

BD Multiwell AutoSampler Additional Features Tutorial

Introduction

This tutorial provides step-by-step instructions on how to use the additional features available in BD Multiwell™ Plate Manager (MPM) software and is similar to that demonstrated in the Additional Features movie. Before starting this tutorial, view the *BD Multiwell AutoSampler Additional Features* movie (located on the BD FACStation™ Software for Mac® OS X CD, version 5.0.1). To best view the movie, your computer monitor resolution should be set to 1024 x 768, 75 Hz. To change the resolution on your monitor go to Apple > System Preferences > Monitors.

This tutorial covers:

- A. View instrument settings
- B. Keywords and choices
- C. Protocol parameters
- D. Export summary table
- E. Print options (not shown in the Additional Features movie)

NOTE: Instructions for daily operation using the additional features in BD Multiwell Plate Manager software can be found at the end of this tutorial.

To perform the steps in this tutorial, you will need:


- A cytometer with the BD Multiwell AutoSampler* option installed
- A third party spreadsheet application, such as Microsoft® Excel (optional)

NOTE: Boldface text indicates key instructions. Additional information is provided in plain text.

Do the following if MPM software is not started.

1. **Click the MPM icon in the Dock to launch BD Multiwell Plate Manager software.**



If the MPM icon  is not in the Dock, do the following:

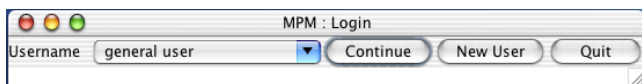
- Drag the MPM icon from the BD Applications folder to the Dock.
- Click the icon in the Dock to launch the software.

*For Research Use Only. Not for use in diagnostic or therapeutic procedures.

2. Choose Analysis Only in the MPM Mode dialog.

Once you select Analysis, it takes a few seconds before the next dialog appears.

3. Choose a name from the Username drop-down menu or create a new user name, and then click Continue.



To create a new user name, click New User, enter a name in the Username field, and click Continue.

Once you click Continue, it takes a few seconds before the main application window appears. The AutoSampler Parameters View appears by default. The window displays one of three views: Keyword Subset View, AutoSampler Parameters View, or Protocol Parameters View. You can choose the view you want by selecting its tab.

A. View Instrument Settings

You can view a currently selected Instrument Settings file or choose to view another settings file by doing the following exercise. This feature can be used as you are setting up the software for acquisition.

1. Select a well in the plate layout View.
2. Choose Acquisition > View Instrument Settings File.
 - If the highlighted well does not have an Instrument Settings file selected, the Instrument Settings and navigation dialogs appear (Figure 1).

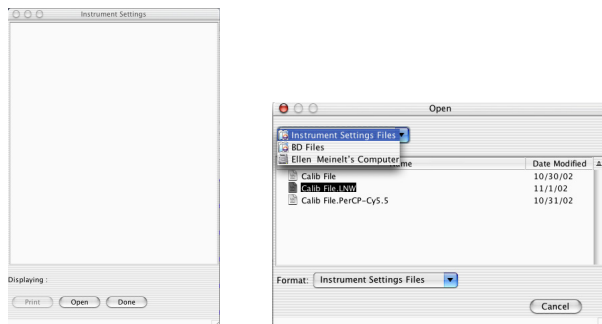
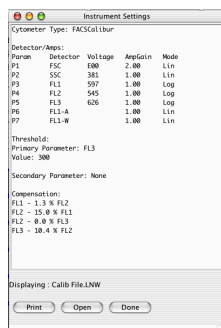


Figure 1 Instrument Settings and navigation dialogs

- If the highlighted well has an Instrument Settings file selected, the Instrument Settings dialog appears with the settings displayed.



2. Navigate to the folder containing the Instrument Settings file you want to view.

For example, do the following:

- Click Open in the Instrument Settings dialog if the navigation dialog is not already opened.
- Double-click the BD Files folder to display its contents.
- Double-click the Instrument Settings Files folder to display its contents.
- Double-click an Instrument Settings file to open it (eg, Calib File).

The Instrument Settings dialog displays the contents of the Instrument Settings file.

3. Print the Instrument Settings, if needed.

Click Print in the Instrument Settings dialog.

4. Open a different Instrument Settings file, if needed.

5. Click Done to exit the Instrument Settings dialog.

NOTE: The previous procedure only allows you to view Instrument Settings. To select Instrument Settings for acquisition, follow the instructions provided in the *Daily Operation* tutorial available from the BD Biosciences website (http://www.bdbiosciences.com/immunocytometry_systems/).

B. Keywords and Choices

There are two kinds of Keywords that exist in BD Multiwell Plate Manager software, predefined and user-defined. Keywords that are predefined are those listed in the AutoSampler Parameters and Protocol Parameters views. Examples of predefined keywords are Sample Volume, Mixing Volumes, and Number of Mixes. User-defined keywords are created in the Master Keywords Set dialog (see Figure 1).

A Choice is a variable of the Keyword. There are also predefined and user-defined Choices. Predefined Choices include the values in the Number of Mixes drop-down menu in the AutoSampler Parameters view. User-defined Choices are created in the Master Keywords Set dialog.

Keywords and Choices are used to assign descriptors to each well before samples are acquired. Once the sample has been acquired, Keywords and Choices can be exported and viewed in a spreadsheet format using third-party software (for example, Microsoft Excel).

In this example, you will create three types of Keywords. The first is free text, the second is a text field with defined choices, and the third is a number field with a numerical range.

For more examples of how to create Keywords and Keyword Subsets, refer to Appendix A on page 21 or refer to the *BD Multiwell AutoSampler User's Guide*.

Creating a Text Keyword

1. Choose Layout > Modify > Master Keywords Set.

The Master Keywords Set dialog appears.

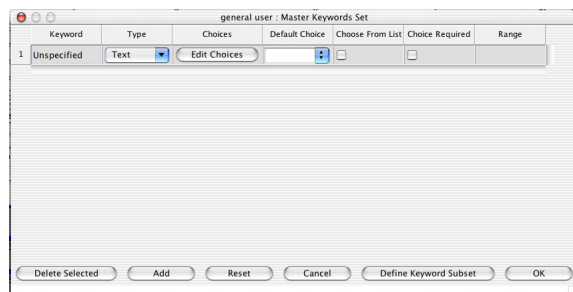


Figure 1 Master Keywords Set dialog

2. Enter *Stimulus* in the field beneath Keyword.

	Keyword	Type	Choices
1	Stimulus	Text	Edit Choices

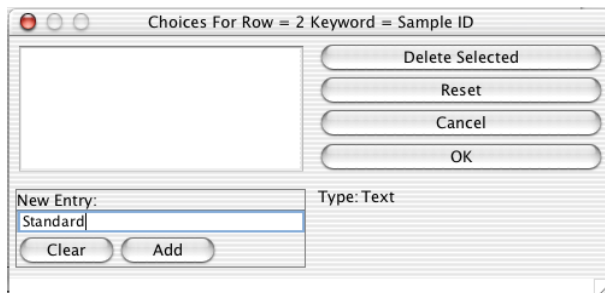
3. Verify that Text is selected from the Type drop-down menu.

Creating a Text Field with Defined Choices

1. Click the Add button at the bottom of the Master Keywords Set dialog to add another Keyword.
2. Enter *Sample ID* in the next Keyword field.

	Keyword	Type	Choices
1	Stimulus	Text	Edit Choices
2	Sample ID	Text	Edit Choices

3. Verify that Text is selected in the Type column for the *Sample ID* Keyword.
4. Click Edit Choices in the Choices column for the *Sample ID* Keyword.
A dialog appears.
5. Enter *Standard* in the New Entry field, and then click Add.



6. Enter *Specimen* in the New Entry field, and then click Add.
7. Click OK to exit the dialog.
8. Click the checkbox in the Choose From List column for the Sample ID Keyword to select it.

Selecting Choose From List prevents you from entering free text. The Sample ID Keyword will include only the choices you specified, in this case, Standard and Specimen. See Figure 2.

	Keyword	Type	Choices	Default Choice	Choose From List
1	Stimulus	Text	Edit Choices		<input type="checkbox"/>
2	Sample ID	Text	Edit Choices	<No Va...	<input checked="" type="checkbox"/>

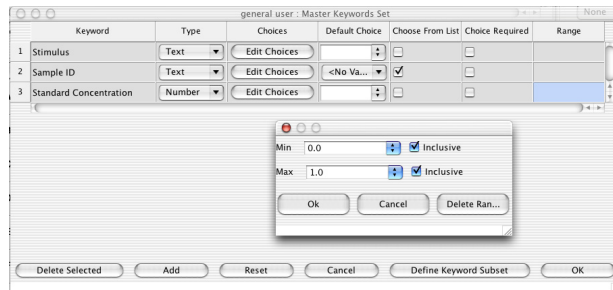
Choose From List checkbox

Figure 2 Selecting Choose From List

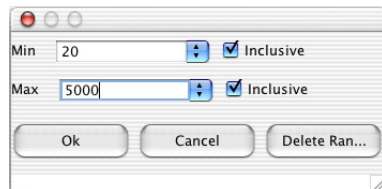
Creating a Number Field with a Numerical Range

1. Click the Add button to add another Keyword.
2. Enter *Standard Concentration* in the next Keyword field.
3. Choose Number from the Type drop-down menu.
4. Click in the Range field of the *Standard Concentration* Keyword.

A dialog appears.



5. Enter 20 in the Min field and 5000 in the Max field; click OK.



The entered range appears in the Range column of the Master Keywords Set. Your Choices for *Standard Concentration* are numerical values between the range of 20 and 5000.

Defining A Keyword Subset Name

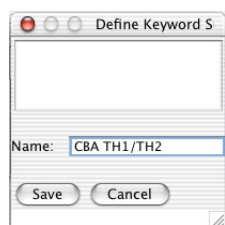
Now that you have defined three Keywords and their Choices, you can select any combination of the defined Keywords to create Keyword Subsets. For example, you can choose the *Sample ID* and *Standard Concentration* Keywords to create a Keyword Subset. For this example, you will create a Keyword Subset containing all three Keywords that you just defined.

1. Select all defined Keywords in the Master Keywords Set dialog.

Hold down the Control key while clicking each row number. All Keywords become highlighted.

2. Click  at the bottom of the Master Keywords Set dialog.

The Define Keyword Subset dialog appears.



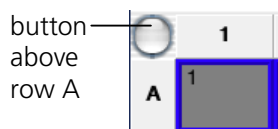
3. Enter *CBA TH1/TH2* in the Name field, and then click Save.
4. Click OK in the Master Keywords Set dialog to exit.

Using the Keyword Subset

Next, you will use the Keyword Subset you just defined to add descriptors to a series of wells.

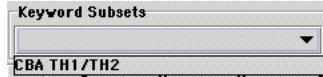
1. Select all wells in the plate layout View.

To select all wells, click once on the button above row A. All the wells become highlighted in blue.



2. Click the Keyword Subset View tab in the Protocol window.

- From the Keyword Subsets drop-down menu, choose *CBA TH1/TH2*.

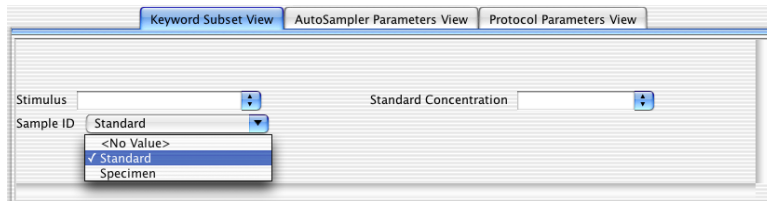


- Select wells A1 through A9.

Drag the mouse over wells A1 through A9.

- Choose Standard from the Sample ID drop-down menu.

Wells A1 through A9 are now assigned the Keyword *Sample ID* and *Standard* as the Choice. Note that aside from No Value, the only available Choices are *Standard* and *Specimen* from the *Sample ID* drop-down menu. You cannot enter free text for *Sample ID* because you selected Choose From List and *Standard* and *Specimen* were the only two Choices defined previously.



- Select wells B1 through H12.

- Choose Specimen from the Sample ID drop-down menu.

Wells B1 through H12 are now assigned with the Choice *Specimen* for the Keyword *Sample ID*.

- Choose well A1 and enter 5000 in the Standard Concentration field.



Well A1 are now assigned the following: *Standard* is the Choice for the Keyword *Sample ID* and 5000 is the Choice for the Keyword *Standard Concentration*. Note that you can enter only a value between 20 and 5000 because these are the minimum and maximum values defined for the Keyword *Standard Concentration*.

9. Enter concentration values for wells A2 through A9.

Enter the following values in the indicated wells:

- A2: 2500
- A3: 1250
- A4: 625
- A5: 313
- A6: 156
- A7: 80
- A8: 40
- A9: 20

10. Select rows B through E and then enter the Choice *PMA* in the Stimulus Keyword field.

The Choice of *PMA* is for the Keyword *Stimulus* is now assigned to the selected wells. Since the Keyword *Stimulus* was defined as a text field without Choices, you can enter anything in this field.

11. Select rows F through H and enter the Choice *SEB* in the Stimulus Keyword field.

The Choice of *SEB* for the Keyword *Stimulus* are now assigned to the selected wells.

The Keyword and Choice assignments for each well is now complete for this example.

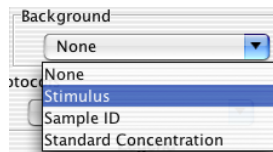
- You have assigned the Choice of *Standard* for *Sample ID* Keyword for wells A1 through A9, as well as a concentration value for each of those wells.
- You have assigned the Choice *Specimen* to the Keyword *Sample ID* and the Choices *PMA* or *SEB* to the Keyword *Stimulus* for wells B1 through H12.

Viewing the Keyword Subset Assignments

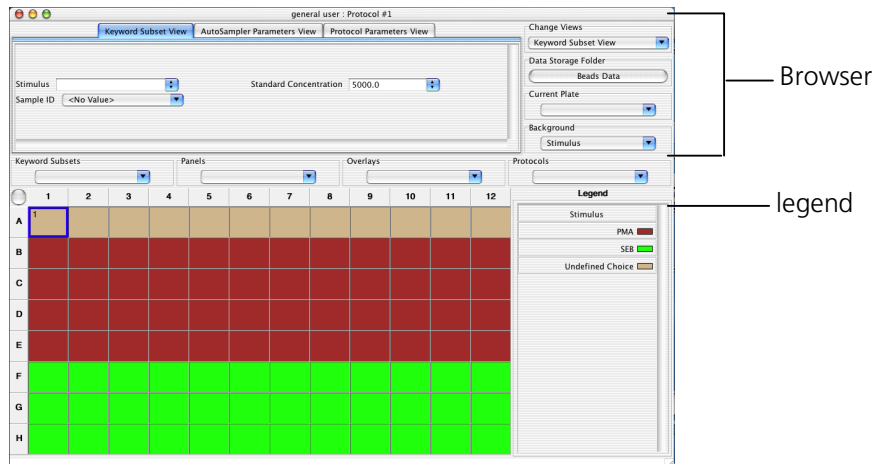
You can view and print the background color and legend for the wells by choosing a Keyword from the Background drop-down menu in the Keyword Subset View.

Printing the Keyword Subset assignment can help you aliquot sample to each well. For example, if wells A1 through A10 were assigned donor 1 as the Keyword, then viewing the Keyword Subset assignment (background color) shows the sample from donor 1 goes into wells A1 through A10.

1. Verify that the Keywords Subset View tab is selected in the main application window, and then choose Stimulus from the Background drop-down menu in the Browser.



The background color changes. The wells are colored according to the Choice (either *PMA* or *SEB*) selected for the Keyword *Stimulus*. A legend is displayed to the right of the wells.



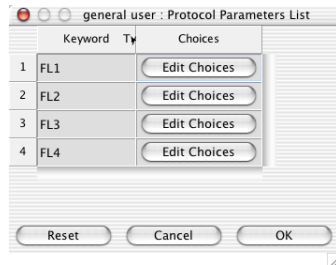
C. Protocol Parameters

Protocol Parameters are predefined Keywords that are shown in the Protocol Parameters View. These Keywords are the fluorescence parameters (FL1, FL2, FL3, and FL4) that are saved by the software. The selection of fluorochrome reagents that can be used for each parameter is Choices.

In this example you will create Choices for each parameter.

1. Choose Layout > Modify > Protocol Parameters List.

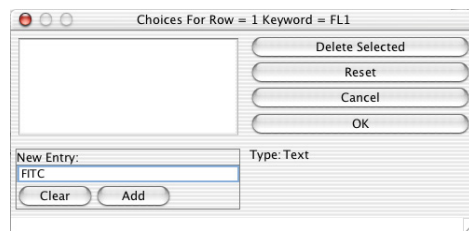
The Protocol Parameters List dialog appears.



2. Click Edit Choices next to the Keyword FL1.

3. Create the following FL1 Choices in the dialog that appears.

- Enter *FITC* in the New Entry field.



- Click Add.
- Enter *IL2-FITC* in the New Entry field.
- Click Add; Click OK.

4. Create the following FL2, FL3, and FL4 Choices.

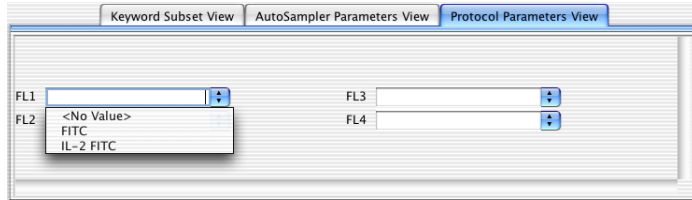
Follow the procedure described in the preceding step to create the following:

- FL2: *PE* and *CD69 PE*.
- FL3: *PerCP-Cy5.5* and *CD4 PerCP-Cy5.5*
- FL4: *APC* and *CD3 APC*

5. Click OK in the Protocol Parameters List dialog.

6. Click the Protocol Parameters View tab.
7. Select the FL1 drop-down menu to display the FL1 Choices.

The following appears.



You can choose a label for each parameter from the available Choices in the drop-down menus or you can enter a different label in the text fields. However, labels entered in the text fields are not saved in the Protocol Parameters List.

D. Export Summary Table

The AutoSampler Parameters, Keyword Subsets, Protocol Parameters, and analysis statistics can be exported to a Microsoft® Excel spreadsheet file. In this example, you will export a summary table using the sample data files located on the BD Multiwell AutoSampler CD.

1. From the File menu, choose Export Summary Table.

The Export Summary Table dialog appears.



2. Click .

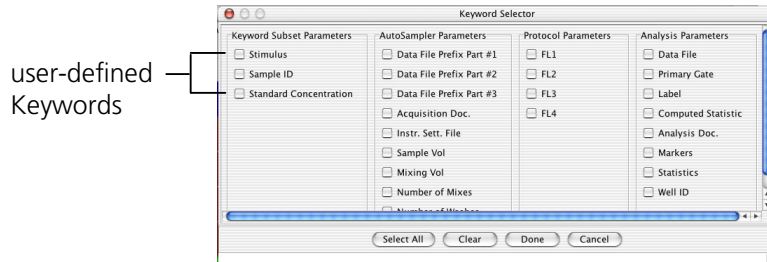
A dialog box appears.

3. Enter *exportfile* as the file name in the Name field.
4. Choose a destination for the file to be saved, and then click Save.

For this example, navigate to your hard drive and create a new folder named *Export Example*; click Save.

5. Click OK in the Export Summary Table dialog.

The Keyword Selector dialog appears. The dialog lists predefined Keywords along with any Keywords you defined. For example, the following figure lists the three Keywords you defined in Keywords Subset Parameters.



NOTE: When exporting a Summary Table from an experiment, you can also export statistics you chose during analysis. Refer to Chapter 2 in the *BD Multiwell AutoSampler User's Guide* for details.

6. Select the Keywords you want to include in your export file.

Make any selection for this tutorial.

7. Click Done when all selections have been made.
8. Click OK in the dialog that appears.



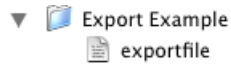
9. View the exported file.

Open the *exportfile* document, if you want. You will need a third-party spreadsheet application, such as Microsoft Excel, to view the exported file as a spreadsheet.

To open the document, choose Hide MPM from the MPM application menu in the upper-left corner of the computer screen. This allows you to view the desktop.



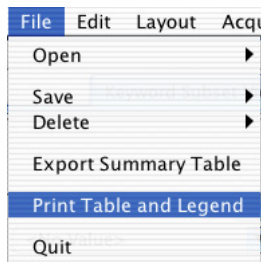
Navigate to the folder where you saved the *exportfile* document and double-click its icon. The spreadsheet application launches and the document opens.



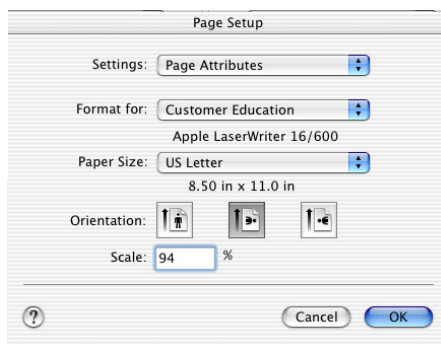
E. Print Options

You can choose to print a Table and Legend. A Table shows the run order of the wells and the well background color for selected Keywords. A Legend shows the selections that were made for each well.

1. Select a Keyword from the Background drop-down menu in the Browser.
2. Choose File > Print Table and Legend.

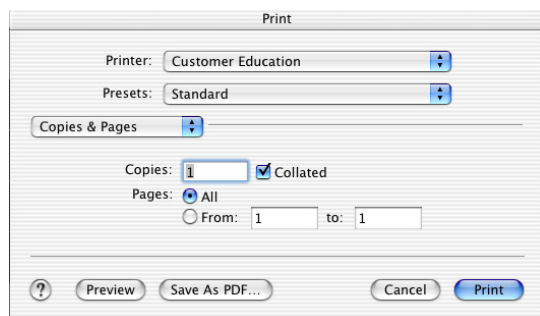


The Page Setup dialog appears.



NOTE: The Orientation and Scale can vary, depending on your printer type. Set these according to your printer.

3. Click OK.
4. Click Print in the dialog that appears.



BD Multiwell AutoSampler Daily Operation Using Additional Features

This section provides instructions on how to perform acquisition with the BD Multiwell AutoSampler using the additional features in BD Multiwell Plate Manager software described in the previous sections. Details are not provided. The following steps are intended to help you run your own experiment once you have already viewed the *Daily Operation and Additional Features* movies, as well as performed the *Daily Operation* and *Additional Features* tutorials.

To perform the following steps, you will need:

- A cytometer with the BD Multiwell AutoSampler option installed
- BD FACSTFlow™ sheath fluid
- A 96-well or 384-well plate

NOTE: Boldface text contains key instructions. Additional information is provided in plain text.

Preparation

- 1. Create and save a BD CellQuest Pro Experiment document to use for acquisition of your samples.**
 - Create Acquisition -> Analysis (dot, density, or histogram) plot(s).
 - Create regions, gates, markers, statistics views, and so on, appropriate for your sample.
 - Choose Event Count or Time as the Collection Criteria in the Acquisition & Storage window in BD CellQuest Pro software.
- 2. Create and save an Instrument Settings file with optimized settings for your sample type.**

Preacquisition

1. Perform system startup by following the startup procedure in Chapter 4 in the *BD Multiwell AutoSampler User's Guide*.
2. Perform instrument QC appropriate for your application.
3. Launch BD Multiwell Plate Manager software and choose Acquisition in the MPM Mode dialog.
4. Click new user or choose from the list of previous users in the Login dialog.
5. Choose a plate type from the Current Plate drop-down menu in the Browser.
6. Specify parameters for each well you want to run.

Specify one or more of the following for each well:

- Data File Prefix (up to three fields can be entered)
 - Sample volume
 - Mixing volume
 - Number of mixes
 - Number of washes
 - BD CellQuest Pro Experiment document
 - Appropriate Instrument Settings file
 - A data storage folder
 - Keywords and Choices
 - Parameter labels
7. Save the Protocol.
 8. Print the Keyword Subset assignment and the Legend, if you want.

Acquisition

Preparing the Instrument

1. Remove the tube of deionized (DI) water from the sample injection port (SIP).
2. Remove the sample injection tube outer sleeve.
3. Install the cytometer interface unit (CIU) over the sample injection tube and place the tube support arm underneath it.
4. Choose AutoSampler > Plate Tray Out.
5. Install the plate with well A1 oriented to the top left in the BD Multiwell AutoSampler, and then close the door.
6. Choose AutoSampler > Plate Tray In.
7. Prime the AutoSampler system at least twice to eliminate any air bubbles.
8. Select a flow rate and press RUN on the cytometer.

Preparing the Software

1. Select the wells in the order to be acquired.
2. Choose Acquisition > Acquire.
3. Click Acquire in the Acquisition Control dialog.

Ending a Run

1. At the end of the run, click OK in the dialog that appears.
2. Choose AutoSampler > Plate Tray Out.
3. Remove the plate.
4. Perform the daily maintenance and shut down procedures as described in Chapter 4 in the *BD Multiwell AutoSampler User's Guide*.


5. Once daily maintenance is complete, place the cytometer in standby and remove the CIU from the SIP.
6. Replace the sample injection tube outer sleeve and install a tube containing no more than 1 mL of DI water on the SIP.

Analysis

Refer to the Multiwell Plate Manager software ReadMe file for information about batch analyzing large sets of files with large files.

If you are analyzing data you just acquired, begin with *Displaying Analysis*.

Specifying the Data Folder

1. Choose File > Open > Experiment.
2. Click  in the Open Experiment dialog.
3. Navigate to the location of the data folder containing data you want to analyze and select the folder.
4. Click Choose, and then click OK in the Open Experiment dialog.
5. Click OK in the Information dialog.

Displaying Analysis

1. After all data has been read in, choose Analysis > Show Analysis View.
2. For each well, specify the BD CellQuest Pro Experiment document you want to use to analyze the data.

You can specify one Experiment document per well. To specify an Experiment document, first select the wells, and then choose the document.

3. Choose the statistics you want to view.

Choose the following in the Analysis View: a primary gate, a marker type, a label (plot) type, and a statistics type.

4. View the data.

The following steps are optional.

1. **Customize the interval type, minimum and maximum cutoffs, number of intervals, or the interval color, if you want.**

Refer to Chapter 7 in the *BD Multiwell AutoSampler User's Guide* for details.

2. **View a more detailed analysis of each individual well.**

Double-click a well to get a view of three plots, similar to the ones created in BD CellQuest Pro software.

2. **Export a summary table.**

See page 11 in this tutorial or refer to Chapter 2 in the *BD Multiwell AutoSampler User's Guide* for details.

4. **Print the table and legend.**

5. **Print the results, if needed.**

To print the results you will need to take a screen shot of the Analysis view. Use one of the following methods:

- Press the Command (Apple) key, shift key, and the number 3 to take a picture of the entire screen
- Press the Command (Apple) key, shift key, and the number 4 to take a picture of a portion of the screen.

The file created is a PDF file. You can then view it by double clicking the file (named picture 1, 2, 3) to launch Adobe® Acrobat® Reader (provided on your BD FACStation™ system). You can rename the file and print it, as needed.

6. **When analysis is complete, exit BD Multiwell Plate Manager software.**

Appendix A

Examples of Keywords and Choices for the BD Multiwell AutoSampler

The following are six examples of how you can use the Keywords and Choices or the Protocol Parameters list in BD Multiwell Plate Manager software.

- To create the Keywords and Choices, choose Layout > Modify > Master Keywords Set.
- To create a Keyword Subset, select the Keywords from the Master Keywords Set dialog and click Define Keyword Subset.
- To use the Keyword Subset, select the wells, and then choose the Subset from the Keyword Subsets drop-down menu in the Protocol window. Click the Keyword Subset View tab in the Protocol window to view the Keyword fields.

See section B, Keywords and Choices (page 4) in this tutorial for details about creating Keywords, Choices, and defining Keyword Subsets.

NOTE: The name of the Keyword Subset is displayed following each table. The Keyword Subset name identifies a group of related Keywords.

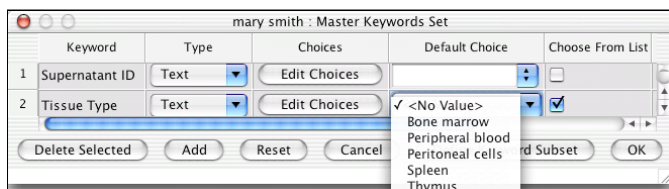
Scenario 1

The lab screens hybridoma supernatants for reactivity on five different tissue suspensions: spleen, peripheral blood, peritoneal cells, thymus, and bone marrow.

Keyword	Type	Choices	Comments
Supernatant ID	Text	Enter the supernatant ID each time, since this will change with each assay.	
Tissue type	Text	Choose from a list: spleen, peripheral blood, peritoneal cells, thymus, bone marrow.	<ul style="list-style-type: none">• Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay.• Click the <i>Choose From List</i> checkbox to restrict Choices to items on the list. If the box is not checked, you can choose from the list or enter new information.

Keyword Subset name: Hybridoma Screen-Hemo

The following figure is an example of the defined Choices for the Keyword *Tissue Type*.



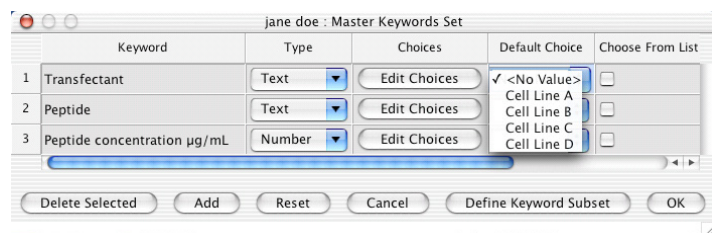
Scenario 2

The lab determines if synthesized peptides at varying concentrations can block antibody binding on three transfected cell lines, each expressing a different epitope of the same antigen, CDX.

Keyword	Type	Choices	Comments
Transfectant	Text	Choose from a list: Line 12, Line 453, Line 233.	<ul style="list-style-type: none"> Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. Check the <i>Choose From List</i> checkbox to restrict Choices to items on the list. If the box is not checked, you can choose from the list or enter new information. If you often use a particular cell line, you can select it under the <i>Default Choice</i> column to make it the default Choice that appears for the <i>Transfectant</i> Keyword.
Peptide	Text	Enter peptide ID.	
Peptide concentration (µg/mL)	Number	Enter the number.	A range can also be specified to restrict what can be entered.

Keyword Subset name: Peptide Inhibition Assay-CDX

The following figure is an example of the defined Choices for the Keyword *Transfectant*.



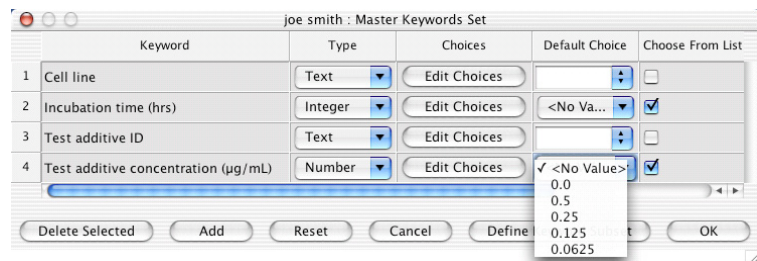
Scenario 3

The lab tests the effect of varying concentrations of factors X, Y, and Z on the proliferation of two cell lines after a 4- or 12-hour incubation. Assume that the assay will eventually be repeated on different cell lines. Cells are first labeled with a membrane dye like PKH2 before being incubated with the factor.

Keyword	Type	Choices	Comments
Cell line	Text	Enter information or choose from a list: HPB-ALL and Jurkat.	<ul style="list-style-type: none"> If you are constantly running new cell lines, free text is best. If you are using a constant set of cell lines, creating a list is best. Enter all the cell line names as Choices, and then pick and choose from the list for each assay.
Incubation time (hrs.)	Integer	Choose from a list: 0, 4, 12.	Use <i>Edit Choices</i> to make the list and check the <i>Choose From List</i> checkbox to restrict the Choices to this list.
Test additive ID	Text	Enter the ID (for example, Control, Factor X, Factor Y, Factor Z).	
Test additive concentration (µg/mL)	Number	Choose from a list: 0, 0.5, 0.25, 0.125, 0.0625.	Use <i>Edit Choices</i> to make the list and check the <i>Choose From List</i> checkbox to restrict the Choices to this list.

Keyword Subset name: Proliferation Assay

The following figure is an example of the defined Choices for the Keyword *Test additive concentration* ($\mu\text{g/mL}$).



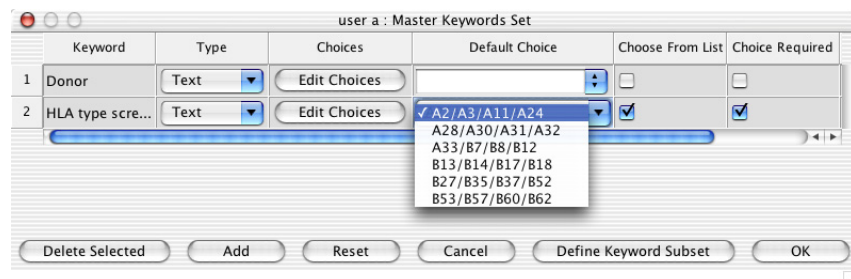
Scenario 4

The lab performs HLA typing.

Keyword	Type	Choices	Comments
Donor	Text	Enter information or choose from a list: Donor A, Donor B, Donor C, etc.	
HLA type screen	Text	Choose from a list: A2/A3/A11/A24, A28/A30/A31/A32, A33/B7/B8/B12, B13/B14/B17/B18, B27/B35/B37/B52, B53/B57/B60/B62.	<ul style="list-style-type: none"> Use <i>Edit Choices</i> to create the list and check the <i>Choose From List</i> checkbox to restrict the Choices to this list. Select <i>Default Choice</i> if one screen is used often. Check the <i>Choice Required</i> checkbox to require a Choice.

Keyword Subset name: HLA Typing

The following figure is an example of the defined Choices for the Keyword *HLA type screen*.

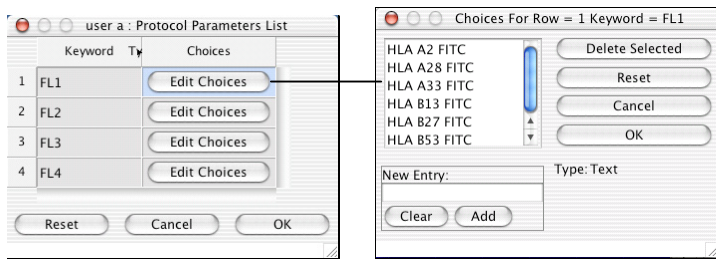


Protocol Parameters List

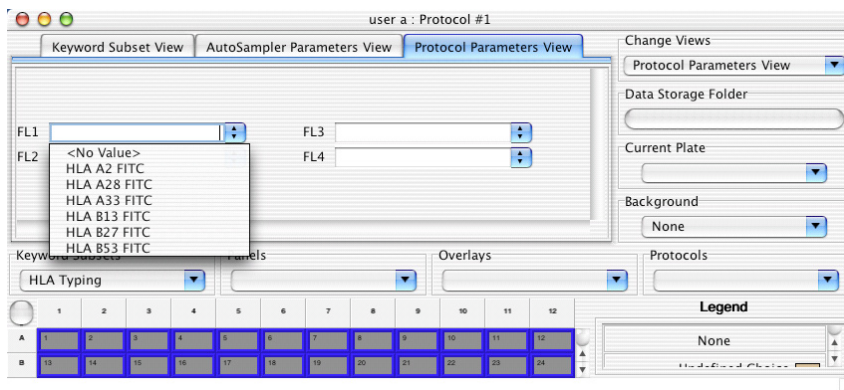
A Protocol Parameters list allows you to predefine labels for the FL1, FL2, FL3, and FL4 parameters. The following is an example of how you can use this feature for scenario 4.

Keyword	Choices	Comments
Parameter label	Examples of labels: HLA A2-FITC, HLA A3-PE, HLA A11-PerCP, HLA A24-APC and so on.	Choose from a list or use free text. Use <i>Edit Choices</i> to create the list.

- Define the labels by choosing Layout > Modify > Protocol Parameters List.
- Click Edit Choices for the parameter you want and enter the label Choices.



The following figure is an example of how the defined labels are displayed in the Protocol Parameters View when the FL1 drop-down menu is displayed.



Scenario 5

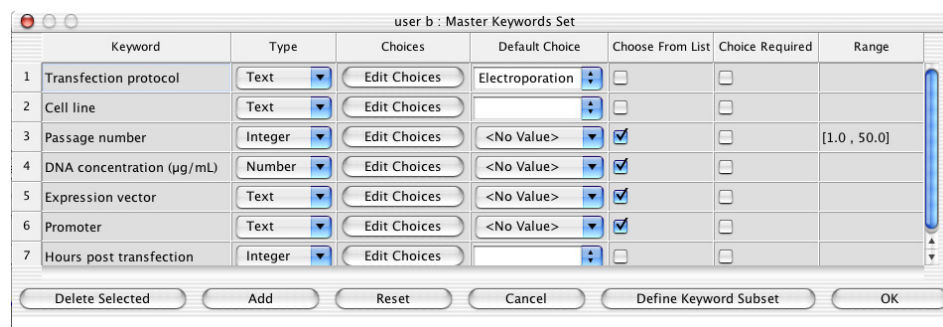
The lab compares mammalian transfection protocols to select the protocol that exhibits the highest transfection efficiency in each of three different transiently transfected cell lines. Transfection efficiency is measured as a function of GFP expression. In addition, optimal concentration of DNA for each transfection protocol and optimal time post-transfection for evaluation of GFP expression by flow cytometry are determined.

Keyword	Type	Choices	Comments
Transfection protocol	Text	Choose from a list: Ca ⁺⁺ Phosphate, Electroporation, DEAE-dextran, CLONfectin™.	<ul style="list-style-type: none"> • Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. • Select the <i>Choose From List</i> checkbox to restrict the Choices to items on the list. If the box is not checked, you can choose from the list or enter new information. • Select a <i>Default Choice</i> if a particular Protocol is used often. • Check the <i>Choice Required</i> checkbox if you want to require a Choice.
Cell line	Text	Choose from a list: eg, 293T cells, COS-1 cells, CHO cells.	Use <i>Edit Choices</i> to create a list. Make other selections in the Master Keywords Set dialog, as appropriate.
Passage number	Integer	Enter a number.	You can also specify a range to restrict what is entered. Make other selections in the Master Keywords Set dialog, as appropriate.
DNA conc. (µg/ml)	Number	Choose from a list: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0.	Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. Make other selections in the Master Keywords Set dialog, as appropriate.

Keyword	Type	Choices	Comments
Expression vector	Text	Choose from a list: pEGFP-N1, pEGFP-N2, pEGFP-C1, pEGFP-C2.	Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. Make other selections in the Master Keywords Set dialog, as appropriate.
Promoter	Text	Enter the text (eg, CMV immediate early promoter).	Make other selections in the Master Keywords Set dialog, as appropriate.
Hours post transfection	Integer	Choose from a list: 24, 36, 48, 72.	Use <i>Edit Choices</i> to create a list if Choices will remain constant from assay to assay. Make other selections in the Master Keywords Set dialog, as appropriate.

Keyword Subset name: Transfection Efficiency-GFP

The following figure is an example of the defined Keywords.



Scenario 6

This scenario is a generic antibody titration format to determine optimal antibody concentration for use in an assay.

Keyword	Type	Choices	Comments
Receptor/ marker	Text	Enter text (eg, CD71).	
Cell type	Text	Enter text (eg, CHO).	Use <i>Edit Choices</i> to create a list if Choices will remain constant from assay to assay.
Cell passage number	Integer	Enter an integer (eg, 7).	You can also specify a range to restrict what can be entered.
Cell concentration cells/mL	Number	Enter a number (eg, 10,000).	You can also specify a range to restrict what can be entered.
Antibody; species/isotype	Text	Choose from a list: eg, mouse mab IgG ₁ , mouse mab IgG _{2a} , mouse mab IgG _{2b} .	<ul style="list-style-type: none"> • Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. • Select the <i>Choose From List</i> checkbox to restrict the Choices to items on the list. If the box is not checked, you can choose from the list or enter new information. • Select a <i>Default Choice</i> if a particular isotype is used often. • Check the <i>Choice Required</i> checkbox if you want to require a Choice.
Antibody concentration (µg/mL)	Number	Choose from a list: eg, 0.0, 0.5, 1.0, 5.0, 10.0, 20.0, 40.0, 80.0.	<ul style="list-style-type: none"> • Use <i>Edit Choices</i> to create a list if the Choices will remain constant from assay to assay. • Specify range to restrict what can be entered.
Antibody incubation time (min)	Integer	Enter an integer: eg, 15, 30, 60, 120.	You can also specify a range to restrict what can be entered.

Keyword Subset name: Generic Antibody Titration

The following figure is an example of the defined Keywords.

The screenshot shows a dialog box titled "user c : Master Keywords Set". It contains a table with the following columns: Keyword, Type, Choices, Default Choice, Choose From List, Choice Required, and Range. Below the table are several buttons: Delete Selected, Add, Reset, Cancel, Define Keyword Subset, and OK.

	Keyword	Type	Choices	Default Choice	Choose From List	Choice Required	Range
1	Receptor/Marker	Text	Edit Choices		<input type="checkbox"/>	<input type="checkbox"/>	
2	Cell type	Text	Edit Choices		<input type="checkbox"/>	<input type="checkbox"/>	
3	Cell passage number	Integer	Edit Choices	<No Va...	<input checked="" type="checkbox"/>	<input type="checkbox"/>	[1.0 , 30.0]
4	Cell concentration/mL	Number	Edit Choices	<No Va...	<input checked="" type="checkbox"/>	<input type="checkbox"/>	[5000.0 , 1.0E7]
5	Antibody species/isotype	Text	Edit Choices	IgG1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
6	Antibody concentration (µg/mL)	Number	Edit Choices	<No Va...	<input checked="" type="checkbox"/>	<input type="checkbox"/>	[0.0 , 80.0]
7	Antibody incubation time (minutes)	Integer	Edit Choices	<No Va...	<input checked="" type="checkbox"/>	<input type="checkbox"/>	[1.0 , 120.0]

Buttons: Delete Selected, Add, Reset, Cancel, Define Keyword Subset, OK