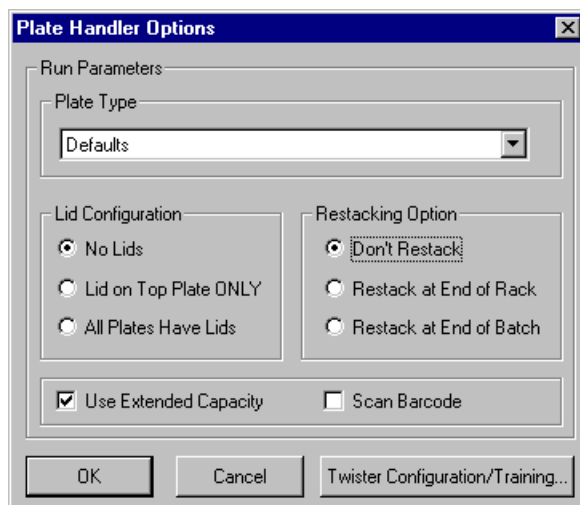


Figure 46. Plate Handler Options dialog box.

From this dialog box you can enter the main Twister control panel by clicking on the *Twister Configuration/Training* button. See the *Twister User Guide* for instructions on configuring the Twister using this panel.

After you exit the Twister control panel, you will have to click on the *Run* button again to reenter the *Plate Handler Options* dialog box.

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After you have defined your plate types and other configurations in the Twister control panel, select the appropriate plate type from the pull-down menu under *Plate Type* in the *Plate Handler Options* dialog box.

The *Restacking Option* buttons allow you to specify whether and how you will restack your plates. *Restack at End of Rack* will restack the plates after each rack is emptied. *Restack at End of Batch* will wait to restack the plates until all the plates in all the racks have been read.

If you have a rack for storing lids on your Twister (specified in the Twister control panel), you can select the appropriate option in the *Lid Configuration* field.

If the Twister Extended Capacity platform has been installed, you can select the *Use Extended Capacity* checkbox. If you do not want to use the Extended Capacity, deselect this checkbox.

If a barcode reader is installed with your Twister, the *Scan Barcode* checkbox will appear checked. If you do not want to scan barcodes, deselect this checkbox. If a plate has a barcode, it will be stored in the plate file and printed on all the reports. (It will not be visible in the reports displayed on the screen.) If a plate has no barcode, the barcode reader will return a blank string.

Number of Plates

If the *Stack Loader* checkbox is selected in the main protocol window, the *Num of Plates* field will be grayed out. As the stack loader progresses through a stack of plates, the *Num of Plates Read* field will indicate the number of plates that have been read.

If you want to load your plates by hand, deselect the *Stack Loader* checkbox and enter the number of plates in the *Num of Plates* field. Then, when you click on *Run*, you will be prompted to insert the next plate in the sequence after each plate has been read.

Filename Field

In the course of a batch of multiple plate readings, each data set is saved as soon as each reading is complete. Before you run a multiple plate protocol, you must specify

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a base file name for the resulting data files. File names are then constructed by appending two digits to the base file name.

Enter the base file name in the *Filename:* field. A file path may be entered as well. Clicking on the button next to the *Filename:* field allows you to browse the directory tree for a directory to save your files in.

For example, if you enter c:\mydata\test and read three plates, the resulting data files will be named test-01.mpm, test-02.mpm, and test-03.mpm, and these will be written to the c:\mydata directory. If you scanned a bar code number on each plate, the data file name will also be appended with that number (e.g., for bar code number ABC123, the resulting file name would be test-01-ABC123.mpm).

Absorbance Delay

Absorbance Delay delays data collection until a well that you select reaches a specified level of absorbance. To access the controls for this feature, click on the *Absorbance Delay* button.

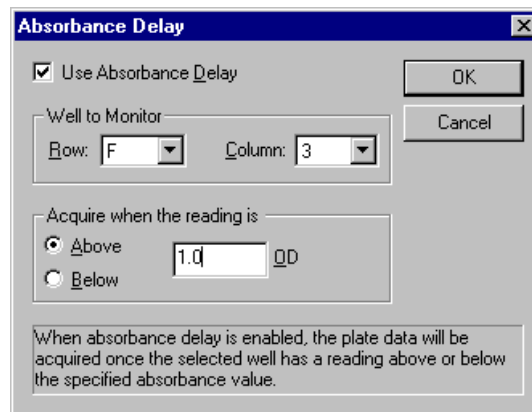


Figure 47. Absorbance Delay dialog box.

In the **Absorbance Delay** dialog box, click on the *Use Absorbance Delay* checkbox to enable this feature. Select the row and column number of the well whose absorbance you want to monitor from the pull-down lists. In the *Acquire when the*

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reading is section, specify an absorbance that is above or below a certain OD value. *Below* will begin data collection when the measured absorbance is below the specified OD. *Above* will begin data collection when the absorbance is equal to or greater than the specified OD.

Click on the *OK* button to implement your changes.

6. Data Formatting and Reports

After you have run your protocol, your data and the associated template will be displayed automatically. You can then organize your data in a variety of reports and save the data as a data file.

To open an existing data file, select *Open...* from the **FILE** menu, select the name of the file you want to open, and click on the *Open* button. Data files have a .mpm extension.

6.1 Summary of Available Reports

When your data is displayed, the available reports will be listed under the **VIEW** menu. These will be different depending on the type of protocol that has been run. Endpoint and multiple-plate protocols produce absorbance data; kinetic protocols produce velocity data. The type of data determines the type of reports that are available.

All protocols can produce the following reports:

- **Raw Image Report (Ultramark only):** This report is available if you have selected a custom template (see section 4.1.2). It displays an image of the microplate with the collected absorbance data (kinetic protocols produce multiple images, one for each absorbance reading).
- **Limit Report:** Given upper and lower data limits, shows which wells had data over the upper limit, which wells had data below the lower limit, and which wells had data within the limits.
- **Matrix Report:** Resembles the Limit Report, except data within the limits are divided into 10 ranges (0–9), and the wells containing the data are numbered accordingly.
- **Control Report:** Same as the Limit Report, except the upper and lower limits are determined by positive and negative controls.

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- **Normalization Report:** Similar to the Limit Report, except that the readings are displayed as percentages relative to the limits.
- **Standard Curve Report:** Displays the interpolation curve defined by your standards, as well as associated statistical analyses. Five different regression methods (linear, quadratic, cubic, cubic-spline, and four-parameter logistic) are available.
- **Unknown Concentrations Report:** Unknown concentrations are computed based on the standards and regression method you have chosen.

The following reports are specific to endpoint and multiple-plate protocols (absorbance data):

- **Raw Data Report:** Displays the raw absorbance values for the microplate wells.
- **Absorbance Report:** Subtracts the mean absorbance of the blank wells from the raw absorbance of each well.

The following reports are specific to kinetic protocols (velocity data):

- **Velocity Report:** Shows the velocity of each well, calculated from the raw data as specified in the Analysis Options dialog box.
- **Adjusted Velocity Report:** Subtracts the mean velocity of the blank wells from the raw velocity of each well.
- **Kinetic Correlation Coefficient Report:** Shows the correlation coefficient of the linear fit for each well.
- **Kinetic Standard Errors Report:** Shows the standard error of the linear fit for each well.
- **Kinetic Plots Report:** Shows the time evolution graph of each well.
- **Kinetic Zoom Plot Report:** Shows the kinetic plots data for a selected well. Shows the fitted equation for the linear regression and its standard statistics.

From within a protocol dialog box, you can select the reports to be automatically displayed after a protocol is run. See section 5.1.6 for instructions.

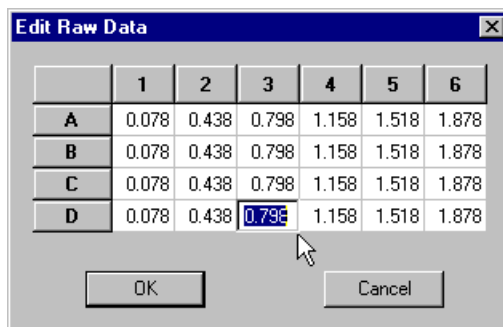
6.2 Saving Your Data

Data is displayed and saved with templates (see Chapter 4 for more information about templates). To save your data, select *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu. This will open a **Save As** dialog box. Enter the name of the data set/template and click on *Save*. The data set/template will be saved with a .mpm extension.

If you try to close a newly created data set before saving, you will be prompted to save the file.

6.3 Manually Editing Data

Microplate Manager allows you to manually edit your raw data. For kinetic data sets, select *Edit Velocities...* from the **EDIT** menu. For endpoint (and multiple-plate) data, select *Edit Raw Data...* from the **EDIT** menu.



	1	2	3	4	5	6
A	0.078	0.438	0.798	1.158	1.518	1.878
B	0.078	0.438	0.798	1.158	1.518	1.878
C	0.078	0.438	0.798	1.158	1.518	1.878
D	0.078	0.438	0.798	1.158	1.518	1.878

Figure 48. Editing raw data.

Your data will be displayed in a spreadsheet format. To change a particular number, select it and overwrite it.

- All changed numbers will be permanently shaded in gray to distinguish them from unedited data.

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You can highlight multiple number fields by dragging your cursor over the fields. You can highlight rows and columns by clicking on the button at the beginning of the row or column. You can cut or copy your data using key commands: Ctrl + x = cut and Ctrl + c = copy

You can paste your data into different rows/columns or into different spreadsheet applications using the key command Ctrl + v.

Click on *OK* to accept your changes.

- For kinetic data, recalculating the velocities will overwrite the manually-entered velocities. Velocities are recalculated whenever the kinetic mode, the time range, or absorbance range is changed. See section 5.3.2 for details.

It is not possible to edit individual absorbance readings of a kinetic data file.

6.4 Identifying Outliers

If some well readings appear to be incorrect and you do not want to include them in your standard curve calculations or reports, you can flag them as outliers.

With your data displayed, select *Edit Outliers...* from the **EDIT** menu. This will open the **Select Outliers** dialog box.

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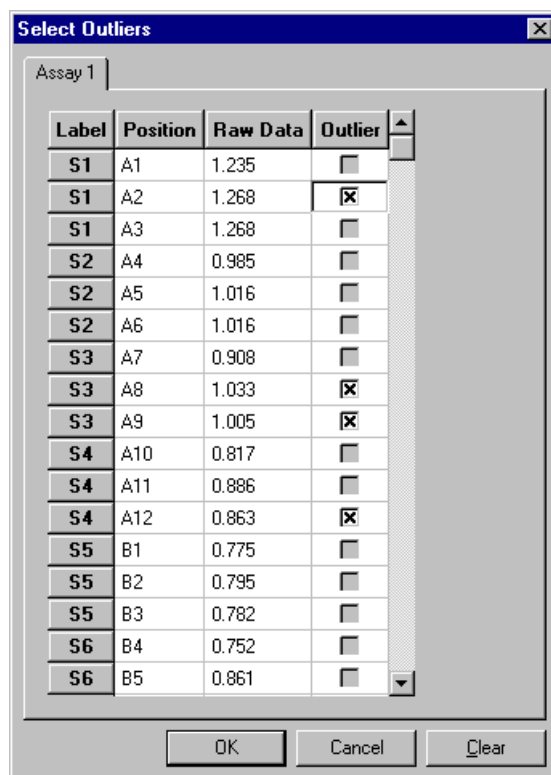


Figure 49. Select Outliers dialog box.

Click on the checkbox next to the well number and associated value that you want to flag as an outlier. This well will not be included in any reports, and will not be used to calculate the standard curve. If an outlier well is part of a replicate group, that well will not be used to calculate the average value for the group.

When you are finished selecting your outliers, click on *OK* to implement the changes. Clicking on *Clear* will uncheck all the boxes.

6.5 Editing or Replacing the Template in a Data File

After a protocol has been run, the template from the protocol file is automatically copied into the data file and displayed (see Chapter 4 for more information about templates). When you save a data set, the template is saved with it. When you open an existing data file, the template is always displayed with the data.

This template may be edited or completely replaced by another template.

Editing a Template in a Data File

To reformat an existing template, simply format it as you would a new template. For instructions on opening, saving, and formatting templates, see Chapter 4.

- Each change you make in a template will automatically update all the open reports. This can greatly slow the processing time of the computer. For this reason, you should close all open reports before changing the template.

Changes to a template will be lost unless the data file is saved again by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

Replacing a Template in a Data File

To replace the template in a data file, open the file with the template to be replaced (if it isn't already open), then choose *Transfer Template...* from the **EDIT** menu and select the data file that contains the desired template.

- The new template must be the same dimensions as the template being replaced.

The new template will be displayed. When the data file is saved by choosing *Save Data/Template* or *Save Data/Template As ...* from the **FILE** menu, the transferred template is saved in the data file with the absorbance readings.

6.6 Reports

With your data displayed, select the report you want to display from the **VIEW** menu. The available reports will be different depending on the type of data you have displayed. Endpoint and multiple-plate protocols produce absorbance data; kinetic protocols produce velocity data.

To close any of the report windows, click on the *Close* box in the upper right corner of the window.

6.6.1 Labels

You can create custom labels to be printed at the top of each report. To create your labels, select *Labels...* from the **EDIT** menu. This will open the **Define Labels** dialog box.

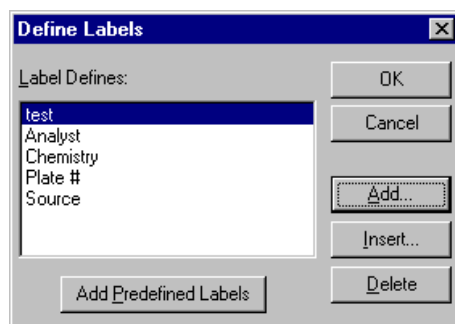


Figure 50. Define Labels dialog box.

You can specify up to six different labels. There are four predefined labels: Analyst, Chemistry, Plate #, and Source. Click on *Add Predefined Labels* to add these labels to your reports. To add some of these labels but not all, first add them all, then select the label(s) you don't want and click on the *Delete* button.

You can also add or insert user-defined labels. To append a label to the bottom of the current list, click on the *Add...* button and enter a name for

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your label in the field in the pop-up box. To insert a label into the label list, select the existing label above which you want to insert the new label, click on the *Insert...* button, and enter a name for your label in the field in the pop-up box.

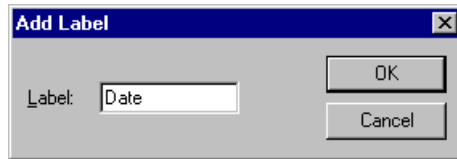


Figure 51. Dialog box for adding or inserting labels.

Click on *OK* to implement your changes.

Back in the **Define Labels** dialog box, to delete a label from the list, select it and click on the *Delete* button.

Fill Labels

After you have created a label, you can enter the information to be included in that label immediately, if desired. Select *Label Contents* from the **EDIT** menu. This opens the **Fill Labels** dialog box.

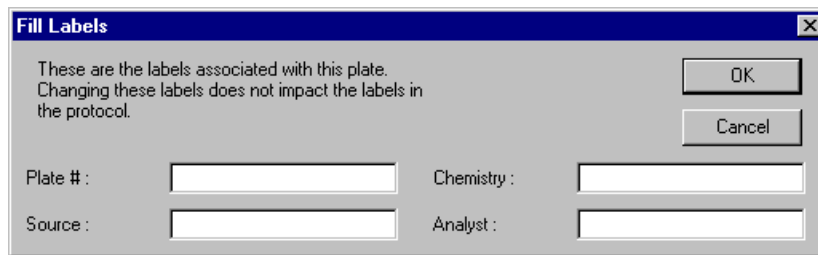


Figure 52. Fill Labels dialog box.

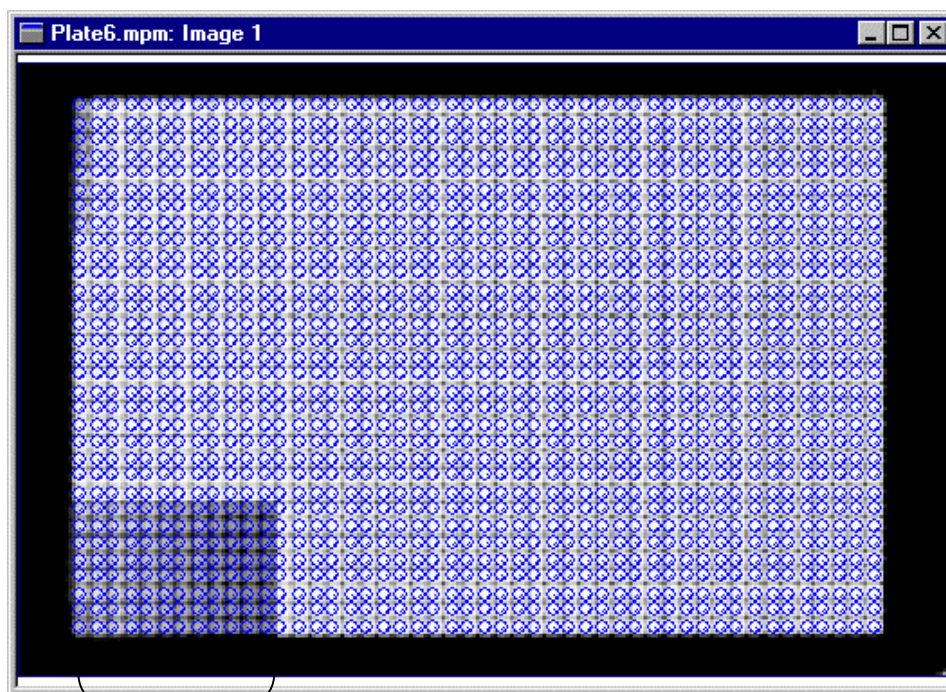
In this dialog, each label name is followed by a text field. Enter the label information to be printed on each report. Click on *OK* when you are finished.

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6.6.2 Raw Image Report (Ultramark only)

The **Raw Image Report** is different from other reports in that it is an actual image of your microplate with the wells and absorbance areas highlighted. This report is only available if you performed a plate reading with the Ultramark using a **custom template**. If you run a protocol using a standard template, well data is collected but no image is generated. (See section 4.1.2 for more information about custom versus standard templates.)

To open an image, select *Raw Image Report* from the **VIEW** menu.



Section of plate with absorbance data

Figure 53. Example of a Raw Image Report (1536-well plate).

- Kinetic protocols will create multiple images: one for each absorbance reading.

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Viewing an Image

The **IMAGE** menu contains special tools for viewing your images.

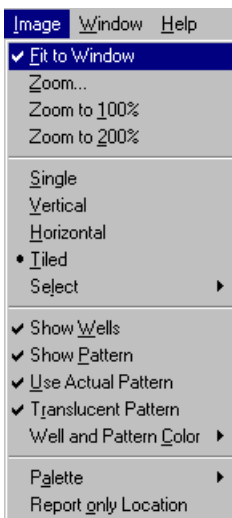


Figure 54. Image menu.

The image is displayed in a window that can be **resized** by dragging the lower right corner of the window in and out. If *Fit to Window* is selected in the **IMAGE** menu, when you resize the window, the image will resize as well. If *Fit to Window* is deselected, the image will not resize when you resize the window, and scroll bars will appear if the image becomes too large to fit in the window.

You can magnify your image by selecting a **zoom** factor from the **IMAGE** menu. Selecting *Zoom...* allows you to zoom in by clicking directly on the image and zoom out by clicking on the image while holding down the Shift key.

Kinetic protocols create **multiple images**: one for each absorbance reading. Under the **IMAGE** menu, you can arrange these vertically by selecting *Vertical* or in rows by selecting *Horizontal*. You can tile the images by selecting *Tile*, or display only one image at a time by selecting *Single*. If you are displaying images one at a time,

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select the one to be displayed by choosing *Select* from the **IMAGE** menu and selecting the appropriate image number.

When you move your cursor over the image, the **cursor location** is displayed in the status bar at the bottom of the Microplate Manager window. When the cursor is over a well, the well number will be displayed next to the cursor and in the status bar. In addition, if applicable, the assay number, well formatting, and current value of the well are displayed in the status bar. If you select *Report Only Location* from the **IMAGE** menu, the coordinates of the cursor and the absorbance value at that point will be displayed, but not the well information.

Image Features

- In studying the features of a microplate image, it is useful to first magnify the image using the zoom tools.

A typical microplate image shows the microplate grid, the outlines of the defined wells, and the defined area of the well that data was collected from.

If *Show Wells* on the **IMAGE** menu is selected, color representations of the individual wells will be displayed. The size and shape of these wells are specified in the **Physical Dimensions** dialog box (see below, and in section 4.1.2).

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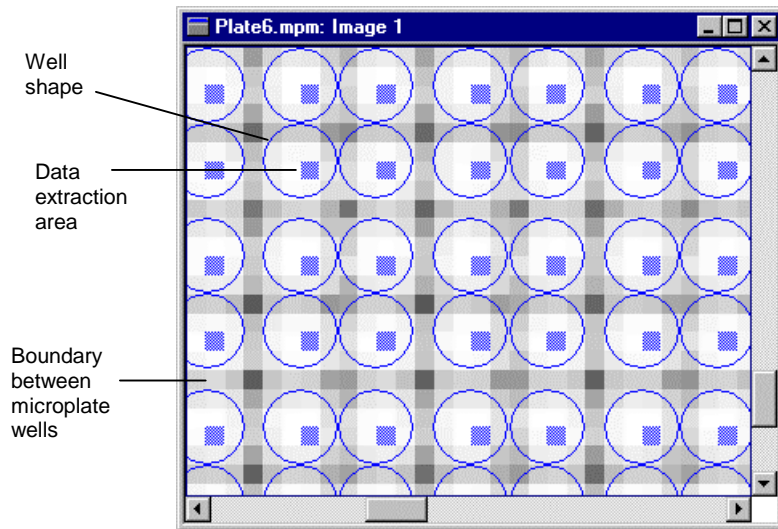


Figure 55. Close-up of a microplate image.

Show Pattern on the **IMAGE** menu displays the area of the well used for data collection (i.e., the extraction area). This too is specified in the **Physical Dimensions** dialog box (see below, and in section 4.1.2).

Show Translucent Pattern toggles the pattern display between transparent and opaque.

Well and Pattern Color allows you to change the color used to display wells and patterns.

Palette allows you to change the color of the entire image.

Formatting the Image

The **Raw Image Report** has a number of special formatting features.

From the **FORMAT** menu, you can change the dimensions of your custom template. Select a different template size by selecting *Plate Size...* or specify different physical

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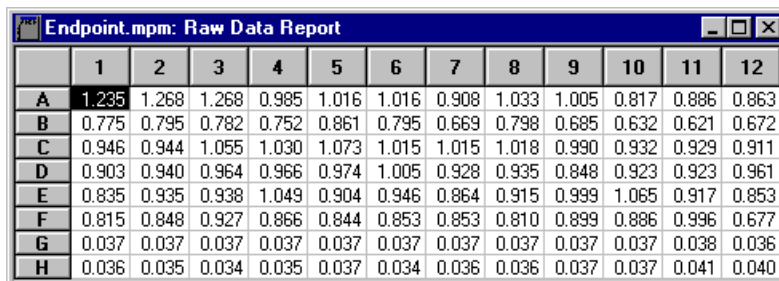
dimensions for your template by selecting *Physical Dimensions....* See section 4.1.2 for details on these features.

Many of these formatting settings affect what part of the image your data is collected from, and thus affect the data itself. For example, if you change the extraction area in the **Physical Dimensions** dialog box, this will change the area of the well that is used for data collection, which will change the data itself.

Because formatting changes can affect your data, **multi-level undo and redo** are supported for the image formatting commands. Select *Undo Plate Format* from the **EDIT** menu to undo each formatting change, or *Redo Plate Format* to redo a formatting change.

6.6.3 Reports Available for Endpoint and Multiple Plate Data Only

Raw Data Report



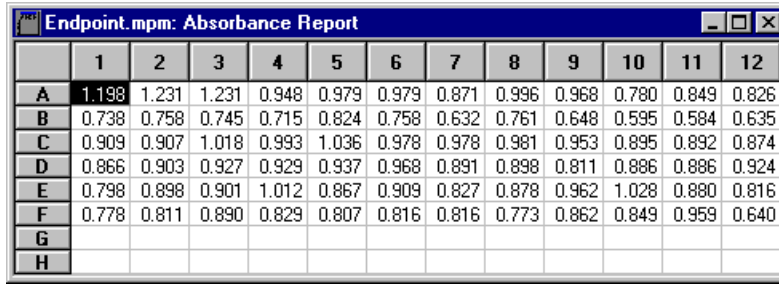
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.235	1.268	1.268	0.985	1.016	1.016	0.908	1.033	1.005	0.817	0.886	0.863
B	0.775	0.795	0.782	0.752	0.861	0.795	0.669	0.798	0.685	0.632	0.621	0.672
C	0.946	0.944	1.055	1.030	1.073	1.015	1.015	1.018	0.990	0.932	0.929	0.911
D	0.903	0.940	0.964	0.966	0.974	1.005	0.928	0.935	0.848	0.923	0.923	0.961
E	0.835	0.935	0.938	1.049	0.904	0.946	0.864	0.915	0.999	1.065	0.917	0.853
F	0.815	0.848	0.927	0.866	0.844	0.853	0.853	0.810	0.899	0.886	0.996	0.677
G	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.036
H	0.036	0.035	0.034	0.035	0.037	0.034	0.036	0.036	0.037	0.037	0.041	0.040

Figure 56. Raw Data Report.

The **Raw Data Report** contains the absorbance readings exactly as they are received from the reader. A missing or out-of-range value is indicated by three asterisks (***)

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Absorbance Report



	1	2	3	4	5	6	7	8	9	10	11	12
A	1.198	1.231	1.231	0.948	0.979	0.979	0.871	0.996	0.968	0.780	0.849	0.826
B	0.738	0.758	0.745	0.715	0.824	0.758	0.632	0.761	0.648	0.595	0.584	0.635
C	0.909	0.907	1.018	0.993	1.036	0.978	0.978	0.981	0.953	0.895	0.892	0.874
D	0.866	0.903	0.927	0.929	0.937	0.968	0.891	0.898	0.811	0.886	0.886	0.924
E	0.798	0.898	0.901	1.012	0.867	0.909	0.827	0.878	0.962	1.028	0.880	0.816
F	0.778	0.811	0.890	0.829	0.807	0.816	0.816	0.773	0.862	0.849	0.959	0.640
G												
H												

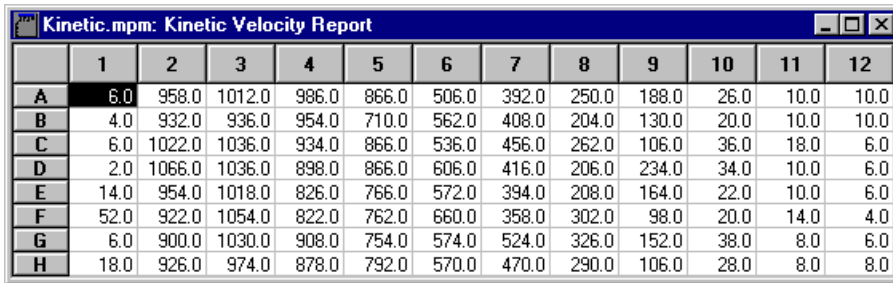
Figure 57. Absorbance Report.

In the **Absorbance Report**, the mean absorbance of all the blank wells in an assay is subtracted from the raw absorbance for each non-blank well in the same assay. A negative value will be reported if the mean absorbance of the blank wells is greater than the absorbance of the unknown well.

Absorbances are displayed only for standards, unknowns, and positive and negative controls.

6.6.4 Reports Available for Kinetic Data Only

Velocity Report



	1	2	3	4	5	6	7	8	9	10	11	12
A	6.0	958.0	1012.0	986.0	866.0	506.0	392.0	250.0	188.0	26.0	10.0	10.0
B	4.0	932.0	936.0	954.0	710.0	562.0	408.0	204.0	130.0	20.0	10.0	10.0
C	6.0	1022.0	1036.0	934.0	866.0	536.0	456.0	262.0	106.0	36.0	18.0	6.0
D	2.0	1066.0	1036.0	898.0	866.0	606.0	416.0	206.0	234.0	34.0	10.0	6.0
E	14.0	954.0	1018.0	826.0	766.0	572.0	394.0	208.0	164.0	22.0	10.0	6.0
F	52.0	922.0	1054.0	822.0	762.0	660.0	358.0	302.0	98.0	20.0	14.0	4.0
G	6.0	900.0	1030.0	908.0	754.0	574.0	524.0	326.0	152.0	38.0	8.0	6.0
H	18.0	926.0	974.0	878.0	792.0	570.0	470.0	290.0	106.0	28.0	8.0	8.0

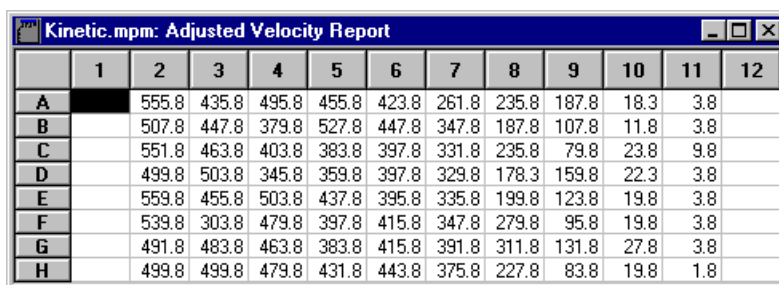
Figure 58. Velocity Report.

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The **Velocity Report** displays the velocities of your well readings, based on the analysis options you selected when you ran your kinetic protocol.

You can reopen the **Analysis Options** dialog box and change the settings by selecting *Analysis Options* from the **OPTIONS** menu. See section 5.3.2 for more information.

Adjusted Velocity Report



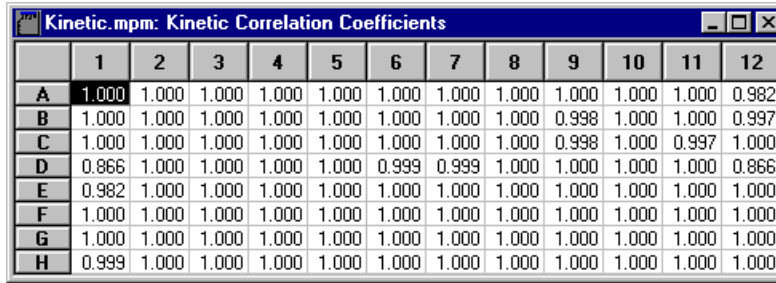
	1	2	3	4	5	6	7	8	9	10	11	12
A		555.8	435.8	495.8	455.8	423.8	261.8	235.8	187.8	18.3	3.8	
B		507.8	447.8	379.8	527.8	447.8	347.8	187.8	107.8	11.8	3.8	
C		551.8	463.8	403.8	383.8	397.8	331.8	235.8	79.8	23.8	9.8	
D		499.8	503.8	345.8	359.8	397.8	329.8	178.3	159.8	22.3	3.8	
E		559.8	455.8	503.8	437.8	395.8	335.8	199.8	123.8	19.8	3.8	
F		539.8	303.8	479.8	397.8	415.8	347.8	279.8	95.8	19.8	3.8	
G		491.8	483.8	463.8	383.8	415.8	391.8	311.8	131.8	27.8	3.8	
H		499.8	499.8	479.8	431.8	443.8	375.8	227.8	83.8	19.8	1.8	

Figure 59. Adjusted Velocity Report.

The **Adjusted Velocity Report** displays the adjusted velocity for each well calculated by subtracting the mean velocity of the blank wells from the raw velocities displayed in the Velocity Report.

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Kinetic Correlation Coefficient Report

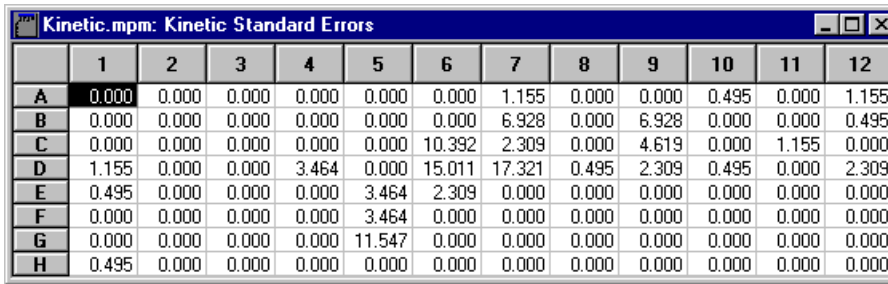


	1	2	3	4	5	6	7	8	9	10	11	12
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.982
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.998	1.000	1.000	0.997
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.998	1.000	0.997	1.000
D	0.866	1.000	1.000	1.000	1.000	0.999	0.999	1.000	1.000	1.000	1.000	0.866
E	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
F	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
H	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Figure 60. Kinetic Correlation Coefficient Report.

The **Kinetic Correlation Coefficient Report** displays the correlation coefficient of the velocity segment for each well. The correlation coefficient, or “r” value, is a measure of the precision of the fit of the linear regression to the raw data. An “r” value of 1.00 represents a perfect correlation. See the discussion in section 6.8.

Kinetic Standard Errors Report



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.000	0.000	0.000	0.000	0.000	1.155	0.000	0.000	0.495	0.000	1.155
B	0.000	0.000	0.000	0.000	0.000	0.000	6.928	0.000	6.928	0.000	0.000	0.495
C	0.000	0.000	0.000	0.000	0.000	10.392	2.309	0.000	4.619	0.000	1.155	0.000
D	1.155	0.000	0.000	3.464	0.000	15.011	17.321	0.495	2.309	0.495	0.000	2.309
E	0.495	0.000	0.000	0.000	3.464	2.309	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	3.464	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	11.547	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.495	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 61. Kinetic Standard Errors Report.

The **Kinetic Standard Errors Report** shows the calculated standard error of the velocity for each well. If you have large standard errors (more than 5–10% of the corresponding velocity), you should reset the limits (under **OPTIONS** > *Analysis Options...*) to focus on the linear portion of the results.

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Kinetic Plots Report

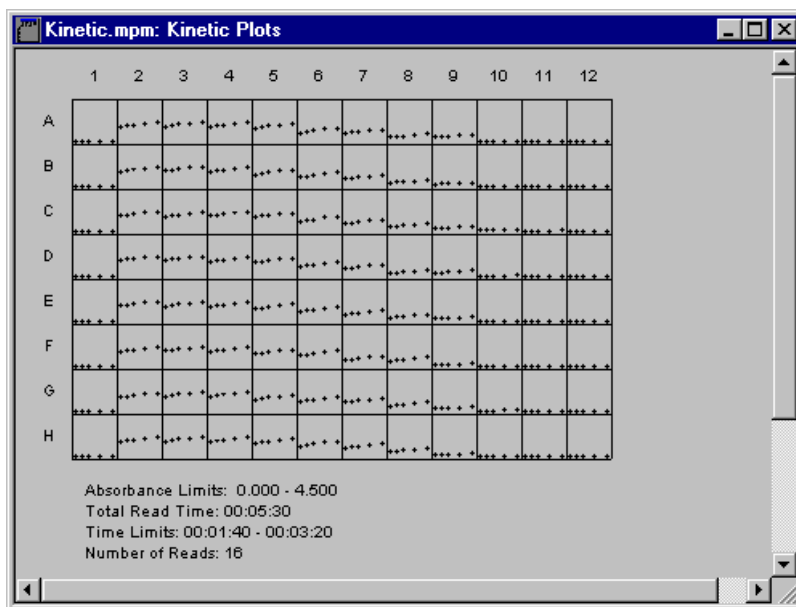


Figure 62. Kinetic Plots Report.

Using the **Kinetic Plots Report**, you can display a plot of the change in absorbance with time for each well. The absorbance limits, total read time, time limits, and number of readings are listed below the chart.

When you move your cursor over the report, the well numbers will appear next to the cursor, and the standard pointer will change to a magnifying glass. To magnify individual wells, click on the wells inside the kinetic plot. A **Kinetic Zoom Plot** window will open for each well you click on.

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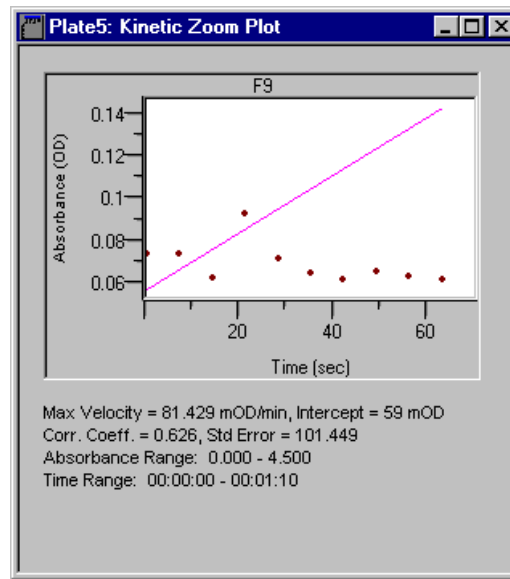


Figure 63. Kinetic Zoom Plot.

This window shows additional velocity information for a single well. It displays a plot of the best linear curve fit and lists the calculated velocity, intercept, correlation coefficient, and standard error for the graph. Below the calculated values, the absorbance range and time range are listed.

- The data in the Kinetic Zoom Plot window is **auto-scaled** based on the data range for that particular well.

6.6.5 Reports Common to all Reading Types

- For the reports that use limits (Limit, Matrix, Control, and Normalization), the value of the lower limit cannot be greater than the value of the upper limit. The program will display an error message if there are conflicting values for the limits.

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Limit Report

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

- : Absorbance (OD) < 0.640
+ : Absorbance (OD) > 1.036
* : 0.640 <= Absorbance (OD) <= 1.036
? : Not available

Figure 64. Limit Report.

If you have defined upper and lower limits for your data, each well is tagged with a “+” if its absorbance/velocity is greater than the upper limit, a “*” if its absorbance/velocity is between the upper and lower limit, and a “-” if its absorbance/velocity is below the lower limit.

Setting Data Limits

The limits can be set or changed by selecting *Limits...* from the **OPTIONS** menu. This will open the **Limits** dialog box.

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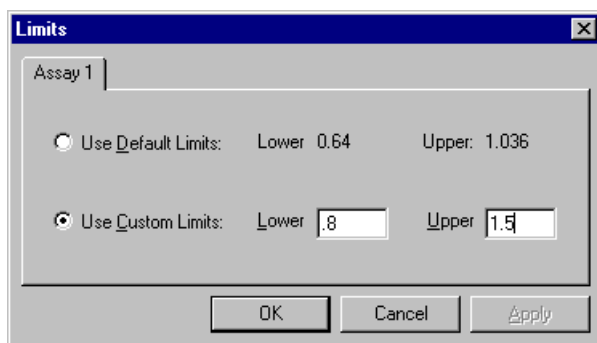


Figure 65. Limits dialog box.

Limits are assigned independently for each assay in a protocol. When there is more than one assay, tabs labeled with each assay number appear in the dialog box. To switch from one assay to another, click on the appropriate tab.

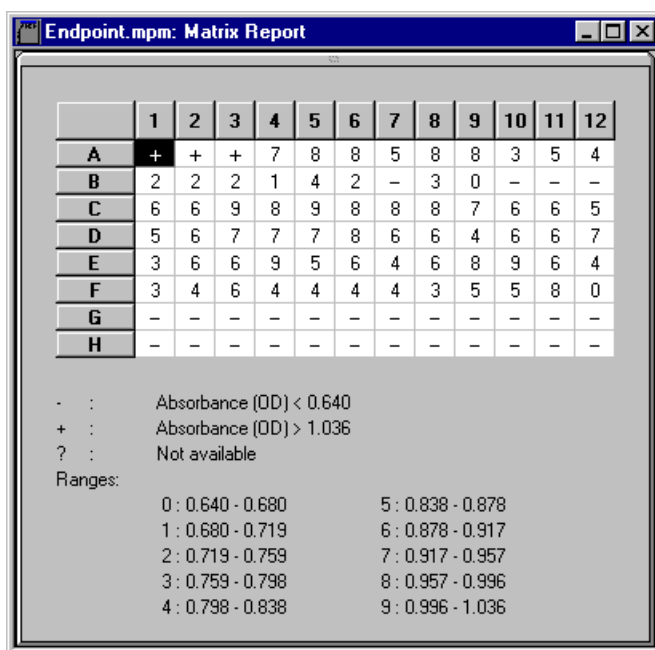
To set your own limits, select the *Use Custom Limits* option and enter numbers in the appropriate fields.

- For kinetic data, your limits will be adjusted velocities; for endpoint data, your limits will be absorbances.

If *Use Default Limits* is selected, the unknown sample with the greatest absorbance (for kinetic reports, the sample with the greatest adjusted velocity) will be used as the upper limit, and the unknown sample with the lowest absorbance (lowest adjusted velocity) will be used as the lower limit. If no unknowns are specified, the default value for both upper and lower limits is 0.000.

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Matrix Report



The screenshot shows a window titled "Endpoint.mpm: Matrix Report". It contains a 12x8 grid of data points. The columns are numbered 1 through 12, and the rows are labeled A through H. The data points are either numbers (0-9), plus signs (+), minus signs (-), or question marks (?). Below the grid is a legend explaining the symbols and a list of 10 numerical ranges.

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

- : Absorbance (OD) < 0.640
+ : Absorbance (OD) > 1.036
? : Not available

Ranges:

0 : 0.640 - 0.680	5 : 0.838 - 0.878
1 : 0.680 - 0.719	6 : 0.878 - 0.917
2 : 0.719 - 0.759	7 : 0.917 - 0.957
3 : 0.759 - 0.798	8 : 0.957 - 0.996
4 : 0.798 - 0.838	9 : 0.996 - 1.036

Figure 66. Matrix Report.

The **Matrix Report** is similar to the **Limit Report** (see above), except that the data between the upper and lower limits are divided into 10 equal ranges, numbered 0–9. Each well with data within the limits is assigned a number, based on which range its data falls into.

See the description of the **Limit Report** for instructions on how to set limits.

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Control Report

	1	2	3	4	5	6	7	8	9	10	11	12
A	97.32	100.00	100.00	77.01	79.53	79.53	70.76	80.91	78.64	63.36	68.97	67.10
B	59.95	61.58	60.52	58.08	66.94	61.58	51.34	61.82	52.64	48.33	47.44	51.58
C	73.84	73.68	82.70	80.67	84.16	79.45	79.45	79.69	77.42	72.71	72.46	71.00
D	70.35	73.35	75.30	75.47	76.12	78.64	72.38	72.95	65.88	71.97	71.97	75.06
E	64.83	72.95	73.19	82.21	70.43	73.84	67.18	71.32	78.15	83.51	71.49	66.29
F	63.20	65.88	72.30	67.34	65.56	66.29	66.29	62.79	70.02	68.97	77.90	51.99
G	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	-0.08
H	-0.08	-0.16	-0.24	-0.16	0.00	-0.24	-0.08	-0.08	0.00	0.00	0.32	0.24

Positive Control : 1.231
Negative Control : 0.000
?: Not available
Values are percentages of the range defined by the positive and negative controls or their default values.

Figure 67. Control Report.

The **Control Report** displays absorbance or velocity data for each well as a percentage of the range between the positive and negative controls in the assay.

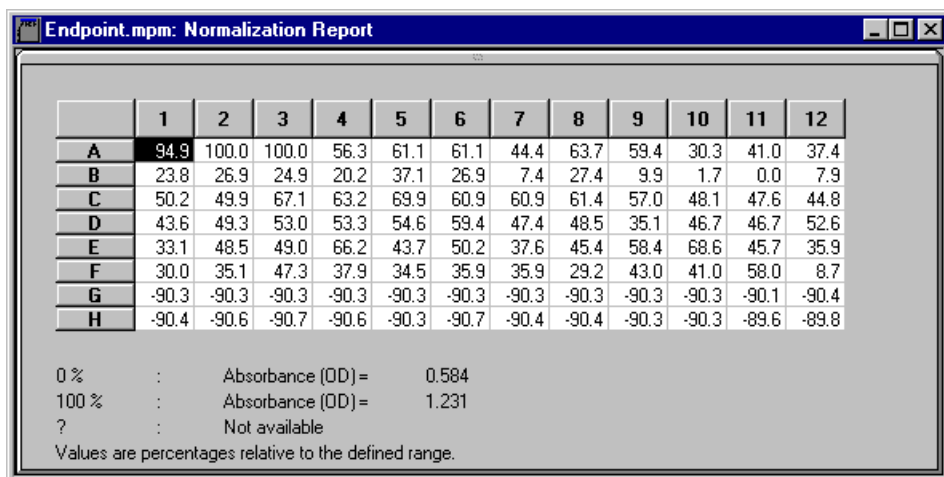
Controls are defined in templates. For more information about controls, see section 4.3.

If no positive control is defined for the assay, the highest absorbance value or highest velocity among the wells defined as unknowns or standards is used. If no negative control is defined, then 0.000 is used as the negative control.

- Controls are different than limits. Any limits you set will not affect this report.

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Normalization Report



The screenshot shows a window titled "Endpoint.mpm: Normalization Report". It contains a table with 12 columns and 8 rows of data. Below the table, there are summary statistics for 0%, 100%, and a question mark, and a note about the values being percentages relative to the defined range.

	1	2	3	4	5	6	7	8	9	10	11	12
A	94.9	100.0	100.0	56.3	61.1	61.1	44.4	63.7	59.4	30.3	41.0	37.4
B	23.8	26.9	24.9	20.2	37.1	26.9	7.4	27.4	9.9	1.7	0.0	7.9
C	50.2	49.9	67.1	63.2	69.9	60.9	60.9	61.4	57.0	48.1	47.6	44.8
D	43.6	49.3	53.0	53.3	54.6	59.4	47.4	48.5	35.1	46.7	46.7	52.6
E	33.1	48.5	49.0	66.2	43.7	50.2	37.6	45.4	58.4	68.6	45.7	35.9
F	30.0	35.1	47.3	37.9	34.5	35.9	35.9	29.2	43.0	41.0	58.0	8.7
G	-90.3	-90.3	-90.3	-90.3	-90.3	-90.3	-90.3	-90.3	-90.3	-90.3	-90.1	-90.4
H	-90.4	-90.6	-90.7	-90.6	-90.3	-90.7	-90.4	-90.4	-90.3	-90.3	-89.6	-89.8

0 % : Absorbance (OD) = 0.584
100 % : Absorbance (OD) = 1.231
? : Not available
Values are percentages relative to the defined range.

Figure 68. Normalization Report.

The **Normalization Report** displays absorbance or velocity data for each well as a percentage of a range defined by the limits.

To set your limits, select *Limits...* from the **OPTIONS** menu. See the description of the **Limit Report** above for more information about setting limits.

If custom limits are not selected in the **Limits** dialog box, the default upper limit is the highest standard, unknown, or control absorbance/velocity value in the assay, and the default lower limit is the lowest standard, unknown, or control absorbance/velocity value in the same assay.

- The default values displayed in the **Limits** dialog box are the ones used by the **Limit Report** and the **Matrix Report**, but the default values used in the **Normalization Report** are determined as described above.

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Standard Curve Report

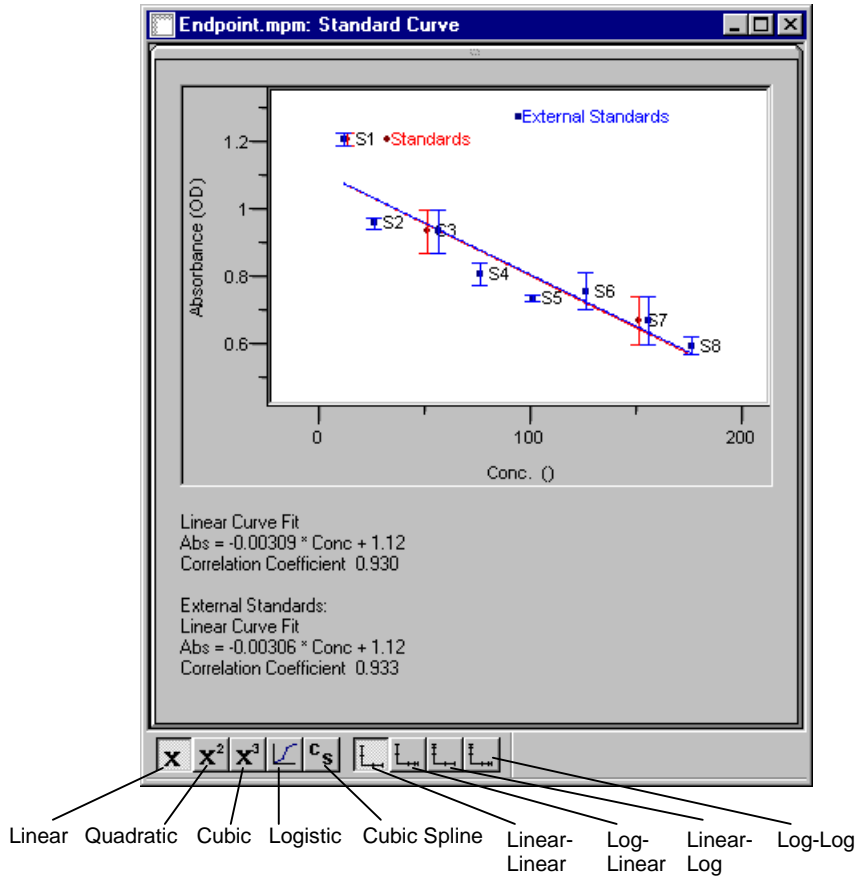


Figure 69. Standard Curve Report.

The **Standard Curve Report** displays the absorbances of the standards plotted against their concentrations, as well as the best curve fit for those data. If replicates have been made of the standards, error bars are also plotted on the graph corresponding to the standard errors.

If more than one assay has been defined on your plate, you can switch between assays by clicking on the appropriate tabs. If both external and internal standards are

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defined, they are plotted together with their respective regression curves (although only the external standards are used to calculate unknown concentrations).

In calculating the curve, you can select among five regression methods: Linear, quadratic, cubic, cubic spline, and logistic. To change the regression method, click on the appropriate button at the bottom of the dialog box, or select the desired method under the **OPTIONS** menu.

- For more information about **standard curve fitting**, see section 6.8 below.

You can also change the axis scale among four different combinations of linear and logarithmic (linear-linear, log-linear, linear-log, and log-log) for every regression method except logistic; the logistic curve fit is constrained to linear-linear axes. To change the axis scale, click on the appropriate button at the bottom of the dialog box, or select the desired scale under the **OPTIONS** menu.

- If you are plotting negative kinetic velocities, then the velocity or maximum velocity axis of the **Standard Curve Report** is constrained to linear unless the *Negative Kinetics* box is checked (logarithms cannot be taken of negative values). See section 5.3.2.

When the mouse is moved within the graph area, the coordinates of the pointer appear in the status bar at the bottom of the Microplate Manager window. (The status bar display is toggled on and off by selecting *Status Bar* under the **VIEW** menu).

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Unknown Concentrations Report

Label	Mean Absorb. (OD)	Std. Dev.	%CV	Conc.	Dilut.
S1	1.220	0.019	1.56	12.000	
S2	0.969	0.018	1.85	25.000	
S3	0.945	0.066	6.94	50.000	
S4	0.818	0.035	4.29	75.000	
S5	0.747	0.010	1.36	100.000	
S6	0.766	0.055	7.17	125.000	
S7	0.680	0.070	10.34	150.000	
S8	0.605	0.027	4.44	175.000	
X1	0.908	0.001	0.16	47.699	1.000
X2	1.005	0.018	1.76	30.929	1.000
X3	1.007	0.041	4.07	30.648	1.000
X4	0.979	0.002	0.22	34.781	1.000
X5	0.924	0.041	4.44	44.423	1.000
X6	0.883	0.013	1.44	53.348	1.000
X7	0.885	0.026	2.96	52.870	1.000

Logistic Curve Fit
Abs = (1.37 - 0.5) / (1 + [Conc / 42.2]^{1.03}) + 0.5
¹ Extrapolating above highest standard.
² Extrapolating below lowest standard.
³ Replicate set includes outliers.

Figure 70. Unknown Concentration Report

The **Unknown Concentrations Report** shows the calculated concentrations of the unknown samples and the dilution and concentration information for all standards. This report also provides statistics for each replicate group of wells, including mean, standard deviation, and coefficient of variation.

At the bottom of the window, the type of curve fit and its equation are shown. If both internal and external standards are defined, then both equations are displayed even though only the external standards are used to calculate the unknown concentrations. The type of axis selected may be inferred by examining the equation. For example, if using a Log-Linear transformation, “Conc” is replaced with “Log(Conc)” on the right-hand side of the equation.

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To change curve fit options or axis types, open the **Standard Curve Report** and select the desired regression method and axis type from the buttons in the dialog box.

6.7 Standard Curve Comparison

When you are using external standards, it is prudent to validate them from time to time. After a large number of readings, you should analyze the standards again and create a new (internal) calibration curve. The internal calibration curve can be compared to the external curve to see if there is a statistically significant difference between the two.

To validate your external standards, first read a plate with both internal and external standards, then select *Standard Curve Report...* from the **VIEW** menu. Both standard curves will be plotted. To compare the two, select *Analysis of Variance...* from the **OPTIONS** menu.

Variation	Degree of Freedom	Mean Square	F
Due to Hypothesis	2	0.015	0.44 ns 34.5%
Due to Error	12	0.033	

Figure 71. Analysis of Variance box.

In the **Analysis of Variance** dialog box, the key statistics are listed in the last column. Within this last column, there are three subcolumns. From left to right, these subcolumns show the F statistic, the significance, and the probability that the difference between the two curves is significant. There are four degrees of significance: not significant, significant, highly significant, and extremely significant. These are indicated by the following symbols: ns, *, **, ***.

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If there is a significant difference between standard curves that should be essentially identical, the user should investigate the source of this difference.

Note that these statistics depend on the type of curve fit chosen. Changing the regression method and axis type will change the analysis of variance.

This information will also be printed at the bottom of the **Standard Curve Report**.

An exhaustive discussion on the analysis of variance is beyond the scope of this manual. The interested user is referred to *Biometry* by Robert R. Sokal and F. James Rohlf, W.H. Freeman and Company, New York, 1981.

6.8 Standard Curve Fitting

The Microplate Manager program provides powerful standard curve fitting and data reduction routines. You can generate the standard curves by five different methods: Linear, quadratic and cubic polynomial regression, cubic spline, and four-parameter logistic curve fitting. For the first four methods, the axes may also be in four different combinations of linear and logarithmic (i.e., linear-linear, log-linear, linear-log and log-log); the logistic curve fit is constrained to linear-linear axes. When used properly, these can greatly enhance and simplify quantitative microplate assay evaluations.

Overview of Regression Methods

Polynomial regression and logistic curve fits are examples of global construction of a calibration function, while the cubic spline is an example of a local approach. The global approach involves using the entire set of standards for calibrating, while the local approach employs a varying subset of the standards. The global approach provides better noise immunity and allows for minor extrapolation when a calibration function is used to analyze a physical process whose mathematical form is well established in the full range or at least the range of interest.

Cubic regression as a global function is useful for interpolating nonsymmetrical functional dependencies with an inflection point (such as the plot of migration distance versus the logarithm of the molecular weight). Higher orders of polynomial regressions are rarely used for calibration if a good fit is not found with linear,

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quadratic, or cubic polynomial regression, because of the oscillatory nature of the resultant fit. In these cases, a local fit (cubic spline) would be preferable.

The concentration of unknowns is determined by substituting the mean absorbance values of the unknowns in the regression equation and solving for the concentration. Microplate Manager performs data reduction without artificial limitations or constraints. This provides added flexibility, but leaves the final interpretation of the results to the user.

In general, you should start with the simplest curve fitting routine (i.e., linear) and examine all four combinations of axes for an acceptable curve fit. If a satisfactory fit is not obtained, then progressively increase the sophistication of the curve fitting until a good fit is obtained.

Ways to Increase Reliability

There are several approaches that you can take to increase the reliability of the results:

- Run an adequate number of standard dilutions to form a broad dose response range. Twelve standards of 1:2 dilutions provide a 3.5 log range of standards, which is usually adequate for most applications.
- Run at least three replicate wells for each dilution of standards and samples.
- Run an adequate number of sample dilutions to ensure that more than one dilution lies in the middle of the curve. Running several dilutions will point out the limitations of the curve-fitting methodologies.

Goodness of Fit

The correlation coefficient (r) gives an indication of how well the polynomial regression (linear, quadratic and cubic) model fits the data. In effect, the correlation coefficient is the square root of the proportion of explained variation to total variation of the regression.

$$r = (\text{explained variation} / \text{total variation})^{1/2}$$

The amount of explained variation increases as the goodness of fit increases. In a perfect fit, all of the variation is explained, the explained variation equals the total

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variation, and the ratio of the explained variation to total variation becomes 1.000. Or, more simply, the closer the correlation coefficient is to 1.000, the better the fit of the regression.

Since the cubic spline is a local function, the r value is meaningless and no goodness of fit may be assigned. (The cubic spline forces the curve through all the data points.)

Limitations of the Methods

Results calculated from mean absorbance data outside the range of the standard curve may be unreliable. (For the purposes of this discussion, the range of the standard curve is defined as the absorbance range between the highest and lowest mean absorbances of the standards used to form the standard curve.) Calculated results that are determined from mean absorbance results outside the range of the standard curve are extrapolations, and should be treated as such when determining the final results of your analysis. Microplate Manager indicates in the **Unknown Concentration Report** when the standard curves are extrapolated to determine the concentration of an unknown.

Results calculated from mean absorbance results at the extremes of the range of the standard curve may also be unreliable. The linear regression, when properly fitted, generally produces reliable results at the extremes of the range of the standard curve. However, the quadratic, cubic, and logistic methods, by their mathematical natures, can produce unreliable results at the extremes of the standard curve. None of the methods will give a unique concentration for a given absorbance if the calibration data is not monotonic in x (i.e., there is more than one x value associated with a unique y value).

7. Data Output

This chapter describes Microplate Manager's functions for printing reports and exporting data.

7.1 Printing

All the reports may be printed to any printer supported by Windows. To print a report when a report window is active, choose *Print...* from the **EDIT** menu. This will open the standard Windows **Print** dialog box.

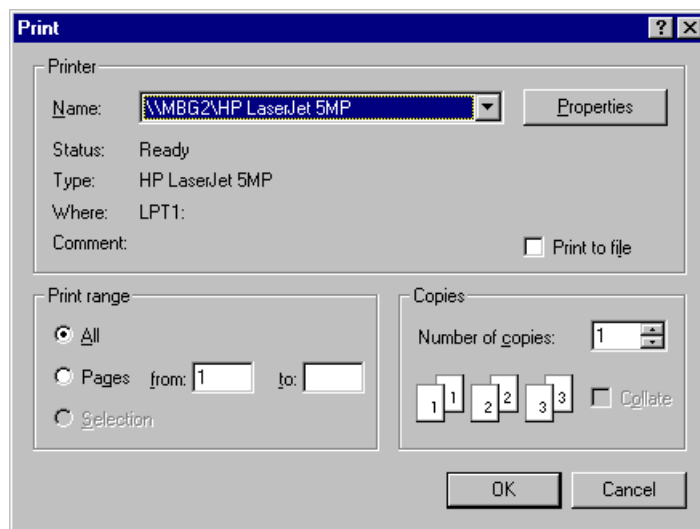


Figure 72. Print dialog box.

For additional settings such as page orientation, paper source, and resolution, click on the *Properties...* button.

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- To automatically print reports immediately after a reading has been performed, click on the *Reports...* button in a protocol dialog box. This will open a list of reports. Click on the appropriate checkbox(es) to print the desired report (s). When the protocol is finished running, the selected report(s) will be printed. See section 5.1.6 for more information.

To select the font that will be used to print the report, select *Page Setup...* from the **FILE** menu.

To preview your printout, select *Print Preview* from the **FILE** menu.

Printed reports include a header with information describing how the results were derived. The following information is printed in the header of each report.

- Title of the report.
- Name of the data file.
- Date and time of data acquisition.
- Assay name. When assay-based reports are printed, each assay will begin on a new page.
- Any labels that have been defined.
- Reading parameters. These vary depending on the read type. In all cases, the measurement and reference (if used) wavelengths are printed.
- Notes (if any).

Note that protocol settings cannot be printed by themselves.

7.2 Data Import and Export

Data in Microplate Manager can be imported and exported in a variety of formats.

7.2.1 Exporting Data

All text-based data and reports may be exported as tab-separated values or as comma-separated values. You can also export the plate template format. The graph-based reports may not be exported.

To export, with your template or text-based data window open, select *Export...* from the **FILE** menu. From the *Save as type:* pull-down list, select the format you want to export in.

Templates can be saved in .eps or .epf format.

Data and reports can be saved as tab-delimited (.txt) or comma-delimited (.csv) values.

There is a third export format (COL) for kinetic data. In this format, all the readings are written to a file. See below for a more detailed description of this format.

Exporting to Excel

If you are exporting your data to Excel, you can select additional formatting options. Select *Export to Excel...* from the **FILE** menu. This will open the **Export to Excel** dialog box.

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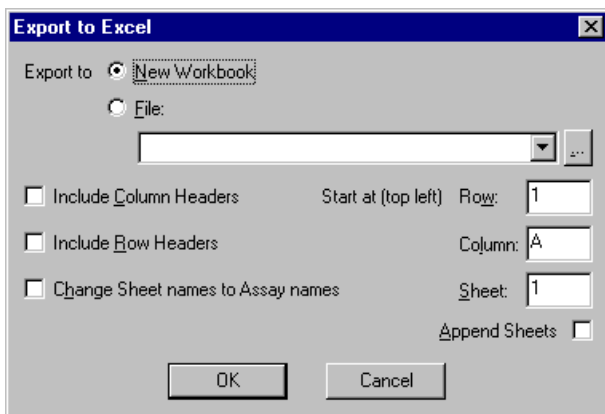


Figure 73. Export to Excel dialog box.

Select the Excel formatting features you want, and click on *OK* to export your data.

7.2.2 Importing Data

Files from the previous version of Microplate Manager can be read into this version of the software. Tab-delimited and comma-separated data are also supported (TXT, CSV, LOT, ASC). From Kinetic Collector PC, data files in matrix format, row format, or column format may be imported (MTX, ROW, or COL).

7.2.3 Kinetic Data Formats

COL Format

The column format consists of $(4 + (\# \text{ of rows}) * (\# \text{ of cols}))$ lines.

Line 1: "Col" (format signature)

Line 2: # of readings (each in its own column)

Line 3: #rows<tab>#cols (if this line is left empty, 12<tab>8 is used)

Line 4: timestamp1<tab>timestamp2<tab>...<tab>timestampN (timestamp format HH:MM:SS)

Line 5: <row1:col1><tab><row1:col1><tab>...<tab><row1:col1>

...

Line (col# + (4 + (row# - 1)*(# of cols))): <row1:colN><tab><row1:colN><tab>...<tab><row1:colN>

Line (col# + (4 + (row# - 1)*(# of cols))): <row2:col1><tab><row2:col1><tab>...<tab><row2:col1>

...

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Line (4 + (# of rows) * (# of cols)): <rowN:colN><tab><rowN:colN><tab>...<tab><rowN:colN>

ROW Format

This format consists of (# of readings + 3) lines.

Line 1: "Row" (format signature)

Line 2: # of readings (each in its own row)

Line 3: #rows<tab>#cols (if this line is left empty, 12<tab>8 is used)

Line 4: timestamp1<tab><row1:col1><tab><row1:col2><tab> ...
<tab><row1:colN><tab><row2:col1><tab> ... <tab><rowN:colN>

Line 5: timestamp2<tab><row1:col1><tab><row1:col2><tab> ...
<tab><row1:colN><tab><row2:col1><tab> ... <tab><rowN:colN>

...

Line (# of readings + 3): timestampN<tab><row1:col1><tab><row1:col2><tab> ...
<tab><row1:colN><tab><row2:col1><tab> ... <tab><rowN:colN>

MTX Format

The format consists of ((# of readings - 1)*(# of rows + 2) + 4) lines.

Line 1: "MTX" (format signature)

Line 2: # of readings (each reading in its own matrix)

Line 3: #rows<tab>#cols (if this line is left empty, 12<tab>8 is used)

Line ((reading# - 1)*(# of rows + 2) + 4): timestamp1

Line ((reading# - 1)*(# of rows + 2) + row# + 4):
<row1:col1><tab><row1:col2><tab>...<tab><row1:colN>

...

Line ((reading# - 1)*(# of rows + 2) + row# + 4):
<rowN:col1><tab><rowN:col2><tab>...<tab><rowN:colN>

Line ((reading# - 1)*(# of rows + 2) + row# + 5): #rows<tab>#cols (if this line is left empty, 12<tab>8 is used)

Line ((reading# - 1)*(# of rows + 2) + 4): timestamp2

Line ((reading# - 1)*(# of rows + 2) + row# + 4):
<row1:col1><tab><row1:col2><tab>...<tab><row1:colN>

...

Line ((reading# - 1)*(# of rows + 2) + row# + 4):
<rowN:col1><tab><rowN:col2><tab>...<tab><rowN:colN>

Line ((reading# - 1)*(# of rows + 2) + row# + 5): #rows<tab>#cols (if this line is left empty, 12<tab>8 is used)

...

Line ((reading# - 1)*(# of rows + 2) + 4): timestampN

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Line $((\text{reading\#} - 1) * (\# \text{ of rows} + 2) + \text{row\#} + 4)$:
`<row1:col1><tab><row1:col2><tab>...<tab><row1:colN>`

...

Line $((\text{reading\#} - 1) * (\# \text{ of rows} + 2) + \text{row\#} + 4)$: `H1<tab>H2<tab>...<tab>H12`

Line $((\text{reading\#} - 1) * (\# \text{ of rows} + 2) + \text{row\#} + 5)$: `#rows<tab>#cols` (if this line is left empty, 12<tab>8 is used)

8. Quick Guides

8.1 Creating a New Template

See Chapter 4 for details.

1. Choose *New Template > 12 x 8* from the **FILE** menu.
2. Choose *Assay* from the **FORMAT** menu (or click on the *Assay* tool of the template toolbar) and define the location of each separate assay.
3. Choose the appropriate well type (*Standard, Unknown, Positive Control, Negative Control, Blank*) from the **FORMAT** menu (or click on the appropriate icon on the toolbar) and define each well in each assay.
4. Set the concentrations of the standards by choosing *Edit Standard Conc...* from the **EDIT** menu.
5. Set the dilutions of the unknowns by choosing *Edit Unknown Dilutions...* from the **EDIT** menu.
6. Choose *Assay Info...* from the **ASSAY** menu to customize the name and display color of each assay and to enter specific notes about each assay that will be printed with reports.
7. For each assay, choose the type of regression and the type of axes used to generate the **Standard Curve** and **Unknown Concentration** reports:
 - a. Select *Standard Curve Report* from the **VIEW** menu.
 - b. Use the tabs to select the assay.
 - c. Choose the desired regression method from the toolbar or **OPTIONS** menu.
 - d. Choose the desired type of axes from the toolbar or **OPTIONS** menu.
 - e. Repeat steps b–d for the other assays.
 - f. Close the **Standard Curve Report** window.
8. For each assay, select the external standards (if any) to be used:

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- a. Select *Standard Curve Report* or *Unknown Concentration Report* from the **VIEW** menu.
 - b. Use the tabs to select the assay.
 - c. Choose *External Standards...* from the **OPTIONS** menu.
 - d. Choose source file for the external standards.
 - e. Choose the source assay from within the source file.
 - f. Repeat steps b–e for the other assays.
 - g. Close the **Standard Curve Report** or **Unknown Concentration Report**.
9. Choose *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu to save the template into a data file.

8.2 Creating and Running a New Kinetic Protocol with a Stored Template

See Chapter 5 for details.

1. Select *New Kinetic Protocol* from the **FILE** menu.
2. Fill in the information in the **Kinetic Protocol** dialog box:
 - a. Specify reading parameters.
 - b. Specify reading sets.
3. Click on *Pick Template...* and select the data file that contains the desired template.
4. Choose all reports to be automatically displayed and/or printed by clicking *Reports...*
 - a. While in the **Reports** dialog box, click on *Labels...* to choose or create labels to print on each report.
 - b. Click on *Fill Labels...* to enter text to be printed along with the labels on each report.
 - c. Click on *OK* to close the **Reports** dialog box and return to the protocol dialog.

5. Click *Analysis Options* to set the kinetic analysis parameters.
6. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.
7. Initiate the protocol by clicking the *Run* button.
8. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

8.3 Creating and Running a New Kinetic Protocol and Creating the Template Simultaneously

See Chapters 4 and 5 for details.

1. Select *New Kinetic Protocol* from the **FILE** menu.
2. Fill in the information for the protocol window:
 - a. Specify reading parameters.
 - b. Specify reading sets.
3. Click on *Show Template* to display a template of 96 undefined wells.
 - a. Follow steps 2–8 of section 8.1 to define the template.
 - b. Click the *Close* box on the template. A prompt will ask you if you want to save the changes. Choose *Yes* and enter a name for the template.
 - c. Click on *Pick Template...* in the kinetic protocol dialog box.
 - d. Select the data file that was saved in step b. The template will be linked to the protocol file.
4. Choose all reports to be automatically displayed and/or printed by clicking *Reports...*
 - a. While in the **Reports** dialog box, click *Labels...* to choose labels to print on each report.
 - b. While in the **Reports** dialog box, click *Fill Labels...* to enter text to be printed along with the labels on each report.

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- c. Close the **Reports** dialog box to return to the kinetic protocol.
5. Click on *Analysis Options* to set the kinetic analysis parameters.
6. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.
7. Initiate the protocol by clicking on *Run*.
8. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

8.4 Creating and Running a New Endpoint Protocol with a Stored Template

See Chapter 5 for details.

1. Select *New Endpoint Protocol* from the **FILE** menu.
2. Fill in the information for the protocol window:
 - a. Specify any initial wait.
 - b. Specify any absorbance delay.
 - c. Specify reading parameters.
3. Click on *Pick Template...* and select data file that contains the desired template.
4. Choose all reports to be automatically displayed and/or printed by clicking *Reports...*
 - a. While in the **Reports** dialog box, click on *Labels...* to choose labels to print on each report.
 - b. Click on *Fill Labels...* to enter text to be printed along with the labels on each report.
 - c. Close the **Reports** dialog box.
5. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.
6. Initiate the protocol by clicking on *Run*.

7. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

8.5 Creating and Running a New Endpoint Protocol and Creating the Template Simultaneously

See Chapters 4 and 5 for details.

1. Select *New Endpoint Protocol* from the **FILE** menu.
2. Fill in the information for the protocol window:
 - a. Specify any initial wait.
 - b. Specify any absorbance delay.
 - c. Specify reading parameters.
3. Click on *Show Template* to display a template of 96 undefined wells.
 - a. Follow steps 2–8 of section 8.1 to define the template.
 - b. Click the close box to close the template. A prompt will ask you if you want to save the changes. Select *Yes* and enter a name for the template.
 - c. In the **Endpoint Protocol** dialog box, click on *Pick Template...*
 - d. Select the data file that was saved in step b. The template will be linked to the protocol file.
4. Choose all reports to be automatically displayed and/or printed by clicking *Reports...*
 - a. While in the **Reports** dialog box, click on *Labels...* to choose labels to print on each report.
 - b. Also click on *Fill Labels...* to enter text to be printed along with the labels on each report.
 - c. Click on *OK* to close the **Reports** dialog and return to the protocol.
5. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.

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6. Initiate the protocol by clicking on *Run*.
7. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

8.6 Creating and Running a Multiple Plate Protocol with a Stored Template

See Chapter 5 for details.

1. Select *New Multiple Plate Protocol* from the **FILE** menu.
2. Fill in the information in the **Multiple Plate Protocol** dialog box:
 - a. Specify any initial wait.
 - b. Specify any absorbance delay.
 - c. Specify reading parameters.
 - d. Indicate if a stack loader is to be used.
 - e. Specify the number of plates (if using a stack loader) or number of times to read plate (if not using a stack loader).
 - f. Specify the root file name for data storage.
3. Click on *Pick Template...* and select data file which contains the desired template.
4. Choose all reports to be automatically displayed and/or printed by clicking on *Reports....*
 - a. While in the **Reports** dialog box, click on *Labels...* to choose labels to print on each report.
 - b. Also click on *Fill Labels...* to enter the text to be printed along with the labels on each report.
 - c. Close the **Reports** dialog box to return to the protocol.
5. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.
6. Initiate the protocol by clicking on *Run*.

7. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

8.7 Creating and Running a New Multiple Plate Protocol and Creating the Template Simultaneously

See Chapters 4 and 5 for details.

1. Select *New Multiple Plate Protocol* from the **FILE** menu.
2. Fill in the user-defined information for the Multiple Plate Protocol window:
3. Fill in the information in the **Multiple Plate Protocol** dialog box:
 - a. Specify any initial wait.
 - b. Specify any absorbance delay.
 - c. Specify reading parameters.
 - d. Indicate if a stack loader is to be used.
 - e. Specify the number of plates (if using a stack loader) or number of times to read plate (if not using a stack loader).
 - f. Specify the root file name for data storage.
4. Click on *Show Template* to display a template of 96 undefined wells.
 - a. Follow steps 2–8 of section 8.1 to define the template.
 - b. Click the close box to close the template. A prompt will ask you if you want to save the changes. Select *Yes* and enter a name for the template.
 - c. Click on *Pick Template...* from the **Multiple Plate Protocol** dialog box.
 - d. Select the data file that was saved in step b. The template will be linked to the protocol file.
5. Choose all reports to be automatically displayed and/or printed by clicking on *Reports...*
 - a. While in the **Reports** dialog box, click on *Labels...* to choose labels to print on each report.

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- b. Also click on *Fill Labels...* to enter the text to be printed along with the labels on each report.
 - c. Close the **Reports** dialog box to return to the protocol.
6. Save the protocol by choosing *Save Protocol* or *Save Protocol As...* from the **FILE** menu.
7. Initiate the protocol by clicking on *Run*.
8. At the end of the run, save the data by choosing *Save Data/Template* or *Save Data/Template As...* from the **FILE** menu.

9. Glossary

Assay: A set of wells grouped together for analysis.

Blank: A well used for background subtraction.

Endpoint analysis: Analysis based on a single absorbance value per well.

Kinetic analysis: Analysis of the rate of change of absorbance for a well over a set period of time.

OD (Optical Density): A measure of absorbance.

Protocol: A group of settings that control a microplate reader. Includes incubator controls, filter settings, mix times, etc. You adjust these settings in a dialog box, and your adjustments may be saved as a file and used repeatedly.

Standard: A well whose concentration is known.

Sample/Unknown: A well whose concentration is to be determined.

Template: A graphical representation of a microplate used by Microplate Manager to identify the numbers and types of wells, different assays, locations of standards, etc. on the microplate

Well: An individual solution container on a microplate.

.MPM: The extension of a data file name.

.EPR: The extension of an endpoint protocol file name.

.KPR: The extension of a kinetic protocol file name.

.MPR: The extension of a multiple endpoint protocol file name.

10. Ordering Information

<u>Catalog Number</u>	<u>Product Description</u>
170-9520	Microplate Manager, Version 5.0
170-9521	PC/Microplate Reader Interface Cable Set with SCSI-2 card.
170-9500	Ultramark Microplate Imaging System
170-6850	Benchmark Microplate Reader
170-6750	Model 550 Microplate Reader

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Appendix A. Menus

The pulldown menus in Microplate Manager change depending on the particular part of the application you are using. For example, if you are editing a template, one set of menu commands are available; if you are preparing a protocols, another set of menu commands will become available.

File

This is the initial state of the **FILE** menu.

New Template accesses a submenu from which you can select a standard template size or (if you are using the Ultramark) open a dialog box in which you can specify a custom template size.

Open opens a dialog box in which you can open an existing data or protocol file.

New Kinetic Protocol opens the default kinetic protocol window.

New Endpoint Protocol opens the default endpoint protocol window.

New Multiple Plate Protocol opens the default multiple plate protocol window.

Import... opens a dialog box in which you can open a data file that is in an external format.

Export... opens a dialog box in which you can save your data in an external format.

Export to Excel... opens a dialog box in which you can save your data in Excel format.

Preferences opens a dialog box in which you can set the default location of data and protocol files.

The last four files opened are listed.

Exit quits the program. You will be prompted to save any open files that have been modified.

This is the appearance of the **FILE** menu when a data file is open or when a template is active. New commands are described below.

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Close closes the current file. All windows associated with the current file are closed. If the file has been modified, you will be prompted to save the changes.

Save Data/Template saves the current data and/or template to a file.

Save Data/Template As... opens a dialog box in which you can save the current data and/or template under a new file name or to a specific directory.

Page Setup... opens a dialog box in which you can select a font for printing.

Print... opens a dialog box from which you can print the currently active report.

Print Preview displays the current report as it will look when printed.

Print Setup... opens a dialog box in which you can select and configure a printer.

When a protocol file is open, *Save Data/Template* and *Save Data/Template As...* change to *Save Protocol* and *Save Protocol As...* and the options for printing (*Page Setup*, etc.) are deleted.

Edit

This is the appearance of the **EDIT** menu when a template is active. At other times (e.g., when one of the protocol windows is active), subsets of the choices shown here are available.

Undo Formatting undoes the last formatting change made to the template.

Redo Formatting undoes the previous "Undo."

Cut, Copy, and Paste are available only in text-based reports. The contents of a report may be copied to the clipboard.

Select All highlights (selects) all values in a report.

Edit Raw Data... displays raw data values for endpoint data sets and allows you to edit them. When a kinetic data set is open, this becomes *Edit Velocities...* Note that manually-entered velocities may be overwritten if the velocity data are recalculated. Velocities are recalculated whenever the kinetic mode, the time range, or absorbance range are changed using the Analysis **Options** dialog box.

Edit Unknown Dilutions... opens a form in which you can enter dilution information for each series of unknowns.

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Edit Standard Conc... opens a form in which you can enter concentrations of standards.

Edit Outliers... opens a dialog box in which you can exclude certain wells from all calculations.

Set Assay... opens a dialog box in which you can change the assay to which the selected well belongs.

Set Replicate Group... opens a dialog box in which you can change the replicate group of the selected well.

Properties... displays the attributes of the selected well.

Labels... opens a dialog box in which you can enter labels to be printed along with the reports.

Label Contents... opens a dialog box in which you can enter values for the report labels above.

Transfer Template... allows you to replace the template in the data file with another template from a saved data file. Only available when a template window is active.

View

Initial state:

Toolbar shows or hides the main toolbar. If you have a smaller monitor, you may want to hide the toolbar to reclaim screen space.

Status Bar shows or hides the status bar. If you have a smaller monitor, you may want to hide the status bar to reclaim screen space.

In addition, when a template (data file) is displayed:

Show Full Size, *Show Compact*, and *Show Tiny* varies the size of the template as it is displayed on your screen. This is useful for larger templates or smaller monitors.

Various reports are also listed. Some reports are available only for kinetic protocols, and others are available only for endpoint and multiple plate protocols. See Chapter 6 for details.

Plate Template displays the template of the active data file.

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Format

This menu is available only when a template is active:

Assay changes the pointer to a tool for defining the area of the microplate in which an individual assay is to be carried out.

Select changes the pointer to a select tool.

Blank–Undefined changes the pointer to tools for setting the well type.

No Autofill turns off the autofilling functions.

Autofill Across allows you to number individual wells or replicate groups sequentially in a row from left to right by dragging across a template.

Autofill Down allows you to number individual wells or replicate groups sequentially in a column by dragging down a template.

Autofill Set Size opens a dialog box in which you can set the size of the replicate groups you want to autofill.

Resequence Well Names renumbers the wells to eliminate any gaps in the numerical sequence.

Plate Size will open the custom plate size dialog box, in which you can select another plate size. (This will only work if you are already displaying a custom plate size.)

Assay

When a template or data file is active:

Assay Info... opens a dialog box in which you can assign custom names and change the highlight colors for assays. Also allows you to enter notes that are printed out with reports.

Options

The initial state of the **OPTIONS** menu has the following items:

Communications Setup... opens a dialog box in which you can select the COM port to which the reader is attached.

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Ultramark Setup... opens a dialog box in which you can enter filters for the Ultramark microplate reader.

Model 550 Setup... opens a dialog box in which you can enter filters for the Model 550 microplate reader.

When the **Matrix, Limit, Control,** or **Normalization Report** window is open:

Limits... opens a dialog box in which you can set the upper and lower limits for the report.

External Standards... opens a dialog box in which you can select another file from which to import standards to be used in calculating unknowns.

When the **Raw, Absorbance,** or **Unknown Concentration Report** window is open, *Limits...* becomes unavailable.

When one of the kinetic report windows is open:

Analysis Options... opens a dialog box in which you can specify time or absorbance limits for data collection. You also can choose the method of calculation of reaction velocities.

When the **Standard Curve Report** window is open:

Various regression methods and axes types become available. You can use these to format the standard curve.

Analysis of Variance... opens a dialog box in which you can compare two different standard curves and create a statistical report.

Window

This menu is only available when a window (template, protocol, etc.) is open.

Cascade arranges all open windows so that they overlap.

Tile resizes and stacks all open windows so that each is fully visible.

Arrange Icons repositions all the iconized windows.

Close All closes all open windows.

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All open windows are listed at the bottom of the menu. The active window is indicated by a check mark. When more than nine windows are open, *More Windows...* is appended to the bottom of the menu. Choosing *More Windows...* opens a dialog box in which you can choose the desired window.

Help

About Microplate Manager displays version information about the application.