Osteopontin

IκB-α

HER-2

Akt

Erk1/2

Stat3

IgG Total

JNK

TGF-β1

MEK1

IL-12p70

NF-κB p65

c-Jun

IL-18

GSK-3α/β

TFF3

VEGFR-2

Stat1

Clusterin

Bio-Plex Manager™ MP Software

User Guide
Version 1.0

BIO·RAD

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User Guide

Version 1.0

10032257 Rev A

BIO·RAD

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The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

CST antibodies exclusively developed and validated for Bio-Plex phosphoprotein and total target assays.

Table of Contents

1 Introduction

Bio-Plex Manager™ MP is an application that allows you to run and maintain your MAGPIX instrument. MAGPIX monitors the state of your instrument and recommends routines to keep the instrument in good working order, and it also recommends maintenance routines to resolve problems. With Bio-Plex Manager MP, you do not have to keep track of when to calibrate your instrument or verify the calibration settings. Its easy-to-use interface facilitates creating and running your protocols. Bio-Plex Manager MP works with any MAGPIX instrument purchased from any vendor.

You will use two software applications, Bio-Plex Manager MP 1.0 and Bio-Plex Manager 6.x to run your experiments and analyze your results. Both software applications are required and are shipped along with the MAGPIX instrument.

Use Bio-Plex Manager MP to do the following:

- Operate the MAGPIX instrument

- Maintain the instrument

- Define protocol parameters and initiate plate readings

Use Bio-Plex Manager 6.x to do the following:

- Review and optimize results

- Export results to Microsoft Excel or other third-party analysis software

- Export results to Bio-Plex Data Pro™ for analysis of your experiment (grouping biological replicates and identifying statistically significant changes)

Bio-Plex Data Pro is an optional software application that allows you to do further analysis of your results:

■ Identify and group biological replicates

■ Determine statistically significant changes

■ Generate and export charts, heat maps, and tables

Bio-Plex Data Pro (catalog #171-001513 and #171-001523) is a separate product that can be purchased from Bio-Rad.

MAGPIX Operation

Data Review
and Optimization

Bio-Plex Manager MP

Bio-Plex Manager 6.x

MAGPIX

Experimental Analysis
and Interpretation

Bio-Plex Data Pro
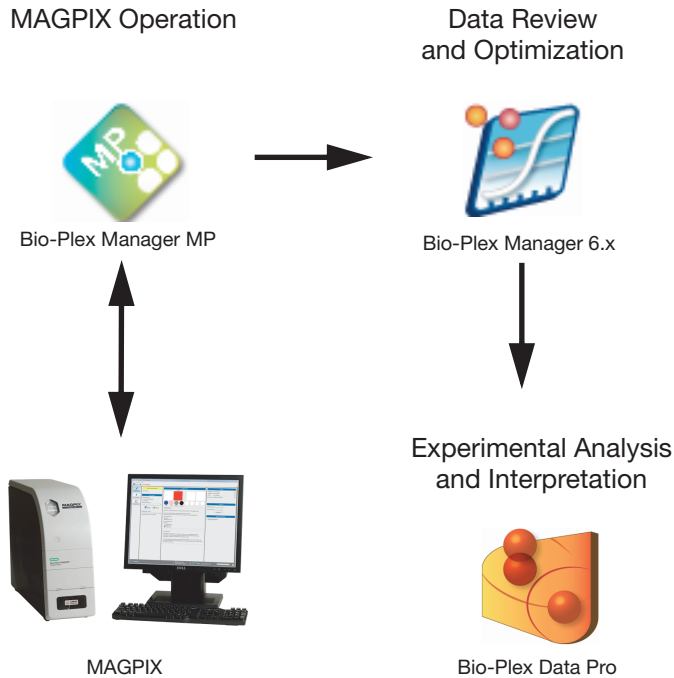
2 Creating Protocols

About Protocols

A protocol contains the parameters of a Bio-Plex Manager™ MP software run. It includes information about the analytes included in the run, the plate wells to be read, the concentrations of standards, and instrument settings. These parameters are saved in the result along with the data from the reading.

You can save a protocol and reuse it or modify it for another reading. Each time you run the protocol, a new result is created and stored in the database.

You set up your protocol from the protocol dialog box. There are three phases to setting up your protocol that correspond to the three panes in the protocol dialog box:

- **Select Analytes** – select an assay panel and the analytes for your experiment.

- **Format Plate** – specify which wells are to be read and the sample type of each well.

- **Standards Info** – specify the concentrations of your standards.

Navigating to the Protocol Dialog Box

Protocols are created from the protocol dialog box. There are a number of ways you can get to the protocol dialog box.

To navigate to the protocol dialog box

1. Click Create/Run Protocols in the navigation bar.

2. Do one of the following:

   - Click New.

   - Click New From and select a protocol from the Select Protocol dialog box.

   - With an open protocol in the Create/Run Protocols view, click Edit.
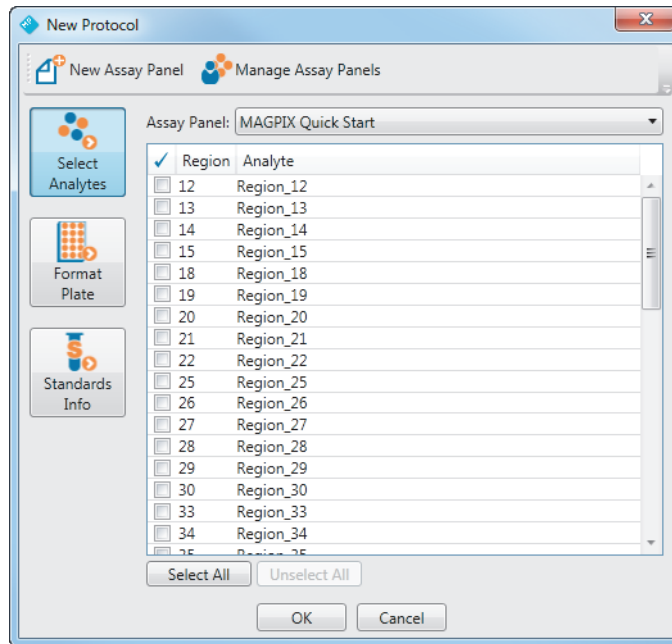
Creating a Protocol

The following are the steps to create a new protocol.

To create a new protocol

1. Click Create/Run Protocols in the navigation bar.

2. Click New to open the New Protocol dialog box.

3. Click the Assay Panel dropdown list and select an assay panel.

4. Select the analytes for your experiment.

5. Click Format Plate.

6. Format the wells to use in the experiment. For more information on formatting wells, see Formatting Plates on page 14.

7. Click Standards Info.

8. From the Standard Lot dropdown list, select the standard lot you want to use with this protocol.

   Note: If no standard lots appear in the list, you must first create a standard lot for your assay panel. For more information, see Creating New Standard Lots on page 50.

9. Select the dilution factor from the Dilution Factor dropdown list. For more information, see Selecting a Standard Lot on page 21.

10. Click OK.

11. (Optional) Click Save As to save the protocol.

    Note: Save the protocol if you want to run it again at a later time or if you want to use it as a template for another protocol. For more information on using the protocol as a template, see Reusing a Protocol on page 23.

12. (Optional) In the Save Protocol dialog box, enter the protocol name and click OK.

Note: Protocols created on one computer cannot be exported and used on another computer running Bio-Plex Manager MP.

About Analytes

Bio-Plex Manager MP software groups analytes by panels. Preconfigured panels of analytes that correspond to off-the-shelf Bio-Plex® assays can be selected. These assays include human, mouse, and rat cytokines and phosphoproteins, as well as the newer human angiogenesis, diabetes, isotyping, and acute phase assays. The following assays are currently ***not*** available for the MAGPIX instrument: nonhuman primate diabetes, canine kidney tox, rat and human kidney tox, angiogenesis, and acute phase. You can add other panels to this list or create custom panels that include only the analytes you use in your experiments.

Note: After you select the assay panel, you select which analytes you want to appear in your reports. During a reading, the array reader detects all the analytes in the sample, including any analytes you excluded from selection. However, only the selected analytes are included in the final reports and tables. After the run is completed, you can always edit the protocol, select any analytes that were previously omitted, and the data for those analytes will appear in the tables.

Each analyte is listed by name and region. The region number refers to the area of a fluorescent color map that identifies the analyte's bead region. Each bead region is embedded with specific quantities of two fluorescent dyes. The array reader detects the combination of these fluorochromes and associates the bead region with a unique region on the color map. Therefore, the location on the map identifies the bead region and its associated analyte.

Each analyte on a Bio-Rad preconfigured panel is identified by a unique region. However, analytes on different panels may occupy the same region. If you pick analytes from more than one panel, only one analyte for a particular region can be selected. Bio-Plex Manager MP warns you if there is a conflict.

Selecting Analytes

After you select the assay panel you want to use in your experiment, you select the analytes you are interested in.

To select an analyte

1. From the protocol dialog box, click Select Analytes.

2. From the Assay Panel dropdown list, select an assay panel.

3. Click the checkboxes to select the analytes to include in your protocol or click Select All to select all analytes.

4. Click OK.

The following figure shows the MAGPIX Quick Start assay panel with analytes in regions 20, 21, and 25 through 27 selected.
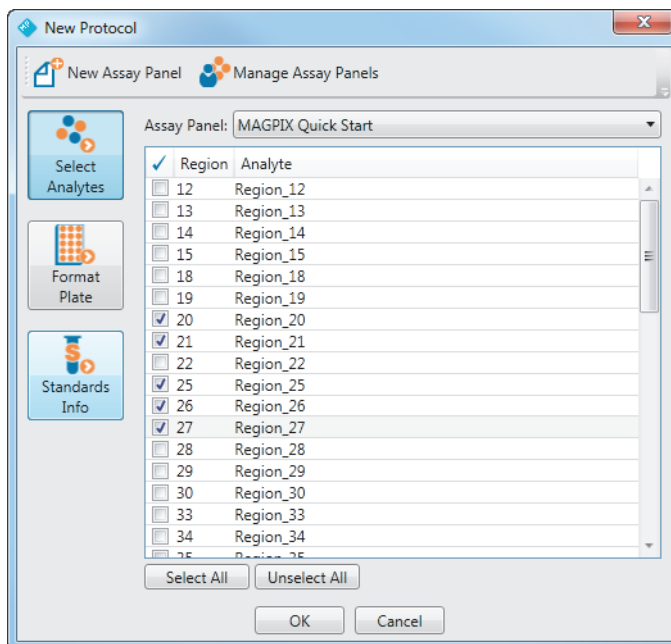
Formatting Plates

The array reader uses the plate formatting to identify which wells are to be read and Bio-Plex Manager MP uses it to determine how to analyze the different samples in each well.

Note: Only formatted wells are read by the array reader. Undefined wells are not read.

To format a plate

1.   Navigate to the protocol dialog box.

2.   Click Format Plate.

3.   Click the button in the toolbar for the type of well you want to format.

4.   Click the wells in the template that you want defined with this well type.

Formatted wells are colored blue with a specific shape to identify the well type. Control, Standard, and Unknown wells are numbered. Blank wells are specified with the letter *B*. For more information on the well types, see Well Types on page 16.
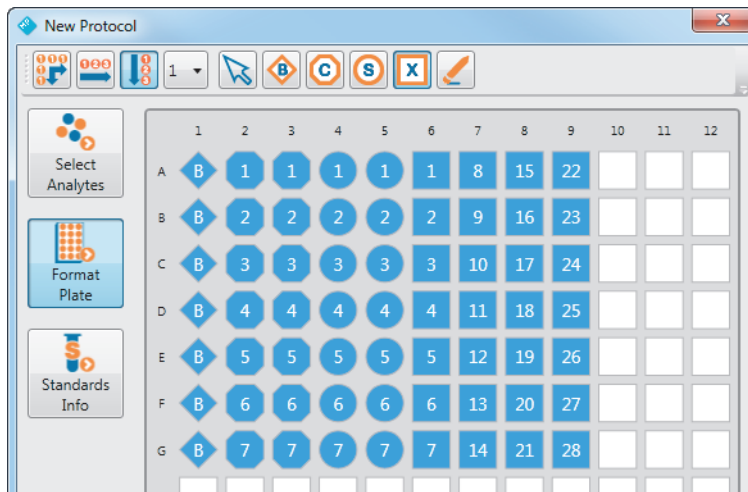
The template diagram in the Create/Run Protocols view displays the formatted wells in your protocol and the raw data table is populated with the well location and sample type.

Create/Run Protocols view

Formatted wells

Name of assay panel

Raw data table
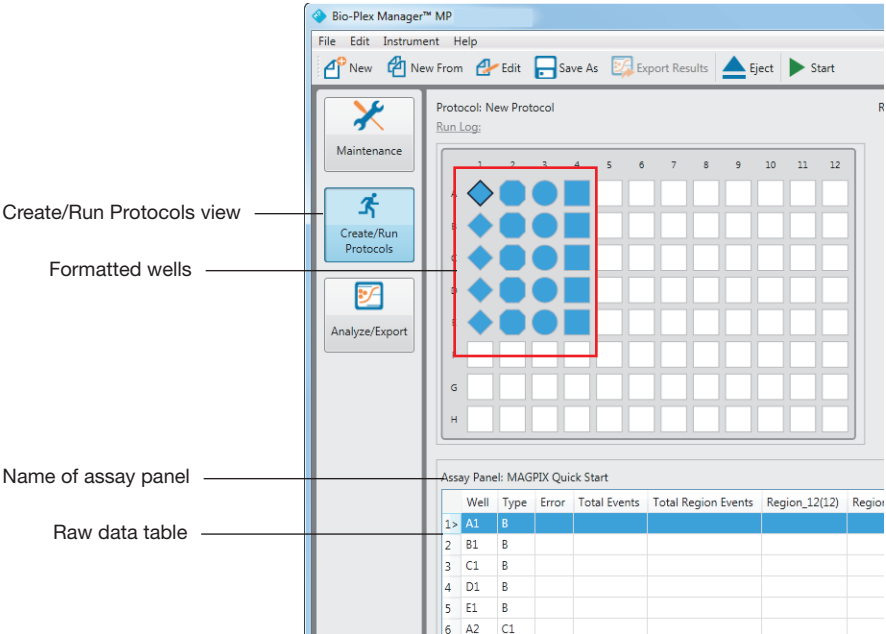
Well Types

The following are different well types that you can specify on the plate:
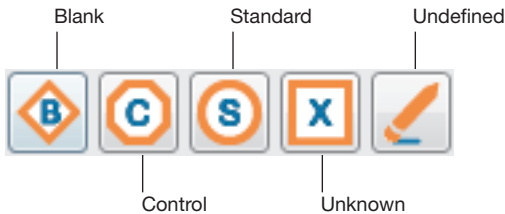
- **Blank Wells**

    In certain types of assays, such as the Bio-Plex phosphoprotein assay, it might be useful to subtract the assay background from the readings of standards, controls, and unknown samples. Prepare blank wells that contain all of the assay components excluding the sample. Blank wells are read along with the rest of the assay. Bio-Plex Manager 6.x then subtracts the mean background reading of these wells from the fluorescence intensity values of the wells containing standards, controls, and unknowns.

- **Control Wells**

    Control wells contain samples of known concentration. The observed concentration of the control wells is compared to the expected concentration and calculated at the end of the reading.

- **Standard Wells**

    Standard wells contain analytes of known concentration. A series of known concentrations of an analyte is used to generate a standard curve of fluorescence intensity compared to analyte concentration. The regression equation for the curve is used to calculate the concentration of analytes in the unknown samples and controls.

- **Unknown Wells**

    The unknown wells contain samples of unknown concentration. These samples are taken from the subjects of your experiments.

- **Undefined Wells**

    Use the Undefined wells button to remove the definition of a well. Undefined wells are not read by the array reader.

Autofilling Well Numbers

Use the Autofill feature to quickly define a well type for multiple wells.

- Autofill Across numbers the wells sequentially from left to right, then top to bottom

- Autofill Down numbers the wells sequentially from top to bottom, then left to right

To use Autofill to format the plate

1. In the New Protocol window, click Format Plate.

2. Click Autofill Across or Autofill Down.

3. Click the well type.

4. Click and drag your cursor in the plate over the wells you want to format.

In the following example, Autofill Down was used to create 20 Unknown wells. Note, the numbering of the wells is top to bottom, then left to right. The arrow shows the path of the cursor as it is dragged across the plate template.
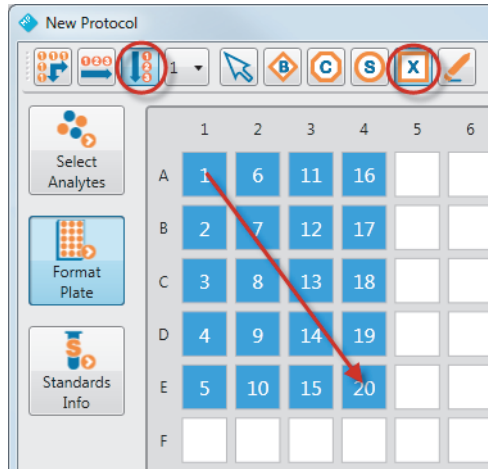
Defining a Replicate Group

You can quickly format a replicate group.

To define a replicate group

1. Click Turn Autofill Off.

2. Click and drag your cursor over the wells that contain the same sample.

    The wells are labeled with the same number.

Autofilling Replicate Groups

You can quickly format multiple replicate groups at one time.

To format a replicate group

1. Click Autofill Across or Autofill Down.

2. Click the Set Replicate Size dropdown list and pick the number of replicates in the group.

   Note: The maximum size you can specify with the Set Replicate Size dropdown list is eight.

   Tip: You can create replicate groups that are larger than eight by clicking Turn Autofill Off, setting the replicate size to *1*, and selecting the number of wells you want in your replicate group.

3. Click the well type.

4. Click and drag your cursor over the wells on your plate.

In the following example, Autofill Across is selected and the replicate size is set to *2*. Eight groups of replicates of Unknown well type were created. Each row represents a replicate group. The arrow shows the path of the cursor as it is dragged across the plate template.
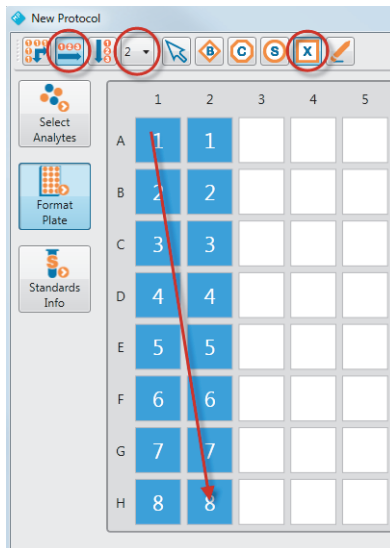
Removing the Well Formatting

Removing the formatting of a well returns it to its unformatted state. Unformatted wells are not read by the array reader.

To remove the formatting of a well

1. Click Undefined.

2. Click and drag your cursor over the wells you want to clear.

   The formatting is removed and the wells return to their unformatted state.

Changing the Well Formatting

You can overwrite the formatting of a well by simply applying another sample type over the wells.

To change the formatting of a well

1. Click the button for the sample type you want to use.

2. Click and drag your cursor over the wells whose format you want to change.

The wells are now formatted with the new well type.

About Standards

Standards are analytes of known concentration. Standards are used to generate a standard curve of values using one of the several regression methods included in Bio-Plex Manager 6.x. This curve is used to calculate concentrations of your unknowns.

In Bio-Plex Manager MP, you define your standard lot, including the starting concentration for each of the analytes. This definition is stored and it can be reused each time you run a plate with those analytes.

Selecting a Standard Lot

Before you can select the standard lot to use with your protocol, you must first select your analytes, format the standard wells, and create a standard lot for the assay panel you are using if one has not already been defined for your analytes.

To select a standard lot for your protocol run

1. From the protocol dialog box, click Standards Info.

   The table displays the analytes you selected on the Select Analytes pane. Each analyte appears as a row in the table. There is a column for each standard well you defined on the Format Plate pane. In this example, eight standard wells were selected on the Format Plate pane, and therefore, there are eight columns: S1, S2, and so on.
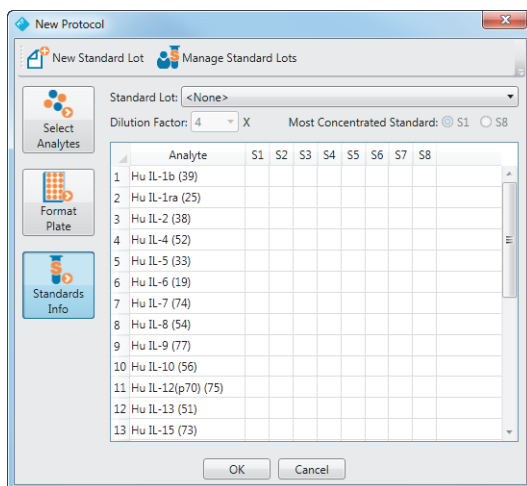
   Note: The Standards Info pane is disabled until you select your analytes and format your standard wells.

2. From the Standard Lot dropdown list, select the standard lot you want to use with this protocol.

Only the standard lots that contain the selected analytes appear in the list. If the list is empty, then there are no defined standard lots that include the analytes you selected and you must create a standard lot. Or if the list does not include the standard lot you are using, then you must create a standard lot definition. For more information on creating standard lots, see Creating New Standard Lots on page 50.

Note: The predefined standard lots are identified by their lot numbers. Included in parentheses is the name of the assay panel and the expiration date. In the example, **5029511** *[Bio-Rad Pro Hu Group 27-Plex]* Exp 2016-03-30, *5029511* is both the name of the standard lot and its lot number.

3. Select the dilution factor from the Dilution Factor dropdown list.

The table autopopulates with the concentrations using the starting concentration specified for that standard lot and the dilution factor.
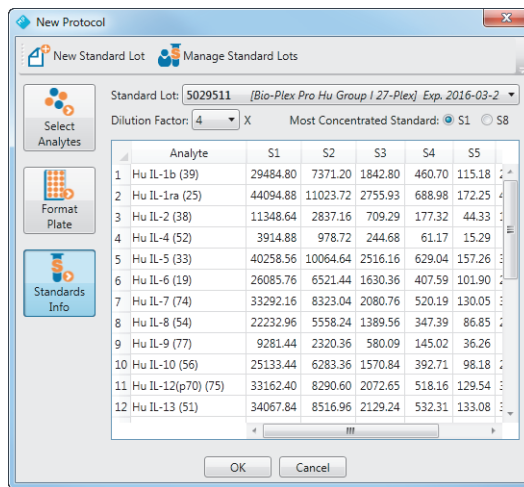
Note: Use Most Concentrated Standard to specify whether the first well or the last well contains the highest concentration of the analyte.

Reusing a Protocol

You can use a saved protocol as a template for your experiment. You can make changes to a copy of the saved protocol — for example, you might change the number of unknown samples.

To reuse a saved protocol

1. Click New From on the toolbar to open the Select Protocol dialog box.

2. Select the protocol you want to use and click OK.

3. In the protocol dialog box, make any changes to the selected analytes, the plate formatting, and the standards information.

4. When you are done making changes, click OK.

Editing a Protocol

The Edit command opens the protocol dialog box for the protocol currently displayed in the Create/Run Protocols view. You might want to edit a protocol in the following situations:

■ You make changes to a saved protocol to match the changes in your experiment. The most common change is to the number of unknown samples in the experiment.

■ You run a protocol and discover that there is a low bead count, indicating that the wrong analytes might have been selected. You can change the selection of analytes in the protocol dialog box.

3 Running Protocols

Once your protocol is created, you are ready to run the protocol. The result is displayed in the raw data table and bead map and it can be exported to Bio-Plex Manager™ 6.x software for analysis. You can interrupt a run at any time, make any necessary changes to the protocol, and resume the run with no loss of data. Bio-Plex Manager™ MP software provides graphical clues and alerts informing you when it detects problems with your run.

Running the Recommended Routine

Run the routine in the Recommended Routine section of the Maintenance view each day you use the MAGPIX instrument. This keeps the instrument free of bubbles and ensures that it is operating according to specifications.

To run the recommended routine

1. Navigate to the Maintenance view.

2. In the Recommended Routine section, click Select to select the routine.

3. Fill the reservoirs and the well strip with the solutions indicated in the Reagent Block.

4. Click Start.

5. When the routine is completed, a dialog box displays indicating that the routine is successfully completed.

You can monitor the progress of the routine; for more information, see .

Plate Handling Guidelines

Before starting the run, note the following plate handling guidelines and warnings:

■   Protect your assay microspheres from light. Once photobleached, the beads are no longer usable. You must care for the microspheres properly to maintain your product warranty.

■   When using a filter plate and wrapping the plate, avoid touching the bottom of the wells. The sample may wick or leak from the well bottom. Should this occur, it can cause problems when reading the plate.

■   Make sure you have added at least 125 μl of sample to all the wells specified in your plate template before starting a protocol run. If the array reader attempts to draw sample from an empty well, air is sucked into the sample loop and injected into the flow chamber. When this happens, bubbles form in the cuvette and interfere with the analysis. Should this occur, perform a Clear Bubbles procedure (see Clear Bubbles Routine on page 77), then rerun the protocol.

■   Shake the microplate on a plate shaker for 30 seconds prior to performing a reading.

For some experiments the temperature of the plate must be maintained at a particular temperature. See Using the Plate Heater on page 28 for more information.

Plate Types

The plate height adjustment procedure adjusts the probe setting for four consumables, the well strip, and the reservoirs in the reagent block. When you run your protocol, you must specify one of the following plate types.

Plate Type	Compatible Consumable	Distance from Platform (mm) *
Flat bottom plate	Bio-Plex Pro™ flat bottom plate (black plate comes with Bio-Plex Pro assays, catalog #171-025001)	3.94
Filter plate	Millipore multiscreen plate (for example, MSBVN1210)	3.05
PCR plate	Bio-Rad low-profile unskirted PCR plate (for example, MLL-9601)	2.79
Auxiliary plate	Nunc PolySorp (for example, 475094), Nunc MaxiSorp (for example, 445105)	3.30

* This is the distance between the platform and the inside bottom of the plate well.

For other plate types, check the manufacturer's specifications to determine the distance from the surface of the MAGPIX platform to the bottom of the well.

The probe backs off 1 mm after it makes contact with the probe height adjustment plate. Therefore, you need to add 1 mm to the distance in the table to get the correct probe height.

For example, if the specification for your plate type is 3.4 mm, you can use the auxiliary plate (3.3 mm) because the actual setting, after running the probe height adjustment, is 4.3 mm. This gives an allowance of 0.9 mm above the plate's well bottom for sample aspiration.

Using the Plate Heater

For some experiments, for example nucleic acid testing, you may need to heat and maintain the temperature of the plate with the MAGPIX plate heater. You can turn the heater on from any view in Bio-Plex Manager MP and set the temperature of the plate from 35–60ºC in 0.5º increments.

Note: While the plate temperature is being set, you cannot run your protocol.

To use the plate heater

1. In the dashboard, select the "Plate heater off" checkbox to turn the heater on.

    The "Turning on" message appears. When the heater is turned on, Set Temp is displayed.

2. Use the left- and right-pointing triangles to set the plate temperature.

    The temperature can be set from 35–60ºC in 0.5º increments.

    When the temperature of the plate falls within range of the set temperature, the thermometer turns green.

3. When you are done, select Set Temp to turn the heater off.

Running the Protocol

After you create your protocol, you are ready to run it. Refer to Chapter 2, Creating Protocols for more information on creating protocols.

Note: You can run only protocols created in Bio-Plex Manager MP. You cannot run protocols created in other versions of Bio-Plex Manager such as Bio-Plex Manager 6.1.

The data from each run are stored as a result in the Bio-Plex Manager MP database. Result names must be unique. You can retrieve the data by exporting the result to Bio-Plex Manager 6.x.
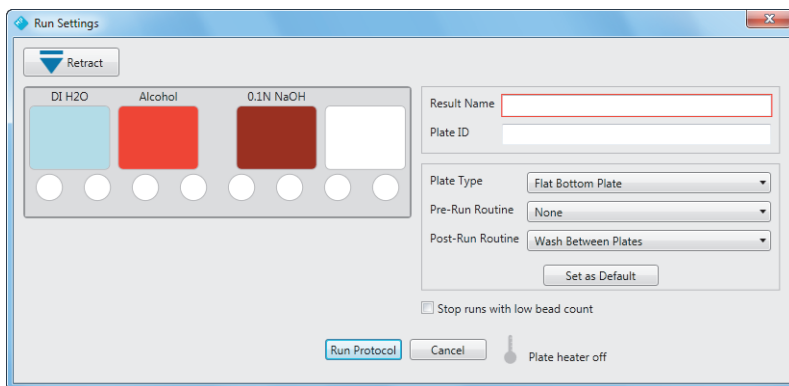
To run your protocol

1. In the Create/Run Protocols view, click Start.

    The microplate platform automatically ejects.

2. In the Run Settings dialog box, enter the name of the result in the Result Name box.

    The result name is the name that the data are stored under in the database. This name is case-sensitive and must be unique. Therefore, *RESULT 1* is not the same as *result 1*.

3. (Optional) Enter the Plate ID.

    You can specify anything here that will help you distinguish your experiments and results.

4. Specify the type of plate in the Plate Type List.

    If you do not see your plate listed here, see Using the Plate Heater on page 28 to determine the equivalent match for your plate type.

5. (Optional) In the Pre-Run Routine list, select any routines you want executed before the protocol run.

    Note: A Rinse routine is always run before a plate is read. This routine is run in addition to any pre-run routine specified here.

If you select a pre-run routine, refer to the Reagent Block diagram and fill the appropriate reservoirs with the specified fluids.

6. (Optional) In the Post-Run Routine list, select any routines you want executed after the protocol run.

   Note: Bio-Rad recommends you run the Wash Between Plates routine after each protocol run to prevent traces of sample or other debris from collecting inside the Bio-Plex® suspension array system.

   If you select a post-run routine, refer to the Reagent Block diagram and fill the appropriate reservoirs with the specified fluids.

7. Accept the default for "Stop runs with low bead count" or clear the checkbox if you do not want the run interrupted.

   By default, the run is interrupted if three consecutive wells have a bead count of less than 20.

8. Click Create/Run Protocols.

   The plate retracts and Bio-Plex Manager MP starts the run.

Reading the Plate

In the template diagram in the Create/Run Protocols view, the blue wells indicate which wells have been formatted. Only formatted wells are read. Each well is read in turn, from top to bottom, then from left to right. A pulsing triangle shows you which well is currently being read.
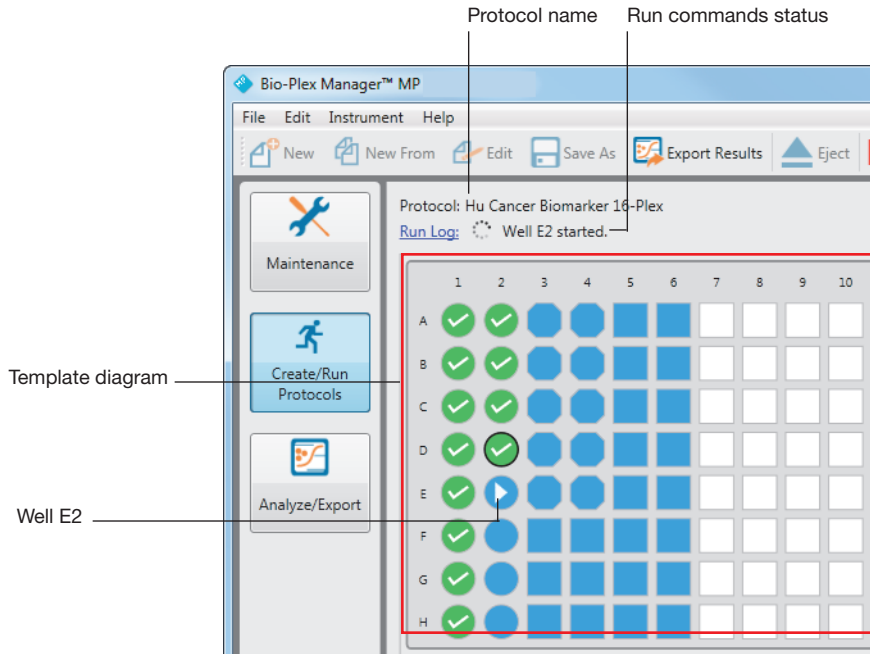
The Run Commands status displays information about the well that is currently being read. In the preceding example, Well E2 is being read. The Run Commands status indicates when the reading begins and ends for the current well.

Once the reading for a well is complete, the pulsing triangle resolves into one of the following symbols indicating the result of the reading.

Symbol	Description
✓	The bead count for all analytes is 20 or higher.
!	The bead count for one or more analytes is below 20 bead events.
✗	No data were found or an error occurred while reading the well.

The results are displayed in the Raw Data table and the Bead Map.

Raw Data Table

As the wells are read, the Raw Data table is populated with the results. The table displays the well location and the well type for all formatted wells. As Bio-Plex Manager MP completes reading each well, the Raw Data table is populated with the results for that well. The fluorescence intensities of the analytes you selected are displayed for each well.

Note: During a reading, the array reader detects all of the analytes in the sample, including any that you have not selected. However, any analytes that are not selected in the protocol are not included in the table, even if they are detected. After a reading you can edit the protocol and change the selection of

your analytes. The new analytes, if detected during the run, will then appear in the table.

Column Heading	Description
Well	Location of the well on the plate template.
Type	Identifies the type of well, Blank, Control, Standard, Unknown, and the sample. For example, C1 is the first control sample and S4 is the fourth standard sample.
Total Events	Total bead events recorded for that well, including unclassified events (events that fall outside the 50 regions). This column can be displayed or hidden using a toggle command.
Total Region Events	Total bead events recorded within the 50 defined regions. This column can be displayed or hidden using a toggle command.
Region_##(##)	When bead region counts are displayed, there are two numbers. The first number is the fluorescence intensity for that analyte. The second number, in parentheses, is the number of bead events for that region.
	If there are low bead counts for any of the selected analytes, the following symbols appear:
	✳ There are fewer than the recommended 50 beads for one or more analytes.
	⚠ There are fewer than 20 beads for one or more analytes.

Bead Map

The bead color map is a density dot plot of the events in a reading for a selected well. Different colored dots in the map represent different numbers of events at those data points. There are 50 available regions in the bead map.

The x-axis is the Classification 1 channel and the y-axis is the Classification 2 channel. These channels measure the embedded fluorochromes in each bead, which are used to identify the bead set and corresponding analyte. The resulting data point clusters in the map represent the different bead sets in the assay and their associated analytes.

Tip: Use Maximize View to expand the bead map to fill the entire window. Once maximized, you can use the Zoom tool to focus further on a particular area in the bead map.

Note: Each bead set generates a cluster of data points, rather than a single point, because of minute variations in fluorochrome levels and intensity among the beads in the set.

The white areas on the map indicate the expected regions of the selected analytes. When you move your cursor over these areas, the name of the analyte appears in a tool tip. The data point clusters should fall within these regions. If one or more clusters do not fall within the white areas, there may be a problem with the beads such as photobleaching. Or the analyte selections in the protocol may be incorrect. If all the data clusters have shifted out of the white regions, there may be microbubbles in the cuvette. The bead map can be used to confirm that the bead sets are correctly selected, measured, and identified.

Interrupting a Run

The results for each run, whether the run is completed or interrupted, are saved to a unique result which can later be retrieved and viewed. Any data that were read up to the point the run is interrupted are saved to the result name you specify in the Run Settings dialog box. When you are ready to run the protocol again, return to the Run Settings dialog box.

To interrupt a run

▶ Click Stop to interrupt a run.

When a sample is being read, the Start button changes to a Stop button.

Note: When you click Stop, Bio-Plex Manager MP finishes reading the current well before it stops the run. Therefore, you may have to wait up to a minute before Bio-Plex Manager MP responds to another command.

Resuming a Run

You can interrupt a run and make changes to your protocol, including changing the analytes you are interested in or correcting the format of your plate. You can run the protocol again and start where you left off or start from the beginning and read the entire plate.

To resume a run

1. From the Create/Run Protocols view, click Start.

2. In the Run Settings dialog box, either accept the default or enter a different name in the Result Name field.

   By default, the Result Name you entered for the first run is appended with a number surrounded by square brackets. For example, if the result name you entered is *Test*, when you return to the Run Settings dialog box to run the protocol again, the result name is *Test [1]*. With each subsequent run, the number is incremented.

3. In the Well Selection diagram, specify the wells you want read.

   Bio-Plex Manager MP reads only selected formatted wells. A black border indicates the selected wells. You can:

   - Read only the wells that were not read on the last run. This is the default, as shown in the figure that follows. In this example, when the run resumes, it will start with well A2.

   - Click Select All to read all the wells.

   - Click on a subset of the formatted wells to read only those wells.

      Tip: Use Shift + Click to select more than one well.
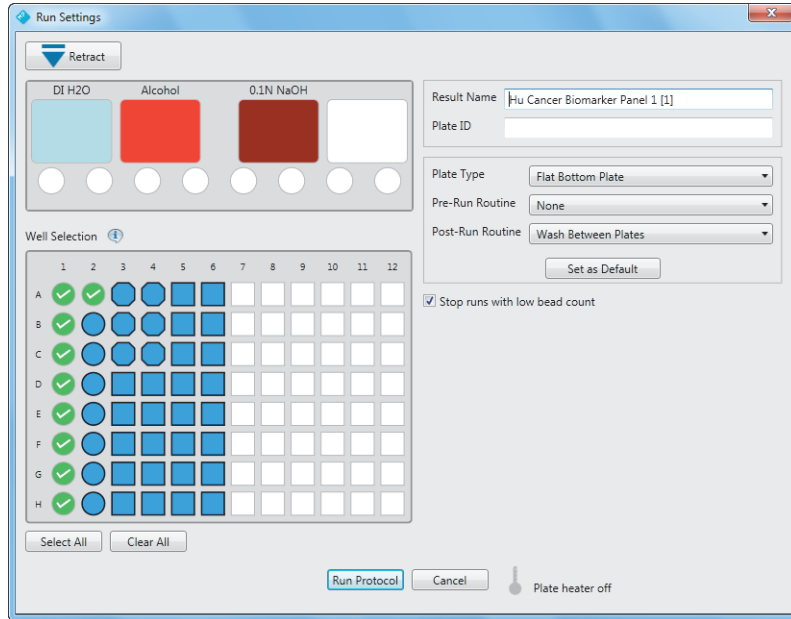
Encountering and Resolving Low Bead Counts

Bio-Plex Manager MP notifies you when a low bead count is detected:

- The well with the low bead count displays a warning symbol.

- A warning symbol is displayed for the region in the Raw Data table if a low bead count occurred for that particular analyte.

You can specify that Bio-Plex Manager MP stop the run if it encounters three or more consecutive wells with low bead count of less than 20. In the Run Settings dialog box, select "Stop runs with low bead count." If a low bead count is encountered, the run is stopped and a dialog box appears with a suggested solution.

In addition, a warning symbol is displayed in the affected wells in the plate template and for the applicable analytes in the raw data table.

LEGEND

1	Warning symbol indicating wells with a low bead count
2	Warning symbol indicating region in which there is a bead count of fewer than 20 beads
3	Asterisk (*) indicating region in which the bead count is lower than the recommended 50 beads

Important: Bio-Plex Manager MP will provide warning messages when a low bead count occurs with recommended steps to resolve the problem. These include reviewing your protocol, performing maintenance on the instrument, and running maintenance routines. It is important that you perform the steps in the recommended order because this increases the likelihood that the problem will be resolved.

Run Results Table

The data from each run are saved as a result in the MAGPIX database. Each result is a row in the run results table.

The following table describes the information in the run results table.

Column Name	Description
Name	Name of the result set
Acquisition Time	Time stamp of when the protocol was run
Assay Panel	Assay panel used in the run
# Analytes	Number of analytes selected in the protocol definition
Description	User-defined description

Tip: Click the column header of any column to sort the table by that column.

Exporting Data to Bio-Plex Manager 6.x

Before data can be analyzed in Bio-Plex Manager 6.x, the Bio-Plex Manager MP run result must first be saved to a file. This file can then be exported to Bio-Plex Manager 6.x.

You can export your results in either of the following ways:

■ Export your results immediately following a protocol run from the Create/ Run Protocols view. For more information, see Exporting Data from the Create/Run Protocols View on page 40.

■ Export your results at a later time from the Analyze/Export view. For more information, see Exporting Data from the Analyze/Export View on page 41.

Note: You can export your data once the plate reading is completed. If you select a post-run routine to be run once the run is completed, you do not have to wait for the routine to complete before exporting your data.

Once the file is saved, it can be viewed in Bio-Plex Manager 6.x in one of the following ways:

■ If Bio-Plex Manager 6.x is installed on the same computer where you are running Bio-Plex Manager MP, then you are prompted to view the result in Bio-Plex Manager 6.x after the file is saved. If you respond *Yes*, the result file is opened in Bio-Plex Manager 6.x.

■ If Bio-Plex Manager 6.x is *not* installed on your computer, then you must copy the saved file to a computer where Bio-Plex Manager 6.x is installed and import it into the software.

Exporting Data from the Create/Run Protocols View

You must first export the result to a file before you can analyze the data.

To export data from the Create/Run Protocols view

1. From the Create/Run Protocols view, click Export Results.

2. In the Export Results dialog box, navigate to where you want the run result to be saved.

3. Enter the name of the file.

   The default file name is the Result Name assigned in the Run Settings dialog box, followed by a time stamp of the time of the export. For example, *Experiment 2013-02-12 13.04.08*. You can accept the default name or enter a different name for the file.

4. Click Save.

   A message appears informing you that the result file has been exported.

   If Bio-Plex Manager 6.x is installed on the same computer where you are running Bio-Plex Manager MP, you are also asked if you want to open the result in Bio-Plex Manager 6.x

5. In the Export Results dialog box, click Yes.

   Bio-Plex Manager 6.x is launched and the run result appears in the Raw Data table.

   Note: You are now in the Bio-Plex Manager 6.x application.
   Bio-Plex Manager MP continues to run and you can switch back and forth between the two application windows.

Exporting Data from the Analyze/Export View

The result from a protocol reading is stored in the run results table. (See Run Results Table on page 38 for more information.) You can export your run result at a later time from the Analyze/Export view.

To export data from the Analyze/Export view

1. Click Analyze/Export in the navigation bar.

2. Click the row of the run result you want to export.

3. Click Export Results.

4. Enter the name of the file.

   The default file name is the run result name, followed by the time stamp of the time of the export. For example, *Experiment 2013-02-12 13.04.08*. You can accept the default name or enter a different name for the file.

5. Click Save.

   A message appears informing you that the result file has been exported.

   If Bio-Plex Manager 6.x is installed on the same computer where you are running Bio-Plex Manager MP, you are also asked if you want to open the result in Bio-Plex Manager 6.x

6. In the Export Results dialog box, click Yes.

   Bio-Plex Manager 6.x is launched and the data result appears in the Raw Data table.

   Note: You are now in the Bio-Plex Manager 6.x application.
   Bio-Plex Manager MP continues to run and you can switch back and forth between the two application windows.

Exporting Data Results Multiple Times

As you analyze your data result in BPM 6.x, you may want to rerun the protocol and generate a new result. As you switch between BPM MP and BPM 6.x, you should be aware of how you name your result files. For example, assume you save your result to a file called *Exp. Region 21*. After you examine the result in BPM 6.x, you decide you want to make some changes to the protocol and run it again. You return to BPM MP, edit the protocol, rerun the protocol, and export the result a second time. When you are prompted to save the result, if you use the *same* file name that you used for the first run (that is, *Exp. Region 21)* you will overwrite the result file from the first run.

Note: Before you export your result from BPM MP to BPM 6.x, you must close the Report Table view for *Exp. Region 21* in BPM 6.x. If you do not close the Report Table view for the first result file, BPM 6.x does not recognize the new result file as an updated version of what is currently displayed, and it does not load the new file. Therefore, you will continue to see the old result.

Tip: Give your result files unique names to avoid running into this problem. By default, a time stamp is appended to the end of the name of the result file, making it unique.

4 Managing Assay Panels and Standard Lots

In Bio-Plex Manager™ MP software, you can select any of the Bio-Rad assay panels for use in your experiments. These panels can be customized for your experiments and reused or shared with colleagues who are using Bio-Plex Manager MP on other computers or in other locations.

Bio-Plex Manager MP makes it easy to manage your standards through predefined standard lots. Standard lots are panels of assays with starting concentrations for Bio-Rad kits. You can use the standard lot as provided or customize it to your experiment. Standard lots can be shared with colleagues.

Creating Assay Panels

You can create assay panels in the following ways from the Manage Assay Panels dialog box:

■ Customize an existing assay panel (Edit button).

■ Create an assay panel by combining one or more existing panels (New From button).

■ Enter your analytes into a new assay panel (New or New From buttons).

■ Import an assay panel (Import button).
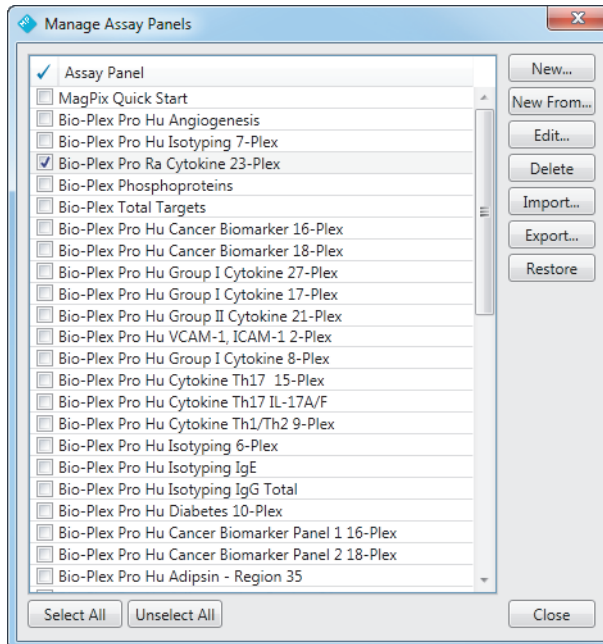
Customizing an Existing Assay Panel

Bio-Plex Manager MP provides a list of all the assay panels it manufactures along with related analytes. In many instances you will take one of these panels and use it as is. If your experiment uses only a subset of the analytes, Bio-Rad recommends you edit the assay panel and delete any analytes you are not interested in and save the panel with a new name. This customized panel makes it easier to run repeated experiments using the same panel. For more information on editing an existing assay panel, see Editing an Assay Panel on page 47.

Creating an Assay Panel by Combining Existing Panels

You can create a new assay panel by combining existing panels. Once this panel is created, you can cull the list and delete any analytes you are not interested in.

To create a new assay panel by combining existing panels

1. From the protocol dialog box, click Select Analytes.

2. Click Manage Assay Panels.

3. In the Manage Assay Panels dialog box, select the panels from the Assay Panel list.

   Note: You can select up to four assay panels.

4. Click New From.

5. In the New Assay Panel dialog box, enter a name for your assay panel.

6. Use the Add and Delete buttons to add or remove analytes from the list.

   Tip: Click the number of the analyte to select the entire row.

7. Use the Up and Down buttons to reorder the analytes.

8. When you are done making changes to the panel, click OK.

   In the Manage Assay Panels dialog box, the new panel appears at the top of the list.

9. Click Close.

From the protocol dialog box, you can now select this new assay panel from the Assay Panel dropdown list.

Importing an Assay Panel

The Import feature can be used to share assay panels among users working on different computers or to add new Bio-Rad assay panels. Once the panel is imported, you can edit the panel or select it in your protocol.

You import an .xpnl file that contains one or more assay panels. If the imported panel has the same name as an existing panel, *Copy* is appended to the name to distinguish the imported assay panel from the existing one; for example, *Assay Panel - Copy*.

To import an assay panel

1. From the protocol dialog box, click Select Analytes.

2. Click Manage Assay Panels.

3. From the Manage Assay Panels dialog box, click Import.

4. In the Import Assay Panels dialog box, navigate to the file you want to import.

5. Select the file and click Open.

    A message informs you of the number of panels that were successfully imported; for example, "Successfully imported 3 assay panels." The imported panels appear at the top of the Manage Assay Panels list.

6. Click Close.

    From the protocol dialog box, you can now select any imported assay panels from the Assay Panel dropdown list.

Creating a New Assay Panel

You can create a new panel by starting with an empty assay panel and entering the details for the assay.

To create a new assay panel

1. From the protocol dialog box, click Select Analytes.

2. Click New Assay Panel.

3. In the New Assay Panel dialog box, enter a name for the assay panel.

4. Enter the region and name of each analyte.

5. Click OK.

   In the protocol dialog box, the new panel is added to the Assay Panel list.

Tip: You can also create a new assay panel from the Manage Assay Panels dialog box.

Editing an Assay Panel

You can make changes to an existing assay panel.

To edit an assay panel

1. From the protocol dialog box, click Select Analytes.

2. Click Manage Assay Panels.

3. Select the panel you want to edit and click Edit.

4. In the Edit Assay Panel dialog box, you can make the following changes:

   ■ Rename the assay panel.

      Tip: Bio-Rad recommends you give the edited panel a new name. This preserves the original Bio-Rad assay panel, and it eliminates problems with overwriting your edited panels if you use the Restore command or import updated panels from Bio-Rad.

■ Use the Add and Delete buttons to add or remove analytes from the list.

   Tip: Click the number of the analyte to select the entire row.

■ Use the Up and Down buttons to reorder the analytes.

5. When you are done making changes to the panel, click OK.

Customizing Your Assay Panel List

The Assay Panel list includes all the assay panels provided by Bio-Rad as well as any panels you create. You may want to delete assay panels from the list because you use only a subset of the published assays. If you delete a Bio-Rad assay panel, you can always return it to the Assay Panel list using the Restore command. For more information on restoring an assay panel, see Restoring an Assay Panel on page 49.

To delete an assay panel

1. From the protocol dialog box, click Select Analytes.

2. Click Manage Assay Panels.

3. From the Manage Assay Panels dialog box, select one or more assay panels and click Delete.

Sharing Customized Assay Panels

If there are multiple users of Bio-Plex Manager MP in your environment, you can customize an assay panel for your environment once and share it with other users. Export the assay panel to a file and have your users import the panels into Bio-Plex Manager MP on their computers. You can also use the Export feature to archive assay panels that you no longer need.

Note: You can export any number of assay panels at a time. All selected assay panels are exported to a single file. When the file is imported, all panels are imported. Users will not be able to selectively import panels from the file.

To export an assay panel

1. From the protocol dialog box, click Select Analytes.

2. Click Manage Assay Panels.

3. Select the panel you want to export and click Export.

4. In the Export Assay Panels dialog box, enter the name of the file and click Save.

Restoring an Assay Panel

You may inadvertently delete an assay panel, or you may want to return to the original version of a Bio-Rad panel that you modified. In these instances, you can restore the assay panels that were originally distributed with the Bio-Plex Manager MP software using the Restore command. Any assay panels you created are not affected.

WARNING!   If any of the Bio-Plex Manager MP assay panels provided by Bio-Rad have been modified, these will be overwritten with the Restore command. If you want to save these edited panels, rename the panels first before you use the Restore command.

To restore the Bio-Plex Manager MP assay panels

1. From the protocol dialog box, click Manage Assay Panels.

2. Click Restore.

About Standard Lots

Standard lots are definitions of analytes and their starting concentration for a particular assay panel. When you create a protocol, you select one of the defined standard lots. The standard lot definitions can be reused, thus eliminating the tedious task of entering the standards each time you run an experiment. Bio-Plex Manager MP comes with a set of predefined standard lots.

A list of the predefined standard lot definitions and any standard lots you create is displayed in the Manage Standard Lots dialog box.
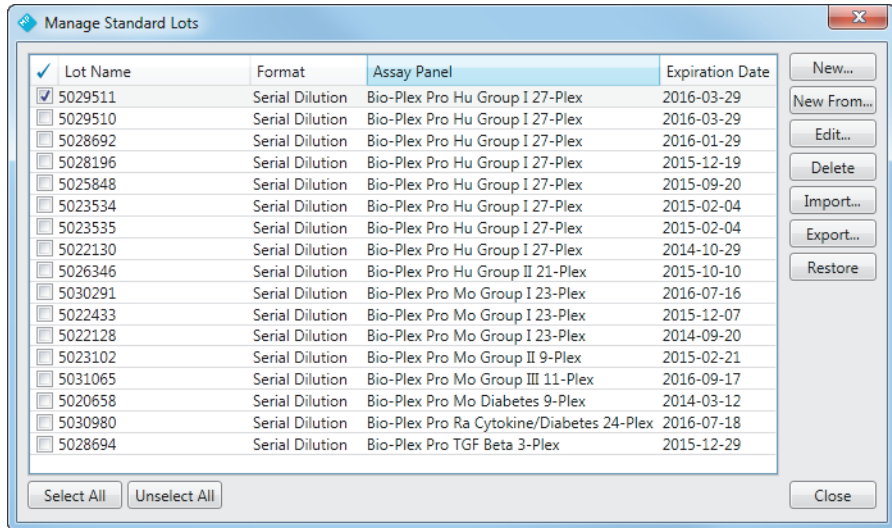
Creating Standard Lots

You can start with a blank standard lot and enter the required information for the standard lot definition. However, you are more likely to take an existing standard lot and modify it. You can share standard lot definitions using the export and import features.

Creating New Standard Lots

To create a new standard lot

1. From the protocol dialog box, click Standards Info.

2. Click Manage Standard Lots.

3. In the Manage Standard Lots dialog box, click New.

4. In the New Standard Lot dialog box, enter a name for the standard lot.

Tip: Bio-Rad recommends you include the lot number of the standards. This will help you select the correct standard to use when running your protocol.

5. In the Expiration Date field, enter the expiration date for this standard lot.

   Tip: Click on the calendar and select a date.

6. From the Assay Panel dropdown list, select the assay panel you are using.

7. Select how the standard lot is defined by choosing one of the Define Standard Lot By options.

   In most instances, you will be using a serial dilution. For information on defining custom concentrations, see Defining a Standard Lot with Custom Concentrations on page 52.

8. Specify the starting concentration for the analytes.

The starting concentrations are specified on a label or product insert in the standard lot kit.

Tip: If the starting concentration for all of your analytes is identical, enter the concentration for the first analyte. Select the entire row by clicking in the first column in the table. Right click and select "Apply starting concentration to all analytes" from the menu to populate the starting concentration for all the analytes with the same number.

Defining a Standard Lot with Custom Concentrations

There may be times when you want to customize the dilutions of your standards. For example, you might start with a dilution factor of 4 for the first three wells, then dilute the standard in smaller increments for the next three wells.

To define custom concentrations for the analytes

1. In the New Standard Lot dialog box, select Custom for "Define Standard Lot By."

    The table displays a column for each standard well.

2. Select the number of standard wells in your protocol.

3. For each analyte, enter the concentration values for each standard.

4. When you are done, click OK.

Creating a Standard Lot from an Existing Lot

You can create a new standard lot from an existing lot. This is useful if you use the same assay panel repeatedly because it saves the time of reentering the starting concentration values.

To create a new standard lot from an existing lot

1. From the protocol dialog box, click Standards Info.

2. Click Manage Standard Lots.

3. In the Manage Standard Lots dialog box, select a standard lot from the list and click New From.

4. Enter a new name for the standard lot.

5. Enter the expiration date for the standard lot.

    Tip: Click on the calendar and select a date.

6. Make any changes to the starting concentration of the analytes.

7. When you are done making your changes, click OK.

Importing a Standard Lot

You can import an .xstds file that contains one or more standard lots. If the imported standard lot has the same name as an existing lot, *Copy* is appended to the name to distinguish the imported standard lot from the existing one; for example, *Standard Lot - Copy*.

To import a standard lot from a file

1. From the protocol dialog box, click Standards Info.

2. Click Manage Standard Lots.

3. In the Manage Standard Lots dialog box, click Import.

4. Navigate to where the standard lot file is, select the file, and click Open.

    Note: The file must have an .xstds extension.

    A dialog box appears informing you that the standard lot is successfully imported and the imported standard lot appears in the list.

Editing Standard Lots

You can make changes to an existing standard lot.

To edit an existing standard lot

1. From the protocol dialog box, click Standards Info.

2. Click Manage Standard Lots.

3. In the Manage Standard Lots dialog box, select a standard lot from the list and click Edit.

4. Make any changes to the name of the standard lot, the expiration date, the method used to dilute the standards, and the starting concentrations of the analytes.

When you are done making changes, click OK.

Deleting Standard Lots

You can delete standard lots you no longer need from the Manage Standard Lots dialog box. Once deleted, you will not be able to select the lot to use with your protocol.

To delete a standard lot

From the Manage Standard Lots dialog box, select the lots you no longer need and click Delete.

Exporting Standard Lots

You can export standard lots to a file, which can then be transferred to another computer and imported into another copy of Bio-Plex Manager MP.

Note: You can export a single standard lot or multiple standard lots at one time. In both instances, the lots are exported to a single file. When the file is imported, all the standard lots are imported. You cannot selectively import standard lots from a file.

To export a standard lot to a file

1. From the protocol dialog box, click Standards Info.

2. Click Manage Standard Lots.

3. In the Manage Standard Lots dialog box, select a standard lot from the list and click Export.

In the Export Standard Lots dialog box, enter the name of the file and click Save.

5 Analyzing Results

Bio-Plex Manager™ MP is used to create and run your protocols. Once the plate has been read, you can review the fluorescence intensities of the analytes you selected and use the bead map to confirm that the bead sets are correctly selected and identified. Further analysis of your results is done in Bio-Plex Manager 6.x. The Bio-Plex Manager MP results are exported to Bio-Plex Manager 6.x. For more information on exporting your results, see Exporting Data to Bio-Plex Manager 6.x on page 39.

Note: Analysis of Bio-Plex Manager MP results is supported only with Bio-Plex Manager 6.0 or higher. Earlier versions of Bio-Plex Manager are not supported.

The following are differences between Bio-Plex Manager MP and Bio-Plex Manager 6.x:

- The Bio-Plex Manager MP results cannot be viewed in a histogram or bead map in either software application. (Bio-Plex Manager 6.x results can be viewed in both a histogram and bead map in the Bio-Plex Manager 6.x software application.)

- Region events are calculated differently in Bio-Plex Manager MP and Bio-Plex Manager 6.x.

    - In Bio-Plex Manager MP, the total region events includes all beads within the 50 regions whether or not they were selected for analysis.

    - In Bio-Plex Manager 6.x, the total region events includes only beads in the selected regions.

    The same data result will reflect this difference in total region events when viewed in the two software programs.

■ In Bio-Plex Manager MP, there are no gated events. MAGPIX is a CCD, camera-based system and the gating of beads in the assay is accomplished through the software without user input.

For more information on analyzing your results in Bio-Plex Manager 6.x, refer to *Bio-Plex Manager Software 6.1 User Guide* and the online help in the software.

6 Maintaining the MAGPIX System

The Maintenance view is used to:

■ Monitor the status of the MAGPIX instrument and to easily identify, at any time, which routines need to be run to keep your instrument operating according to the manufacturer's specifications

■ Run the maintenance routines, including calibration and verification

■ Import the calibration and verification kits

■ Adjust the probe height
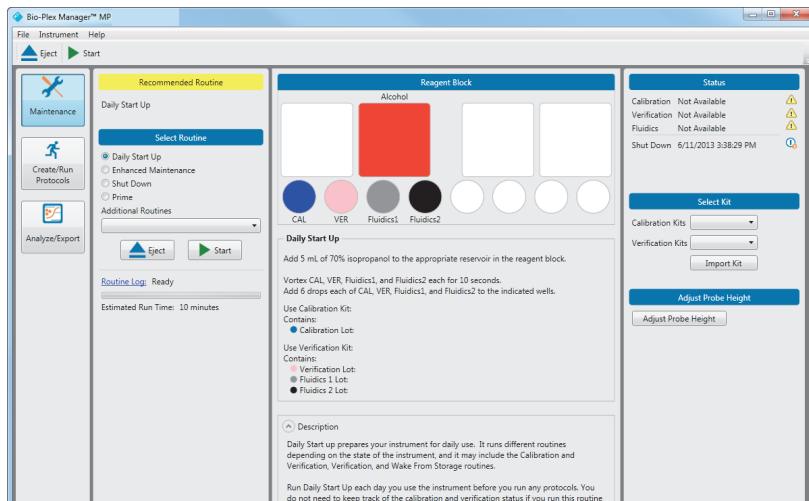
Recommended Routine

Bio-Plex Manager™ MP software monitors the MAGPIX instrument and identifies what routines need to be run to keep the instrument in good working order. You do not have to keep track of whether you have run the correct routines at the right time. Bio-Plex Manager MP informs you what routine needs to be run in the Recommended Routine section. When all recommended maintenance has been performed, *None* is displayed.
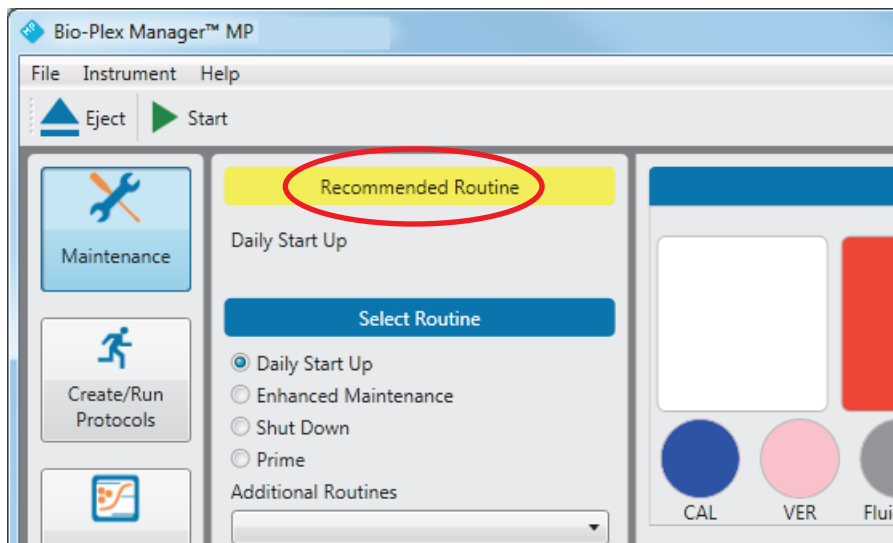
Best Practice

■   At the start of each day of operation, Bio-Rad recommends that you run the recommended routine.

■   Before you run a protocol, check to see that all maintenance is up to date and the Recommended Routine section displays *None*.

Daily Maintenance Routines

Bio-Rad recommends that you run the following routines each day you use the MAGPIX instrument.

At the Beginning of the Day

Run the routine in the Recommended Routine section. One or both of the following routines are recommended each day:

- Daily Start Up

- Enhanced Maintenance

Continue to run the recommended routine until maintenance is up to date and *None* is displayed in the Recommended Routine section.

At the End of the Day

At the end of each day, run the Shut Down routine before you shut down the instrument.

Daily Start Up Routine

The Daily Start Up routine runs different procedures depending on the state of the instrument. Bio-Plex Manager MP detects whether the instrument needs to be verified, calibrated, or both, and it runs the appropriate commands as part of Daily Start Up. This routine can take 4–12 minutes.

Note: When you turn on the MAGPIX instrument, Daily Start Up is generally the routine that is recommended. However, there may be times when Enhanced Maintenance is recommended, for example, if calibration and verification previously failed. Run whatever routines are recommended until maintenance is up to date and no routines appear in the Recommended Routine section.

Tip: If verification, calibration, or both routines fail after successive attempts, contact Bio-Rad Technical Support for assistance.

Enhanced Maintenance

The Enhanced Maintenance routine will be recommended, for example, if calibration or verification failed on the previous day. Once this routine is successfully completed, it is followed with a recommendation to run the Daily Start Up routine.

Shut Down Routine

If you do not run the Shut Down routine at the end of the previous day, when you start up the instrument the next day, the Status section in the Maintenance view shows that Shut Down is not current. Run the Shut Down routine first. This routine takes 2 minutes. Once that routine has successfully completed, then run the routine in the Recommended Routine section.

Running a Routine

In addition to running the recommended routines as part of daily maintenance, all routines may be selected and run at any time.

To run a routine

1. From the Maintenance view, select the routine from the Select Routine list.

2. If the routine does not appear in the list, click the Additional Routines dropdown list and select the desired routine.

3. Fill the reservoirs as indicated in the Reagent Block.

   The Reagent Block is colored with the reservoirs and wells that need to be filled for the selected routine. For more information on filling the reservoirs, see Setting Up the Reagent Block on page 64.

4. If indicated in the Reagent Block, fill the well strip with the appropriate reagents.

   For more information on filling the well strip, see Setting Up the Reagent Block on page 64.

5. If the routine involves calibration and/or verification, select the kit in the Select Kit section.

       a.   Click the Calibration Kits dropdown arrow to select the correct calibration kit.

       b.   Click the Verification Kits dropdown arrow to select the correct verification kit.

Note: The lot number on the MAGPIX Calibration Kit or the MAGPIX Verification Kit CD must match the lot number of the kit to which the reagents belong.

For more information on calibration and verification kits, see Calibration and Verification Kits on page 66.

6.   Click Start.

Once the routine is completed, a dialog box appears informing you that the routine is completed.

Note: You cannot run a protocol while a routine is running. You must wait for the routine to complete.

Monitoring the Routine

From the Maintenance view, you can monitor the status of the routine in any of several ways.
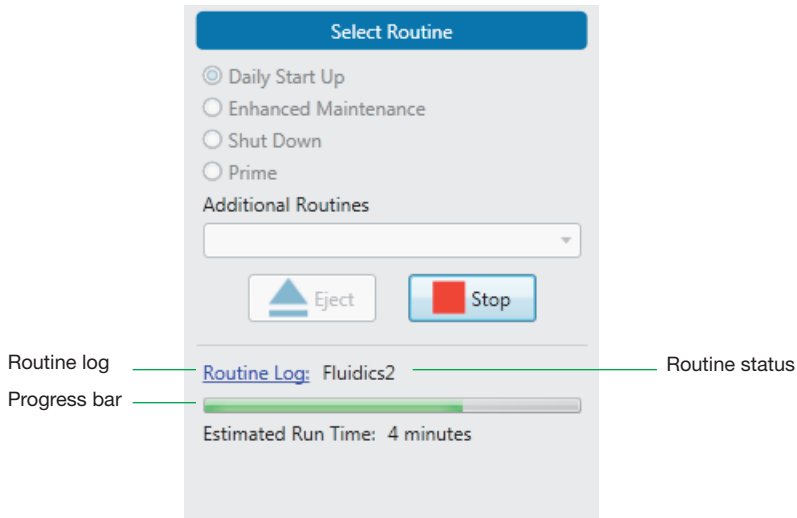
The following table describes the methods for monitoring the routine status.

Routine status	The progress of the commands in the routine are displayed here. The status changes, displaying each command as it is being executed. When the routine is finished running, the status displays *Completed*.
Routine log	Click the Routine Log link to open a Routine Commands dialog box displaying a complete list of all the commands in the routine, the reagents used, and the status of the commands.

Progress bar	The progress bar is a visual display of the duration of the routine. The progress bar fills from left to right as the routine is run. When it is completely filled in, the routine is finished running.

The following figure is an example of the Routine Commands dialog box.
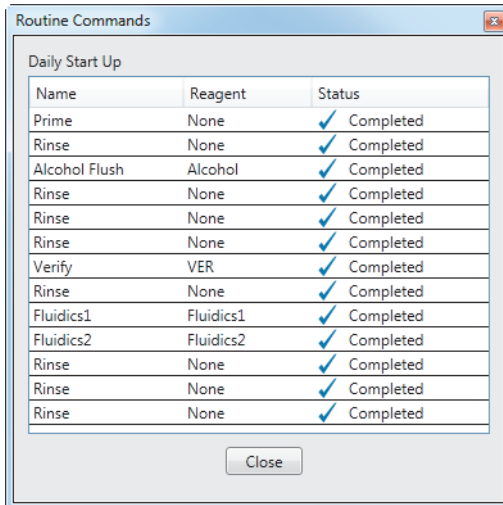
Setting Up the Reagent Block

The Reagent Block section of the Maintenance view provides you with the information you need to set up the reagent block before you run your routine.

1	The colored squares indicate which reservoirs to fill and specify the appropriate liquids.
2	The wells are color-coded to correspond to the reagents specified in the reagent block instructions. The colors match the color of the bottle covers of the reagents.
3	Routine name
4	Instructions on which liquids and reagents are required, any preparation required, and the amounts of the liquids and reagents to use.
5	Calibration or verification kit lot numbers
6	Reagent lot numbers
7	Description of the routine. Click the arrow to show or hide the description.

There is a diagram of the MAGPIX reagent block and well strip.

Important: The reservoirs and wells in the diagram correspond to those in the MAGPIX reagent block and well strip as seen when you stand facing the ejected plate.

When you select a maintenance routine, one or more reservoirs and wells are filled in.

The Reagent Block section displays the lot number of the calibration kit and verification kit currently selected. It also displays the lot numbers of the reagents that belong to the calibration and verification kits. Use this information to ensure that you are using the correct reagents.

Important: Do not mix reagents from different verification kits. All of the reagents must belong to the same lot.

If you select a different kit from the Select Kit section, the lot information in the Reagent Block section automatically updates with the new kit information.

To set up the Reagent Block

1. Fill any colored reservoir with the indicated liquid.

2. For each colored well in the well strip, locate the appropriate calibration or verification kit.

3. Vortex each bottle for at least 10 sec.

4. Fill the well with 6 drops of the solution from the bottle with the matching cap color.

Calibration and Verification Kits

Calibration and verification kits are sold separately. Each calibration kit comes with the following:

- 1 bottle of MAGPIX calibrator

- 1 calibration kit CD

- 1 pack of well strips

Each verification kit comes with the following:

- 1 bottle of MAGPIX verifier

- 1 bottle of MAGPIX fluidics 1

- 1 bottle of MAGPIX fluidics 2

- 1 verification kit CD

- 1 pack of well strips

About Kit Lot Numbers

The lot number on the box identifies the kit. Use this lot number to coordinate the reagents with the calibration or verification values (on the CDs). The bottle for each reagent is *not* identified with the lot number for the kit. Each bottle is identified with its own lot number, but this number is different from the lot number for the kit.

Important: The reagents must be used with the calibration or verification values with the same lot number. If the values and reagents have different lot numbers, the calibration and verification routines will fail.

Tip: When you open a new kit, continue to store the bottles in the original box or write the lot number of the kit on each of the reagent bottles. This will make it easier to keep track of which reagents are associated with a particular lot.

Importing the Calibration or Verification Values

The calibration kit CD and the verification kit CD contain values required to calibrate the MAGPIX instrument and verify the results. Only one set of calibration values and one set of verification values can be used at a time.

Best Practice

- Import the calibration or verification values as soon as you open a new kit.

To import the calibration or verification values

1. Navigate to the Maintenance view.

2. In the Select Kit section, click Import Kit.

3. Load the kit by doing one of the following:

    - If you have the CD, place the calibration or verification kit CD in the DVD drive and click Load from CD.

    - If the file is on your computer, navigate to where it is located on the computer, select the file, and click Open.

The lot number of the calibration kit or the verification kit appears in the Reagent Block section of the Maintenance view. Use this number to verify that you are using the correct calibration or verification values with your reagents.

Checking the Status of the Instrument Maintenance

The Status section of the Maintenance view displays the status of the following routines:

- Calibration

- Verification

- Fluidics

- Shut Down
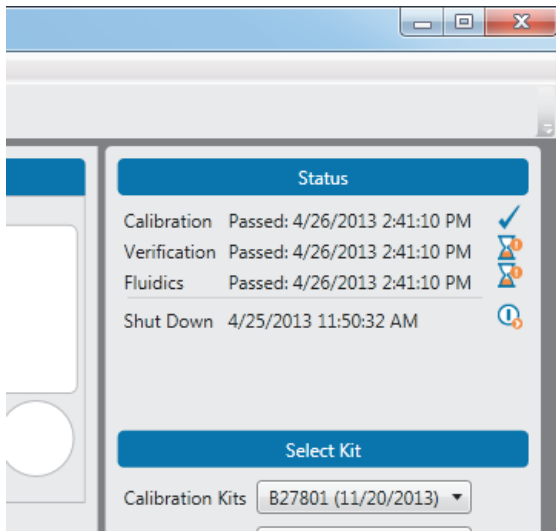
The Status section displays information about the last time the routine was run. It shows whether the routine was successfully completed or not (Passed or Failed) and a time stamp.

Status also shows the current status of the routine with the following symbols:

Symbol	Description
	This routine is current and up to date.
	This routine is not current or the routine failed.
	The Shut Down routine is in one of two states: ■ It has either never been run since the software was installed. ■ It has not been run in the current day. This is a reminder to run the Shut Down routine at the end of the day before you turn the instrument off.

If the Calibration, Verification, or Fluidics routines display the hourglass icon, then run all routines in the Recommended Routine section. Once the routine is successfully completed, the routines in the Status pane will display as current.

If the Shut Down routine displays the hourglass icon, then run the Shut Down routine before running any recommended routines.

Responding to a Failed Routine

A routine may fail due to instrument problems or user error. When failure occurs, follow the instructions in any messages that appear and perform the recommended routines.

Calibration Failures

If the calibration routine fails, Bio-Rad recommends you perform the following steps in the order shown. Performing the steps in order increases the likelihood that when you run the calibration routine again, it will succeed.

To correct a calibration failure

1. Check that the beads are in the correct wells.

2. Check that the correct calibration control values are being used.

3. Clean the sample probe.

4. Check that the waste container is not full.

5. Run the Adjust Probe Height procedure.

6. Run the recommended maintenance routine.

7. Run the Calibration and Verification routine.

 If calibration fails a second time, contact Bio-Rad Technical Support.

Verification Failures

If the verification routine fails, Bio-Rad recommends you perform the following steps in the order shown. Performing the steps in order increases the likelihood that when you run the verification routine again, it will succeed.

To correct a verification failure

1. Check that the beads are in the correct wells.

2. Check that the correct verification and/or calibration control values are being used.

3. Clean the sample probe.

4. Check that the waste container is not full.

5. Run the Adjust Probe Height procedure.

6. Run the recommended maintenance routine.

7. Run the Calibration and Verification routine.

    If verification fails a second time, contact Bio-Rad Technical Support.

Interrupting a Routine

You can interrupt a maintenance routine before it has finished, for example, if you forgot to fill the reagent block with the necessary liquids and reagents.

To interrupt a maintenance routine

▶   Click Stop.

Note: When you click on the Routine Log link while a routine is running, the Routine Commands window opens. You must close the Routine Commands window before you will be able to click Stop to interrupt the routine.

Preparing Your Instrument for Nonuse

When the MAGPIX instrument is not used for a period of time, microbes may grow in the water reservoir and traces of liquids and reagents in the lines can crystallize. Therefore, if you do not plan to use your instrument for 14 or more days, Bio-Rad recommends you prepare your instrument for a period of nonuse. Taking these steps before the instrument is idle will minimize the maintenance required when you resume using it.

To prepare your instrument for nonuse

1. Run the Prepare for Storage maintenance routine.

2. Remove the drive fluid container from the instrument.

3. Remove and empty the waste container.

4. Empty any liquids from the reagent block. Wash and dry the reagent block.

5. Empty any liquids from the well strip.

Adjusting the Probe Height

The probe must be calibrated in order to work correctly with the different plate types. Adjust the probe height in the following situations:

- After you set up the MAGPIX instrument and the computer, you must first adjust the probe height before you run any protocols.

- After you remove and clean the probe, you must adjust the probe height.

- If you experience sample problems, adjusting the probe height may resolve these errors.

To adjust the probe height

1. From the Maintenance view, click Adjust Probe Height.

   The Adjust Probe Height dialog box appears.

2. Empty the reagent block and the well strip.

   The rightmost reservoir in the reagent block and the rightmost well in the well strip must be empty.

3. Insert the MCV plate, well strip, and reagent block.

4. Click Adjust Probe Height.

   When the calibration is completed, a dialog box appears confirming that the adjust probe height procedure is completed.

5. Click OK.

Weekly Maintenance

Clean the sample probe once a week to keep it free of any debris. Whenever you remove the sample probe from the probe assembly, you must recalibrate the probe before you read another plate. Therefore, cleaning the sample probe must always be followed up with running the Adjust Probe Height maintenance routine.

Cleaning the Sample Probe

To clean the sample probe

1. Turn off the MAGPIX instrument and unplug the power cord.

2. Remove the sample probe.

3. Use either or both methods to clean the sample probe:

   - Bath sonicator — place the tip of the sample probe in the bath sonicator for 2–5 min.

   - 10 ml syringe — force distilled water through the tip of the sample probe to its large end.

4. Replace the sample probe. Screw the black fitting on top of the sample probe until it clicks.

5. From the Maintenance view, run the Adjust Probe Height procedure.

MAGPIX Maintenance

Within the first year of use, Bio-Rad recommends that you perform the 6-month and 12-month maintenance. The maintenance kits are:

- Bio-Plex® MAGPIX™ 6-Month Preventive Maintenance Kit — cleans the air filters and replaces the syringe seals (catalog #171-012010).

- Bio-Plex MAGPIX 12-Month Preventive Maintenance Kit — replaces the sample probe tube and the drive fluid filter (catalog #171-012012).

7 Maintenance Routines

The maintenance routines keep the MAGPIX instrument in good working order and operating according to the manufacturer's specifications. Bio-Rad recommends that you run the recommended routine at the start of each day to minimize having to keep track of running the correct routines at the right time. In addition to running the recommended routine, you can run any of the maintenance routines as necessary.

Calibration and Verification Routine

Description of Routine

Calibration normalizes the operation of the MAGPIX analyzer when it acquires data. Once calibrated, the values remain until you recalibrate. Verification verifies the operation of the MAGPIX analyzer.

When to Run this Routine

Bio-Plex Manager™ MP software adds calibration and verification to the Daily Start Up routine once a week in accordance with the manufacturer's recommendation. Therefore, if you run the Daily Start Up routine daily, you will be running the Calibration and Verification routine as recommended.

In addition, recalibrate the system if the any of the following is true:

- The delta calibration temperature exceeds ±5ºC.

- The instrument has been moved.

- The instrument undergoes hardware maintenance, for example, a part replacement.

Estimated Run Time

10 minutes

To Run the Calibration and Verification Routine

Before you run the Calibration and Verification routine, you must import the calibration and verification kit information. For more information, see Calibration and Verification Kits on page 66.

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Calibration and Verification from the Additional Routines dropdown list.

3. Add 5 ml 70% isopropanol to the appropriate reservoir in the reagent block.

4. Vortex CAL, VER, Fluidics1, and Fluidics2 each for 10 sec.

5. Add 6 drops each of CAL, VER, Fluidics1, and Fluidics2 to the indicated wells.

6. Click Start.

Clear Bubbles Routine

Description of Routine

The Clear Bubbles routine performs a series of fluidics operations to force out air bubbles that may be trapped in the cuvette.

When to Run this Routine

If you suspect there is air in the MAGPIX system, run the Clear Bubbles routine.

Estimated Run Time

3 minutes

To Run the Clear Bubbles Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Clear Bubbles from the Additional Routines dropdown list.

3. Add 5 ml distilled or deionized water and 5 ml 70% isopropanol to the appropriate reservoirs in the reagent block.

4. Click Start.

Daily Start Up Routine

Description of Routine

Daily Start Up prepares your instrument for daily use. It runs different routines depending on the state of the instrument. Daily Start Up may include the Calibration and Verification, Verification, and Wake From Storage routines.

When to Run this Routine

Run Daily Start Up each day you use the instrument before you run any protocols. You do not need to keep track of the calibration and verification status if you run this routine at the start of each day.

Estimated Run Time

4–12 minutes

To Run the Daily Start Up Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Daily Start Up.

3. Fill the reservoirs and the well strip with the solutions indicated in the Reagent Block diagram.

4. Click Start.

Enhanced Maintenance Routine

Description of Routine

The Enhanced Maintenance routine clears any bubbles from the system and clears any clogs from the probe and fluidics.

When to Run this Routine

This routine is recommended when a low bead count occurs during a protocol run or when calibration or verification fails.

Estimated Run Time

14 minutes

Before Running the Routine

Before you run the Enhanced Maintenance Routine, Bio-Rad recommends that you check the following:

1. Check that the correct calibration and control lots are being used.

2. Clean the sample probe.

3. Check that the waste container is not full.

4. Run the Adjust Probe Height procedure.

To Run the Enhanced Maintenance Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Enhanced Maintenance.

3. Add 5 ml distilled or deionized water, 5 ml 70% isopropanol, and 5 ml 0.1N NaOH to the appropriate reservoirs in the reagent block.

4. Click Start.

Fluidics Preparation Routine

Description of Routine

The Fluidics Preparation routine prepares the fluidics system for daily use.

When to Run this Routine

Run the Fluidics Preparation routine when you replace an empty drive fluid container or if the drive fluid line is not aspirating drive fluid because the line has become dislodged.

Estimated Run Time

2 minutes

To Run the Fluidics Preparation Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Fluidics Preparation from the Additional Routines dropdown list.

3. Add 5 ml 70% isopropanol to the appropriate reservoir in the reagent block.

4. Click Start.

Prepare for Storage Routine

Description of Routine

The Prepare for Storage routine clears all fluids from the instrument.

When to Run this Routine

Run the Prepare for Storage routine if you will not be using the instrument for more than two weeks or before you ship the instrument to Bio-Rad.

Estimated Run Time

6 minutes

To Run the Prepare for Storage Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Prepare for Storage from the Additional Routines dropdown list.

3. Add 5 ml distilled or deionized water, 5 ml 0.1N NaOH, and 5 ml 10% bleach to the appropriate reservoirs in the reagent block.

4. Click Start.

Prime Routine

Description of Routine

The Prime routine primes the fluidics system by forcing drive fluid through the fluidics lines.

When to Run this Routine

If the Unpack MAGPIX routine fails during the initial instrument set up, then run the Prime routine.

Estimated Run Time

1 minute

To Run the Prime Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Prime from the Additional Routines dropdown list.

3. Click Start.

Shut Down Routine

Description of Routine

The Shut Down routine cleans the fluidics lines and prevents a buildup of debris within the system.

When to Run the Routine

Bio-Rad recommends that you run the Shut Down routine at the end of each day that you use the instrument. Refer to the Shut Down entry in the status section in the Maintenance view to determine whether the Shut Down has been run that day.

Estimated Run Time

2 minutes

To Run the Shut Down Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Shut Down.

3. Add 5 ml distilled or deionized water and 5 ml bleach to the appropriate reservoirs in the reagent block.

4. Click Start.

Unclog Routine

Description of Routine

This procedure performs a series of fluidics operations to remove possible obstructions from the fluidics lines.

When to Run this Routine

If you suspect there is an obstruction in the MAGPIX system, run the Unclog routine.

Estimated Run Time

2 minutes

To Run the Unclog Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Unclog from the Additional Routines dropdown list.

3. Add 5 ml distilled or deionized water and 5 ml 0.1N NaOH to the appropriate reservoirs in the reagent block.

4. Insert the plate in the microplate platform and click Start.

Verification Routine

Description of Routine

The Verification routine ensures that the calibration settings are valid and ensures that well-to-well cross talk does not occur.

When to Run this Routine

Run the Verification routine when its status in the Maintenance view is not up to date. Bio-Plex Manager MP adds verification to the Daily Start Up routine in accordance with the manufacturer's recommendation. Therefore, if you run the Daily Start Up routine daily, you will be running the Verification routine as recommended.

Estimated Run Time

4 minutes

To Run the Verification Routine

Before you run the Verification routine, you must import the verification kit information. For more information, see Calibration and Verification Kits on page 66.

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Verification from the Additional Routines dropdown list.

3. Add 5 ml 70% isopropanol to the appropriate reservoir in the reagent block.

4. Vortex VER, Fluidics1, and Fluidics2 each for 10 sec.

5. Add 6 drops each of CAL, VER, Fluidics1, and Fluidics2 to the indicated wells.

6. Click Start.

Wake from Storage Routine

Description of Routine

The Wake from Storage routine prepares the instrument to be used after it has been idle for a long period of time.

When to Run this Routine

Run the Wake from Storage routine if the instrument has been idle for 2 weeks or longer or if it has been prepared for storage.

Bio-Plex Manager MP adds Wake From Storage to the Daily Start Up routine if it detects that the instrument has been idle for 2 weeks or longer. Therefore, if you run the Daily Start Up routine daily, you will be running this routine as recommended.

Estimated Run Time

2 minutes

To Run the Wake From Storage Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Wake From Storage from the Additional Routines dropdown list.

3. Add 5 ml distilled or deionized water and 5 ml 70% isopropanol to the appropriate reservoirs in the reagent block.

4. Click Start.

Wash Between Plates Routine

Description of Routine

The Wash Between Plates routine performs a series of fluidics operations to prevent traces of sample or other debris from collecting inside the Bio-Plex® suspension array system.

When to Run this Routine

Bio-Rad recommends that you wash the fluidics lines after each plate reading.

Note: In the Run Settings dialog box, the default is to run the Wash Between Plates routine after the reading.

Estimated Run Time

6 minutes

To Run the Wash Between Plates Routine

1. Navigate to the Maintenance view.

2. In the Select Routine section, select Wash Between Plates from the Additional Routines dropdown list.

3. Add 5 ml distilled or deionized water, 5 ml 70% isopropanol, and 5 ml 0.1N NaOH to the appropriate reservoirs in the reagent block.

4. Click Start.

A License Activation

To have access to all of the features of Bio-Plex Manager™ MP software, you must activate the product with a valid product license. You will need a separate license for each computer on which you install Bio-Plex Manager MP.

Note: If you purchased a new MAGPIX instrument, the computer included with the instrument comes with the software already activated. Therefore, you can skip this step.

Activating the Bio-Plex Manager MP License

You can activate the product either online (if you have access to the Internet) or through Technical Support (if you do not have Internet access).

Note: To activate your license, you will need the product activation code, which can be found in the Bio-Plex Manager MP CD folder.

Activating the License Online

Follow the procedure below if the computer on which Bio-Plex Manager MP is installed has an Internet connection. With this method, the software uses your activation code to automatically activate your software.

To activate Bio-Plex Manager MP online

1. Start the Bio-Plex Manager MP software.

2. If the product license has not been activated, the Product Activation Required window appears.
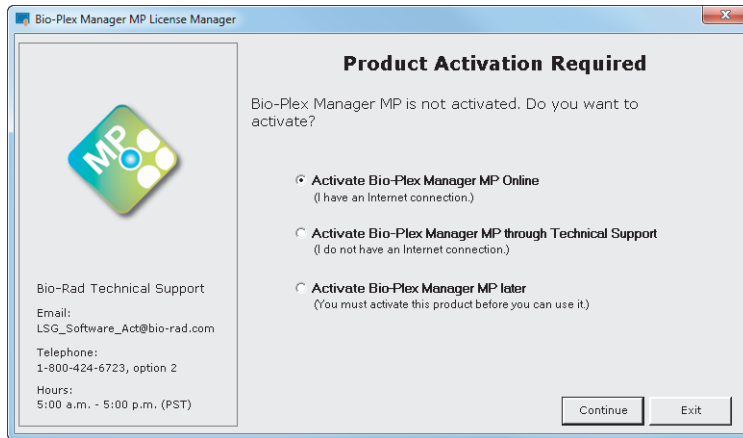
3. Select Activate Bio-Plex Manager MP Online and click Continue.

4. Enter your activation code in the text box and click Activate.

    Note: The activation code can be found in the CD folder.
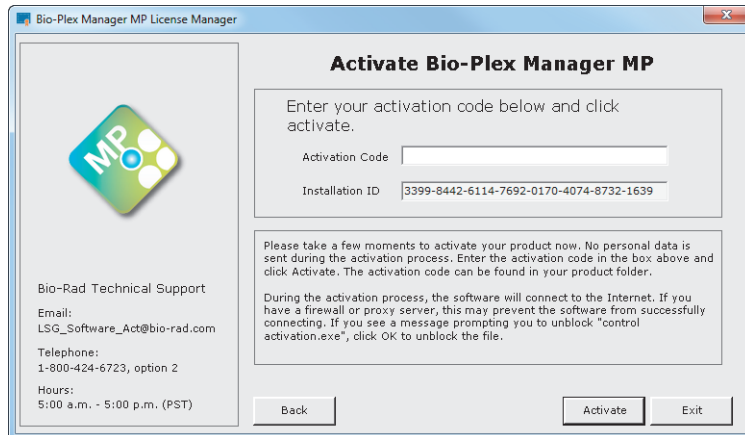
The Activation Successful window appears confirming that the software has been activated.
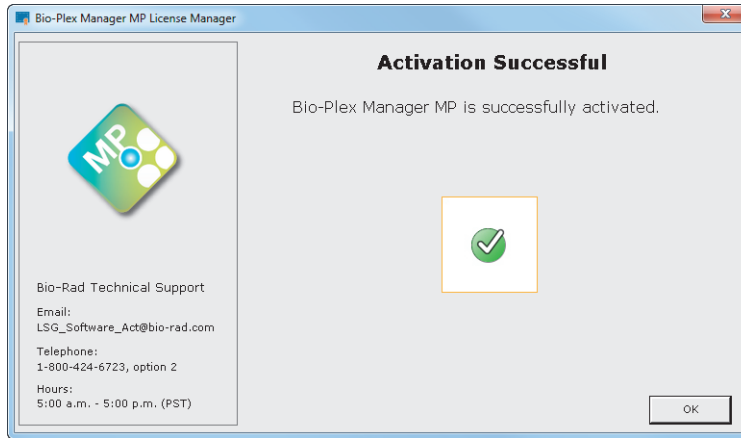
5. Click OK to exit the activation software and start Bio-Plex Manager MP.

Activating the License through Technical Support

If the computer on which Bio-Plex Manager MP is installed does not have an Internet connection, you can request a product license from Bio-Rad Technical Support to activate the software. This is a two-phase process:

1. Generate a credentials file and email it to Technical Support.

   Technical Support uses the credentials file to generate a license and emails the file back to you.

2. Use the license file to activate your software.

The procedures that follow describe this two-phase process.

Generating the Credentials File

To generate the credentials file

1. Start the Bio-Plex Manager MP software.

2. If the product license has not been activated, the Product Activation Required window appears.

3. Select Activate Bio-Plex Manager MP through Technical Support and click Continue.
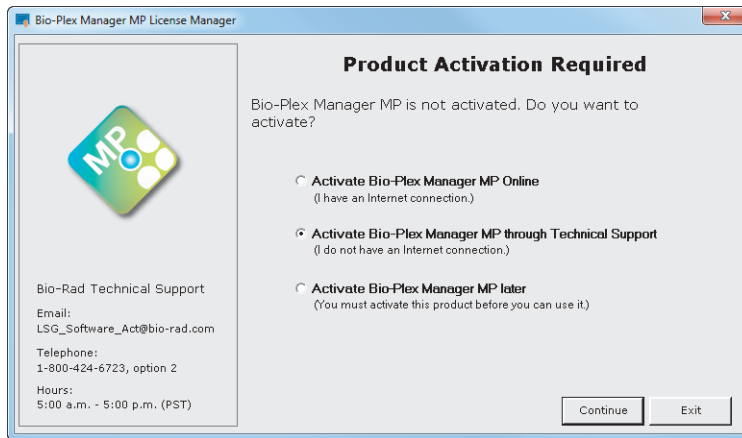
4. Select Send credentials to Technical Support and click Continue.
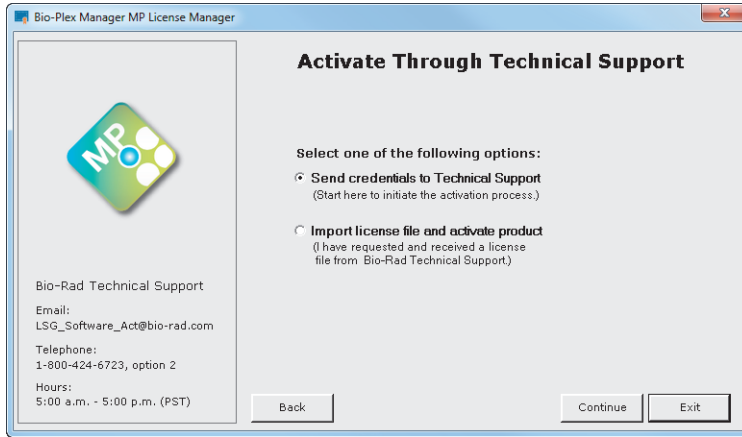
5. Enter your activation code in the text box and click Save.

   Note: The activation code can be found in the CD folder.
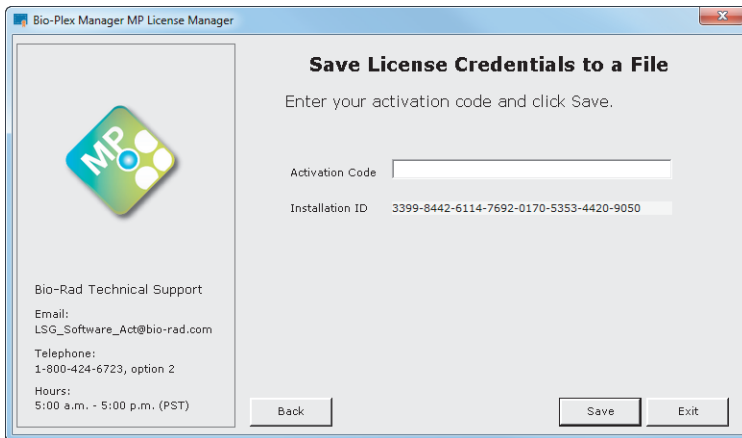
6. In the Save Licensing Credentials to a File dialog box, enter the name of the file and click Save.

The File Successfully Saved window appears with instructions on how to send the credentials file to Technical Support.
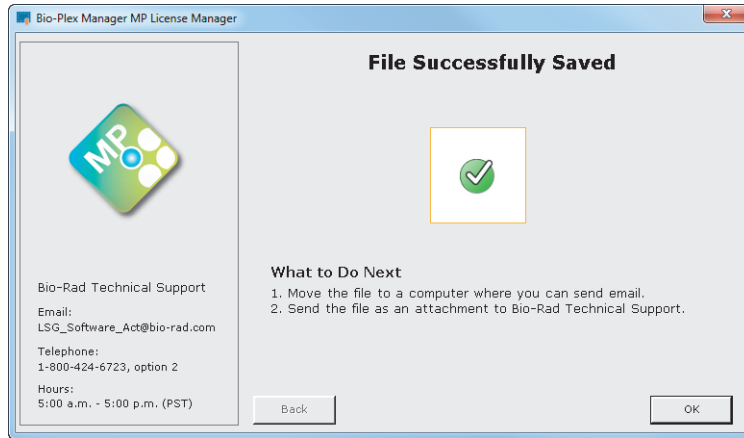
7. Move the credentials file to a computer where you can send email, and email the file to Bio-Rad Technical Support (LSG_Software_Act@bio-rad.com).

Activating the Software with a License File

Technical Support will generate a file with the license and email the file to you. When you receive the email, transfer the file to the computer where Bio-Plex Manager MP is installed. The file can be copied to a local hard drive, flash drive, or network drive.

To activate Bio-Plex Manager MP with the license file

1. Start the Bio-Plex Manager MP software.

2. In the Product Activation Required window, select Activate Bio-Plex Manager through Technical Support and click Continue.
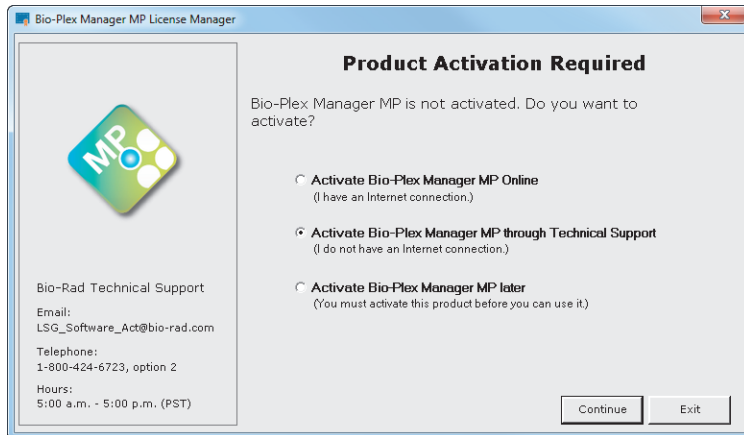
3. Select "Import license file and activate product" and click Continue.
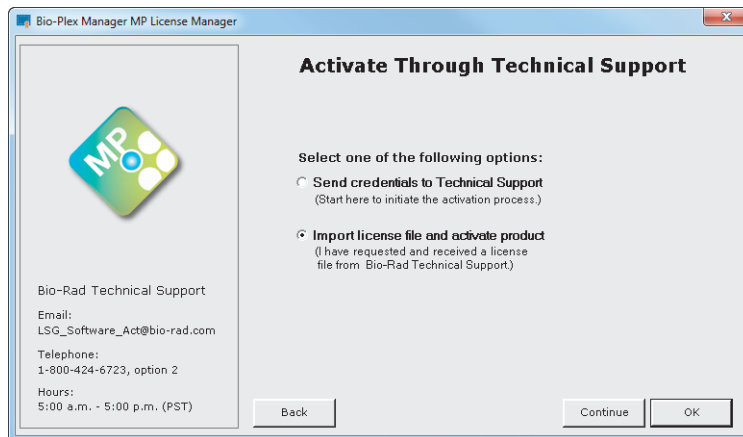
4. Click Activate.
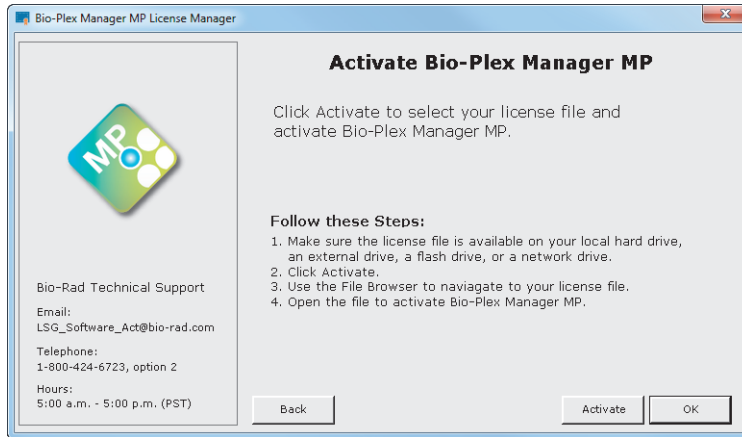
5. Navigate to where the license file is located, select the file, and click Open.

The Activation Successful window appears confirming that the software has been activated.
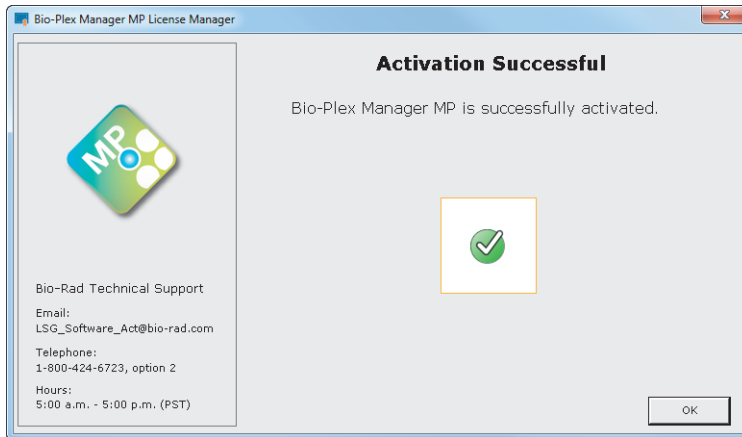
6. Click OK to exit the activation software and start Bio-Plex Manager MP.

Deactivating the Bio-Plex Manager MP License

You may want to deactivate the Bio-Plex Manager MP license for one of the following reasons:

■ You want to move the license from one computer to another.

   This can occur, for example, if you are replacing your computer. You must first deactivate the license from the first computer and then activate it on the second computer.

■ You want to remove the license from the computer.

Deactivating the License Online

To deactivate Bio-Plex Manager MP online

1. Start Bio-Plex Manager MP.

2. From the Help menu, click Manage License.

3. Select Deactivate Bio-Plex Manager MP Online and click Continue.
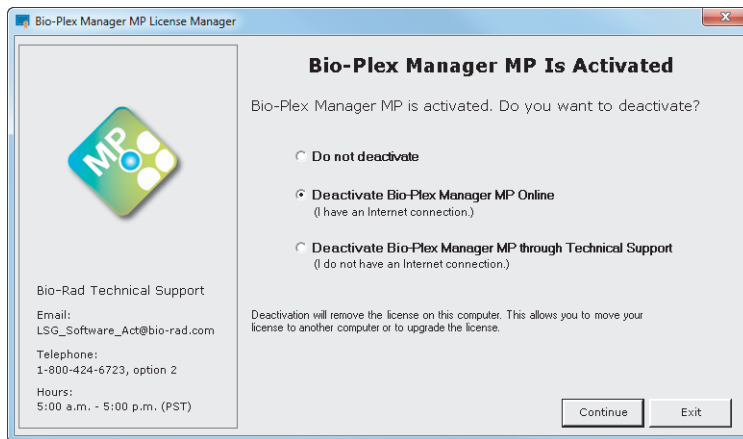
4. Enter your activation code and click Deactivate.

Note: The Activation Code field will be populated with the activation code. If the field is empty, you can find the activation code in the CD folder.
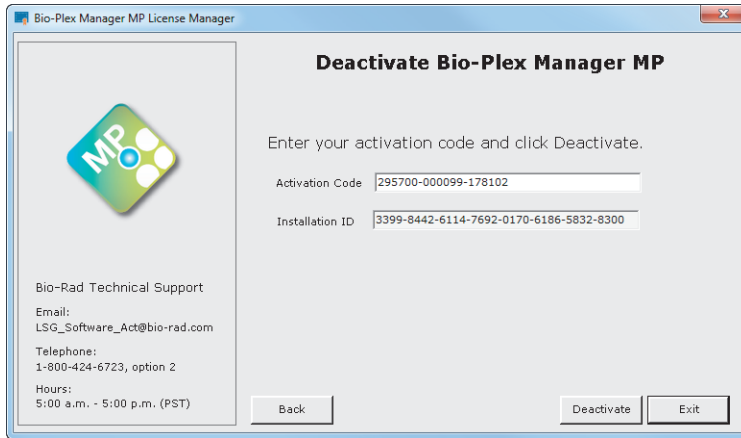
The Deactivation Successful window appears confirming the software has been deactivated.
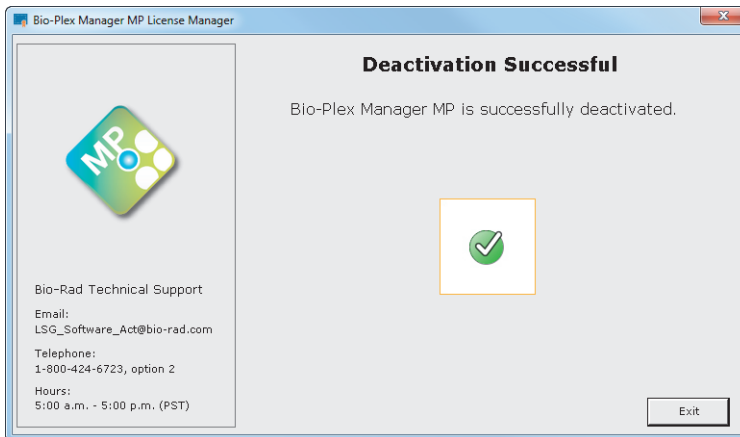
5. Click Exit.

Deactivating the License through Technical Support

If the computer on which Bio-Plex Manager MP is installed does not have an Internet connection, you can deactivate your license through Bio-Rad Technical Support. This is a two-phase process:

1. Generate a deactivation certificate file and email it to Technical Support.

2. Technical Support informs you that the license has been deactivated.

Important: Until Technical Support sends you a confirmation that the license is deactivated, you will not be able to move the license to another computer or reactivate it on the current computer.

Exporting the Deactivation Certificate

To export the deactivation certificate

1. Start Bio-Plex Manager MP.

2. From the Help menu, click Manage License.

3. Select Deactivate Bio-Plex Manager MP through Technical Support and click Continue.
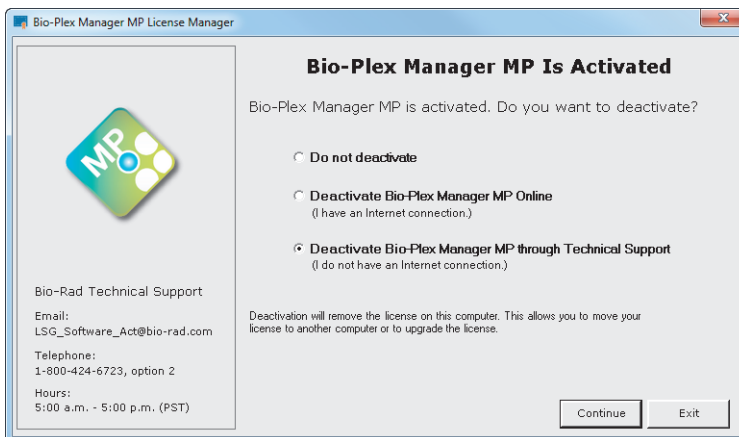
4. In the Export Deactivation Certificate window, click Save.
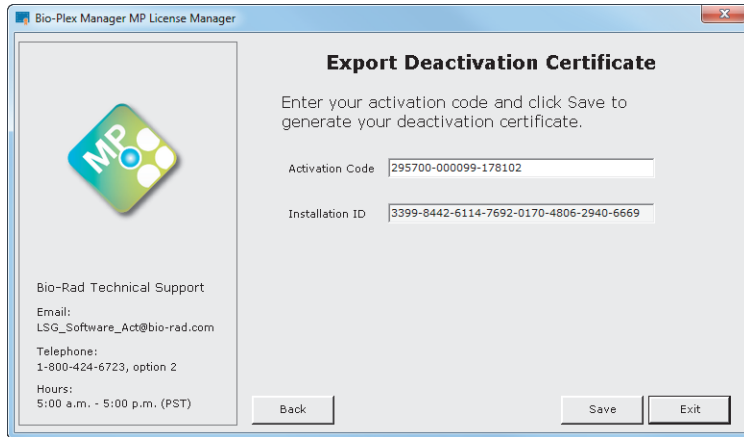
A dialog box appears asking you to confirm the deactivation.

5. Click Yes.

6. In the File browser, navigate to where you want to save the deactivation certificate.

7. Enter the name of the file and click Save.

The File Successfully Saved window appears with instructions on how to send the deactivation certificate file to Technical Support.
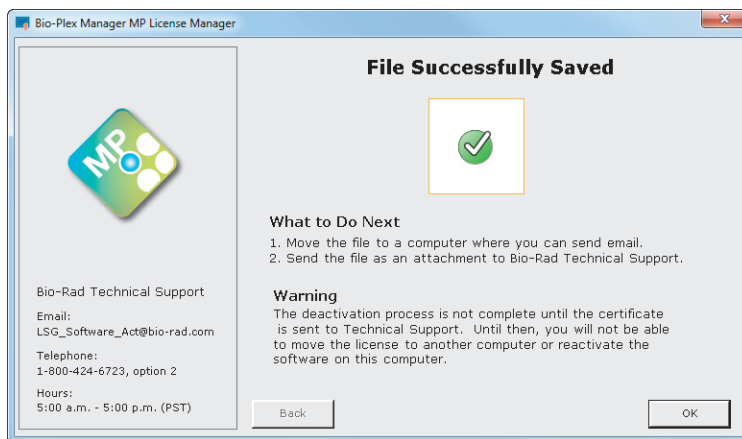
8. Click OK.

9. Move the deactivation certificate file to a computer where you can send email, and email the file to Bio-Rad Technical Support (LSG_Software_Act@bio-rad.com).

Technical Support will reply with an email informing you that the license has been deactivated. Once you receive this email, the license has been removed from the computer. You have the option to reuse the license and activate it on any computer.

A | License Activation

B Backing Up the Database

There are two databases that need to be backed up on a regular basis:

- xPONENT database
- Bio-Plex Manager™ MP database

Backing up the xPONENT Database

Bio-Rad recommends that you back up the xPONENT database on a regular basis. You can manually back up the xPONENT database or you can schedule automatic backups. The default is manual backups. Database backups are managed with the Archive Utility.

Unless you specify otherwise, you will get the following reminder to back up your database:

Please initiate a Scheduled Archive or you will eventually run out of disk space and not be able to run Batch in xPONENT. Click Yes to open the Archive tool.

You can also run the utility by navigating to the application.

To open the Archive Utility

1.   Navigate to the following location: All Programs\Luminex\Common

2.   Click xPONENT Archive to start the utility.
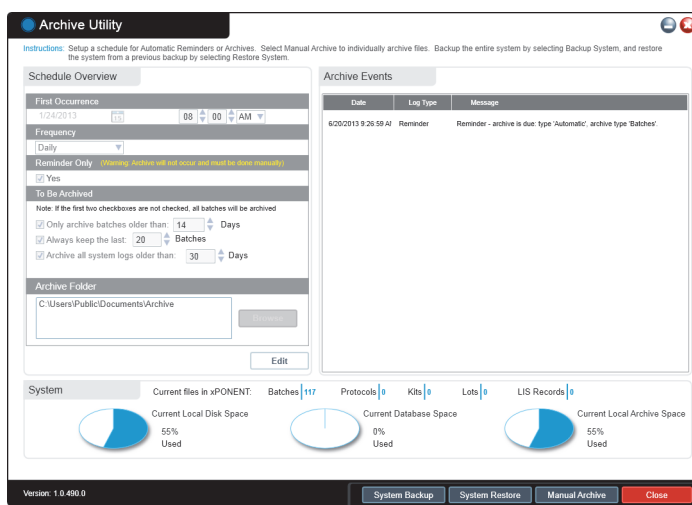
You can specify the frequency with which you receive reminders to back up the database or you can schedule automatic database backups. For more information on the Archive Utility, see xPONENT for MAGPIX 4.2 Software User Manual available on the Luminex web site.

Backing up the Bio-Plex Manager MP Database

It is a best practice to back up the database frequently and keep the backup copies in a safe location. A USB or network hard drive is a good backup solution. Keep in mind that it can take several minutes to back up a large database.

To back up the database

1. Exit the Bio-Plex Manager MP software.

2. Navigate to C:\ProgramData\Bio-Rad\Bio-Plex Manager MP.

    Note: If you do not see the ProgramData directory, the directory may be hidden and you must show the hidden files. See Showing Hidden Files on page 105.

3. Copy the database file, BioPlexManagerMP_214.db3, to a backup location.

Showing Hidden Files

If you do not see the directory specified in the database path, the cause may be that the directory is hidden from view. You must display the hidden directory before you can back up your database.

To display hidden files

1. Go to the Windows Start menu, select Settings, and select Control Panel.

2. Click Folder Options.

3. In the Folder Options dialog box, click the View tab.

4. Under Advanced Settings, click Show hidden files, folders, and drives.

5. Click OK.

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C Troubleshooting

Problem	Possible Cause	Solution
Instrument is not connected.	The connection to the MAGPIX instrument has been lost.	Try each of these suggestions in order until you reconnect to the instrument: 1. Confirm that the instrument is turned on. 2. Reconnect to the instrument. From the Instrument menu, select Reconnect Instrument. 3. Turn off the instrument and exit Bio-Plex Manager™ MP. Turn the instrument on and restart the software. 4. Restart the computer. Restart Bio-Plex Manager MP.
Region Selection/ Severe Clog	One or more of the analytes selected has little or no data.	Perform the following steps: 1. Stop the run. 2. Review your data and verify that you have selected the correct analytes in the protocol definition. Make any necessary changes to the selected analytes. 3. Clean the probe and run the Enhanced Maintenance routine. 4. Run the protocol again.

Problem	Possible Cause	Solution
Low Bead Count	Three or more consecutive wells have bead counts lower than the recommended number.	Perform the following steps: 1. Stop the run. 2. Clean or replace the sample probe. 3. Perform the Adjust Probe Height procedure. 4. Perform the Enhanced Maintenance routine. 5. Run the protocol again.
You empty the waste container but continue to see the following error message: The waste container is full. Empty the waste container and replace it with a dry waste container.	The instrument detects some moisture in the waste container and triggers an alert.	When emptying the waste container, replace it with a **different** waste container that is dry. The sensors on the emptied waste container may not be completely dry, causing the instrument to incorrectly detect the waste container as full.

Problem	Possible Cause	Solution
A Windows security alert dialog box appears with the message: Windows Firewall has blocked some features of this program.	The user's profile and permissions are set such that the user must grant access to xPONENT.	Click "Allow access."

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