

Bio-Plex Data Pro™ Software User Guide

Version 1.0



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1 Introduction

Bio-Plex Data Pro™ software provides tools for annotating and analyzing the results of experiments conducted in Bio-Plex Manager™ software. When you import Bio-Plex Manager data into Bio-Plex Data Pro, you can edit samples, view results, and perform statistical analysis on data. These actions take place within a Bio-Plex Data Pro project.

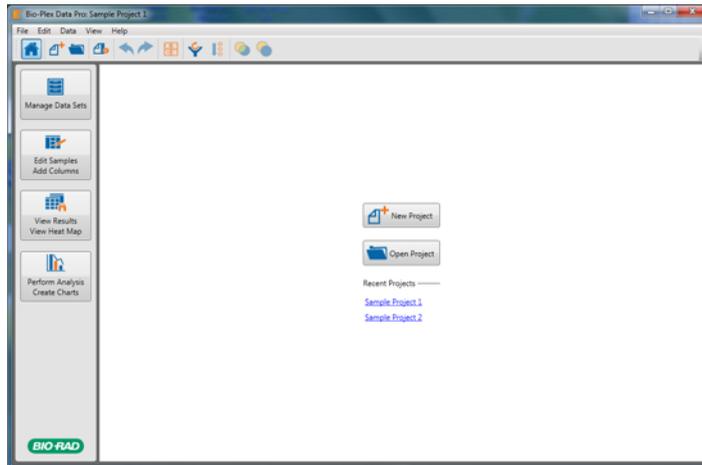
Basic Workflow

The basic workflow in Bio-Plex Data Pro consists of the following steps:

1. Create a project.
2. Import your data sets (Bio-Plex Results files [.rbx]).
3. Edit your samples.
4. View your results.
5. Analyze your data.

This guide explains how to use Bio-Plex Data Pro to perform the steps in this workflow.

The Workspace



The Bio-Plex Data Pro software Home view displays buttons to create a new project or to open an existing project. You can also open a recent project by clicking the project name in the Recent Projects list. The left pane displays the project workflow, starting at the top with Manage Data Sets and ending with Perform Analysis/Create Charts. Once a project is open, clicking each button in the left pane takes you to the view where you complete the step.

Toolbar Commands

Toolbar buttons give you quick access to basic commands. You can also access these commands from the menus.



Home



New Project



Open Project



Import Bio-Plex Results File



Undo



Redo



Select Sample Type for analysis



Clear All Analysis Filters



Reset Sample Lists to Default Sort



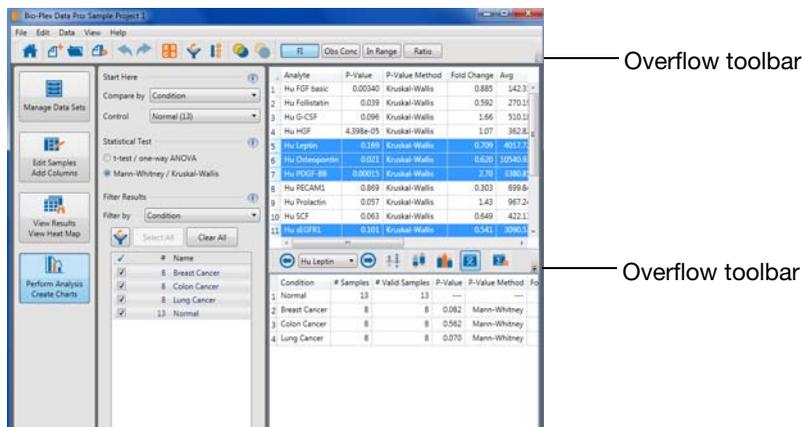
Merge samples run on different panels/assays



Undo Merge Samples

Overflow Toolbar Commands

Clicking the triangle displays an overflow toolbar of hidden commands. Overflow toolbars are enabled whenever the screen size does not allow all toolbar commands to be displayed.



File Menu Commands

The following commands can be accessed from the File menu:

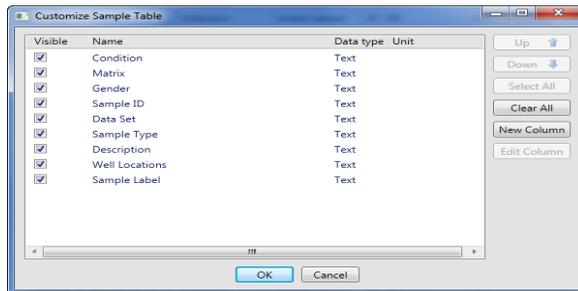
- **New Project** — opens a dialog box in which you specify project details
- **Open Project** — opens a dialog box in which you can open an existing project and organize your projects
- **Import Bio-Plex Results File** — opens a dialog box, in which you can browse to a data set and import it
- **Exit** — closes Data Pro software

Edit Menu Commands

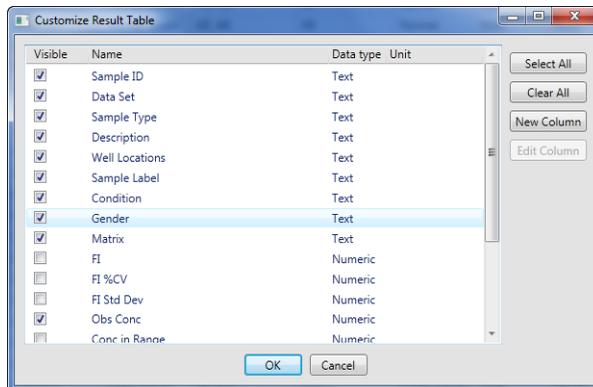
The following commands can be accessed from the Edit menu:

- **Undo** — removes the effect of an action you performed.
- **Redo** — restores the effect of an action you undid.
- **Cut** — copies contents of selected cells to the clipboard and deletes contents of selected cells that are not read only.
- **Copy** — copies the contents of selected cells to the clipboard.
- **Paste** — pastes clipboard contents into the table. Read-only cells are skipped. When clipboard content is a single value, its content is copied to each selected cell in the table.

- **Customize Sample Table** — opens a dialog box in which you can select or clear columns or add new columns to the sample table.



- **Customize Result Table** — opens a dialog box in which you can hide or display columns, add new columns, and reorder columns.



- **Copy Table to Clipboard** — copies the entire table to the clipboard, including the column titles.
- **Copy Table to Clipboard Full Precision** — copies the data with full precision to the clipboard.
- **Copy Plot to Clipboard** — copies selected scatter plot or box and whisker plot to the clipboard.
- **Export Plot** — exports plots or graphs to a Portable Network Graphics (.png), JPEG, bitmap, or TIFF format.

In addition to Edit menu commands, you can use the following commands to copy, paste, and delete data in tables.

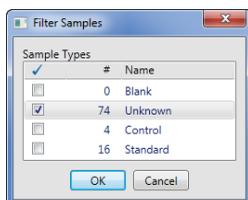
CTRL+A Select All selects the entire table in tables that allow multiple selections.

Copy Table to Clipboard	Right-click the table and select Copy Table to Clipboard on the context menu to copy the entire table to the clipboard, including the column titles.
CTRL+C	Copies the contents of selected cells to the clipboard.
Delete	Deletes the content of selected cells if they are not read-only.
CTRL+X	Copies contents of selected cells to the clipboard and deletes content of selected cells that are not read-only.
CTRL+V	Pastes clipboard contents into the table. Read-only cells are skipped. When clipboard content is a single value, its content is copied to each selected cell in the table.

Data Menu Commands

The following commands can be accessed from the Data menu:

- **Select Sample Type for analysis** — opens a dialog box in which you select sample types to use in your analysis. These sample types are the standards, controls, blanks, and unknowns imported from Bio-Plex Manager.



- **Clear All Analysis Filters** — clears all filters in the project.
- **Reset Sample Lists to Default Sort** — clears all sort settings in the project.
- **Merge samples run on different panels/assays** — opens a dialog box that allows you to merge samples from different data sets (.rbx files) that share the same identity. Choose the column that contains the information you need to match samples.
TIP: Use Merge Samples when you notice samples listed several times that you want analyzed as a single sample.
- **Undo Merge Samples** — restores the sample row settings.

View Menu Commands

From the View menu you can access the following:

- Imported data sets in the Data Set Table
- Individual sample data in the Sample Table
- Results in the Results Table (Single Analyte), Multi Analyte Table, and Heat Map
- Analyze data in Scatter Plot, Box and Whisker Plot, Bar Graph, Summary Table, Export to Statistics Software table, and Results by Sample table

2 Working with Projects

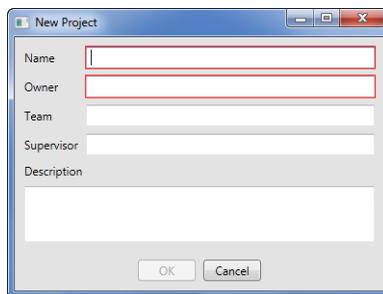
A project is the container for your data. After you create your project, you import data into the project. You can have as many projects as you like.

Creating a Project

Follow the procedure for creating a new project.

To create a project:

1. From the Home view, click New Project.
The New Project dialog box appears.

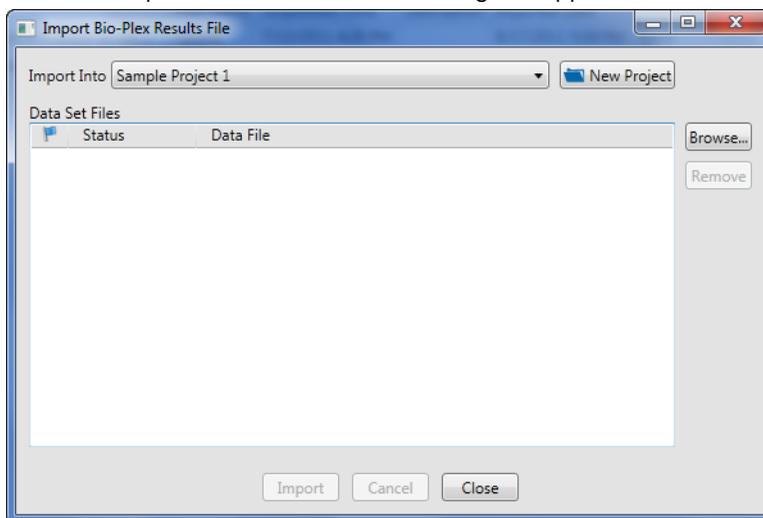


Note: You can also create a new project by selecting New Project from the File menu or clicking New Project in the toolbar.

2. Type a name for the project in the Name box.

3. Type the name of the project owner in the Owner box.
4. (Optional) Type the name of the team.
5. (Optional) Type the name of the supervisor.
6. (Optional) Type a description for the project (up to 255 characters).
7. Click OK.

The Import Bio-Plex Results File dialog box appears.



Note: The name of the project appears in the Import Into list.

8. Click Browse and navigate to the data sets you want to import.
9. Select the data sets you want to import and click Open.
10. In the Import Bio-Plex Results File dialog box, click Import.
The data sets are copied into the database. When the Status displays Done, the data sets have been successfully copied.
11. Click Close.
The project opens in the Manage Data Sets view and displays the Data Sets table.

Data Sets Table

The Data Sets table displays the details for the data sets imported from RBX files at the plate level. It is most useful immediately after data import, when you can use it to verify that the imported data are what you want to analyze.

Name	User Name	Acquisition Time	Description	Imported Date	Imported by	Comment
1 Normal vs. Disease plasma 1	vgupta	7/13/2011 4:08 PM		8/17/2011 5:39 PM	tyeck	
2 Normal vs Disease serum 1	vgupta	7/13/2011 4:26 PM		8/17/2011 5:39 PM	tyeck	

The following information is displayed for each data set:

Name — displays the name of the data set; by default this is the name of the imported Bio-Plex Results file (.rbx file).

User Name — displays the name associated with the data set as specified in the general information page of Bio-Plex Manager.

Acquisition Time — displays the date and time the data were collected on the Bio-Plex instrument.

Description — displays the data file description entered in the Bio-Plex Manager RBX file.

Imported Date — displays the date the file was imported into the system.

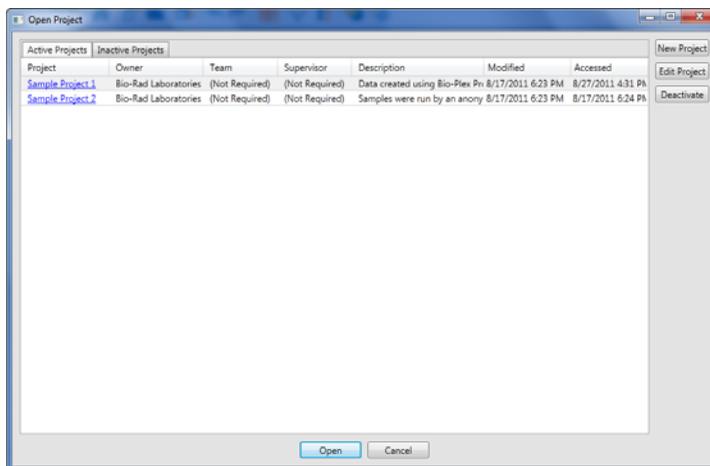
Imported by — displays the name of the Windows user logged in when import occurred.

Opening a Project

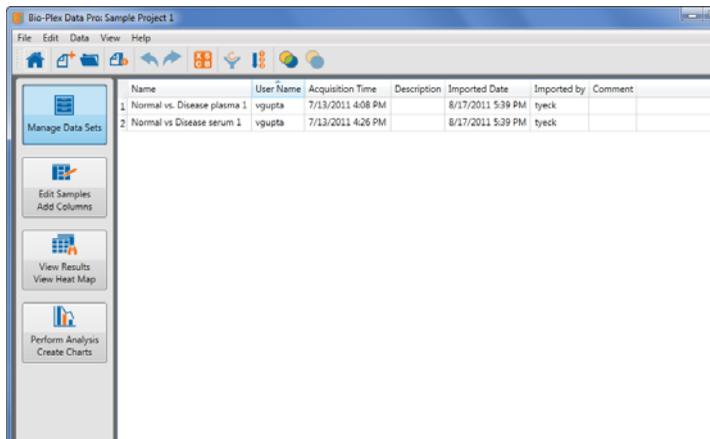
You can open a project in the Home view by clicking on the project name in the Recent Projects list or by clicking Open Project.

To open a project:

1. In the Home view, click Open Project.



- In the Open Project dialog box, click on the project name.
If the project contains data, the project is opened in the Manage Data Sets view. It displays all the data sets contained in the project. See Importing Data Sets on page 15 for more information about data sets.



If the project does not contain data, the Import Bio-Plex Results File dialog box appears. See Importing Data Sets on page 15 for information on importing data into your project.

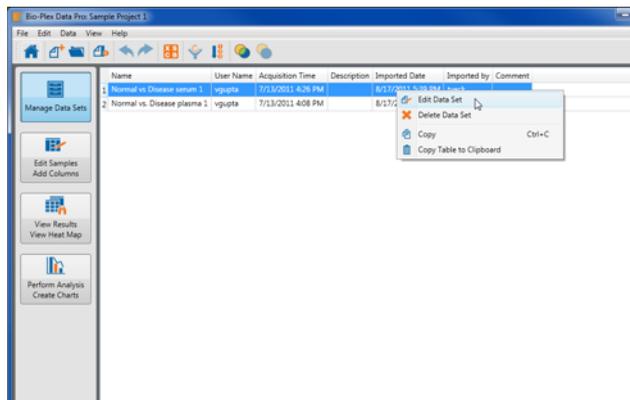
Editing a Project

You can edit project attributes in the Edit project dialog box.

To edit a project:

1. Click Open Project.
The Open Project dialog box appears, displaying a list of projects.
2. Right-click in the row of the project you want to edit, and click Edit Data Set.

TIP: Do not click the project name; this will open the project.



The Edit project dialog box appears.

3. Edit the project settings and click OK.

Organizing Your Projects

Over the course of time, you will create multiple projects, and you may not be working with all of them at the same time. You can organize your projects by removing projects that you are not currently working on from the list of active projects and moving them to the Inactive Projects list. You can perform any of the commands on an inactive project that you perform on an active project; you can open and edit the project, view the data, and perform statistical analysis on the data, and so on. You can return inactive projects to the Active Projects list at any time.

To move a project to the Inactive Projects list:

- From the Active Projects tab in the Open Projects dialog box, select the project you want to move to the Inactive Projects list and click Deactivate.

To return a project to the Active Projects list:

- From the Inactive Projects tab in the Open Projects dialog box, select the project you want to move to the Active Projects list, and click Activate.

3 Working With Data Sets

Bio-Plex Manager™ software results files are imported into Bio-Plex Data Pro™ software for analysis. You can view the data set in the Manage Data Sets view. You can also edit the properties of the data set and delete data sets if you no longer need them for your analysis.

Importing Data Sets

Data are imported into projects from the Import Bio-Plex Results File dialog box. You can select an existing project or you can create a new project into which the files are imported.

You control the data that are imported in one of the following ways:

- Add data files using the Import Bio-Plex Results File command.
- From Bio-Plex Manager, import your results directly into Bio-Plex Data Pro.

All files that appear in the Data Set Files list are imported into the selected project. You can combine data from multiple experiments into a project. You can import up to ten data sets (RBX files) into a single Bio-Plex Data Pro project. Each RBX file represents one data set. You can import data set files into multiple projects by selecting another project.

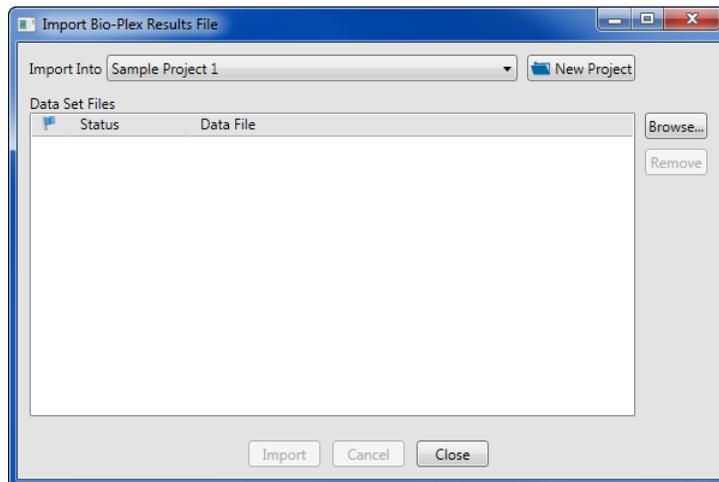
The procedure for importing data in Bio-Plex Data Pro differs slightly from importing through Bio-Plex Manager. See *Importing Data into Bio-Plex Data Pro Software* on page 16, and see *Importing Data Directly from Bio-Plex Manager Software* on page 17 for more information.

Importing Data into Bio-Plex Data Pro Software

The following procedure describes how to import data into Bio-Plex Data Pro.

To import data sets into a project:

1. From Bio-Plex Data Pro, click Import Bio-Plex Results File in the toolbar.
The Import Bio-Plex Results File dialog box appears. 
2. Select the desired project from the Import Into drop-down list, or create a project by clicking New Project.
3. Click Browse to navigate to and select the data sets you want to import.
4. Click Open.
The Data File field displays the file name, including the path to the file.
The Status displays as Ready.



5. Click Import.
The system imports all data files in the list. Each file displays a status as it is imported:
Done — the data set was successfully imported.
Exists — if you try to import a data set that was previously imported into the project, Bio-Plex Data Pro does not reimport the data. It displays the status Exists to indicate the data has already been imported into the project.

Ext. Standards — the data were successfully imported. The unknown values were calculated using an external standard curve.

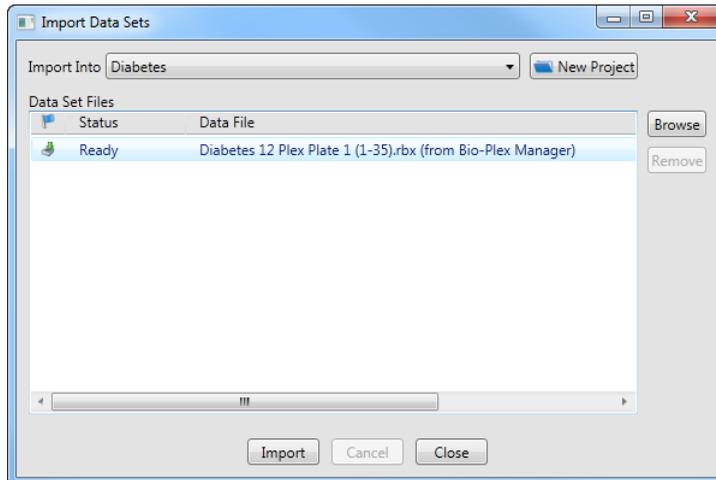
Renamed — the data were successfully imported. The name of the file being imported is the same as a file previously imported into the project, but the data are different. To distinguish between the two data sets, the file name of the data being imported is appended with a number, for example *001* is appended at the end of the file name *Bio-Plex Phosphoprotein 11-Plex 001*.

Note: If the data being imported are identical to data previously imported but they are exported from a different version of Bio-Plex Manager, Bio-Plex Data Pro imports the data and displays the Renamed status.

6. You can click Browse and import additional data sets, or click Close if you are done importing data.

Importing Data Directly from Bio-Plex Manager Software

The Export Results to Bio-Plex Data Pro feature in Bio-Plex Manager allows you to import data directly into Bio-Plex Data Pro. You initiate the data export from Bio-Plex Manager. Then the Import Bio-Plex Results File dialog box in Bio-Plex Data Pro displays automatically. If the Bio-Plex Data Pro program is not already running, it launches automatically.



The Data File field displays the file name and indicates the source as Bio-Plex Manager. You may import this data set just as you would any RBX file. You can add more data sets to this list by using the Browse button. You may also return to Bio-Plex Manager to export another data set.

For more information on this feature, see the *Bio-Plex Manager Software 6.1 User Manual*.

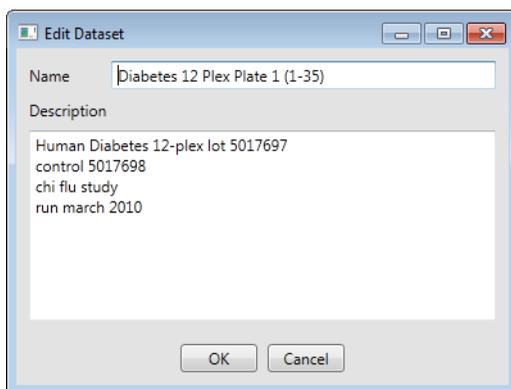
Editing a Data Set

You can edit the name and description of a data set.

To edit a data set:

1. Open the project whose properties you want to edit.
2. In the Data Sets table, right-click the data set you want to edit, and then click Edit Data Set in the context menu.

The Edit Dataset dialog box appears.



3. Edit the name and description and click OK.

Deleting a Data Set

The following procedure describes how to delete a data set from your project.

To delete a data set:

1. In the Data Sets table, select the data sets you want to delete.
2. Right-click and select Delete Data Set in the context menu.

Deleting a Data Set

A message asks you to confirm that you want to delete the data set.

3. Click Yes.

The data sets are deleted from the project.

4 Organizing the Data

After data sets are imported into Bio-Plex Data Pro™ software, you can manage the data that is used in your analysis. You can add data to your samples with data from your experiments. You can filter your data and include or exclude samples. Finally, data can be copied or exported to other applications for analysis.

Selecting Samples

When you import Bio-Plex Manager™ software data sets into Bio-Plex Data Pro, all of the samples in your experiment are imported. Typically, you will want to exclude some samples, for example Blank and Standard samples. Use “Select Sample Type for analysis” in the toolbar to specify which samples to include. This is a global setting that applies to all views of the project in Bio-Plex Data Pro.

Note: By default, the Blank and Standard samples are excluded from the analysis.

To select the samples to be used in your analysis:

1. Click “Select Sample Type for analysis” in the toolbar.
2. In the Filter Samples dialog box, clear the checkboxes for the samples you want to exclude from your analysis.
3. Click OK.

Viewing and Editing Samples

The Edit Samples/Add Columns view displays a tabular list (Sample table) of all unique samples in a project in the raw form imported from the RBX files. In this view, you add columns to the Sample table, which allows you to define your biological replicate groups and assign other important information such as demographic data, strain, or gender associated with each sample.

Editing the data adds descriptive information that enables you to make meaningful comparisons when you analyze the data. This descriptive information is a prerequisite for data visualization and statistical calculations. Annotation assists you in identifying data groupings.

You can edit the data on a plate-by-plate basis using the data set selector. You can copy, paste, or edit data into table columns from a spreadsheet. You can also change the order of the columns. Columns and order selections persist until you change them. Once you add columns, you can add data from a spreadsheet to each cell, one row or one column at a time, or all at once.

The Sample Table

When you import data into Bio-Plex Data Pro, the data are displayed in a Sample table.

	Condition	Matrix	Gender	Data Set	Sample Type	Description	Sample Label
1	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X1
2	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X2
3	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X3
4	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X4
5	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X5
6	Normal	Plasma	Male	Normal vs. Disease plasma 1	Unknown	Normal	X6
7	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X7
8	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X8
9	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X9
10	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X10
11	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X11
12	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X12
13	Normal	Plasma	Female	Normal vs. Disease plasma 1	Unknown	Normal	X13
14	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X14
15	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X15
16	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X16
17	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X17
18	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X18
19	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X19
20	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X20
21	Breast Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	breast cancer	X21
22	Colon Cancer	Plasma	Male	Normal vs. Disease plasma 1	Unknown	colon cancer	X22
23	Colon Cancer	Plasma	Male	Normal vs. Disease plasma 1	Unknown	colon cancer	X23
24	Colon Cancer	Plasma	Male	Normal vs. Disease plasma 1	Unknown	colon cancer	X24
25	Colon Cancer	Plasma	Male	Normal vs. Disease plasma 1	Unknown	colon cancer	X25
26	Colon Cancer	Plasma	Female	Normal vs. Disease plasma 1	Unknown	colon cancer	X26

The toolbar in the Edit Samples/Add Columns view displays additional icons.



Select Sample Type for Analysis — opens a dialog box in which you select the sample types to use in your analysis. These sample types are the standards, controls, blanks, and unknowns imported from Bio-Plex Manager. This selection is a global setting.



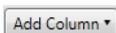
Merge Samples run on different panels/assays



Undo Merge Samples

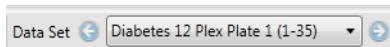


Customize Sample Table — opens a dialog box in which you can select columns that will be visible in the Sample table.



Add Column — opens a dialog box in which you can select a column to add or type a column heading.

The toolbar also displays a drop-down list of all the data sets in the project.



Left and right arrows facilitate scrolling through the list. This list enables you to view just the data set you want to edit. The choice of data set persists for the duration of the session.

The Sample table displays the following columns by default.

Sample ID — this field can be used to assign a unique ID to a sample and its replicates.

Data Set — one source data set; if the sample belongs to multiple data sets, the sample is listed multiple times.

Sample Type — Unknown, Control, Standard, Blank, or External Standard.

Description — displays information that was entered in the sample description in the originating Bio-Plex Manager Results file. You can edit this field.

Well Locations — the well or wells on the plate where the experiment was run, for example, A1, A2 or C1, D1.

Sample Label — name of the sample, for example, S1–Sn, X1–Xn, C1–Cn, eS1–eSn. Replicates identified in Bio-Plex Manager are merged.

About the Sample and Results Tables

The Sample and Results tables are synchronized, so changes you make to one table are immediately reflected in the other. For example:

- Filtering data from one table filters it from the other.
- Sorting data in one table sorts it in the same order in the other.
- Adding a column of data in one table adds it to the other.
- Changing the order of the columns in one table changes the column order in the other.

Filtering Data

Data can be included or excluded from the project dynamically. This is referred to as filtering. You can choose the types of samples being used in your analysis at any time by using the filter tools. These filters are available in most views throughout the software. All filters apply across the entire project. For example, filter settings you apply to the Sample table are reflected in the Results table and Perform Analysis/Create Charts view. They can be cleared at any time using the Clear All Analysis Filters command.

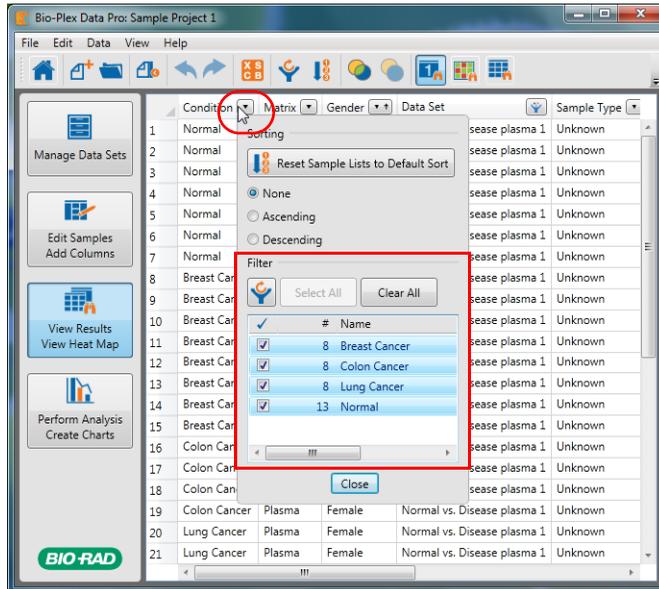
Filtering Data in the Sample and Results Tables

Contents of each column, including custom columns, can be filtered using the drop-down list in its column heading. By default, all rows are included.

Note: Filters cannot be applied to the Multi Analyte table in the View Results/View Heat Map view.

To filter data:

1. Click the triangle in the column header to open the drop-down menu. In the Filter section, there is a list of the different sample types for that column.



2. Select the checkboxes of the samples you want to include, and clear the checkboxes of the samples you want to exclude.

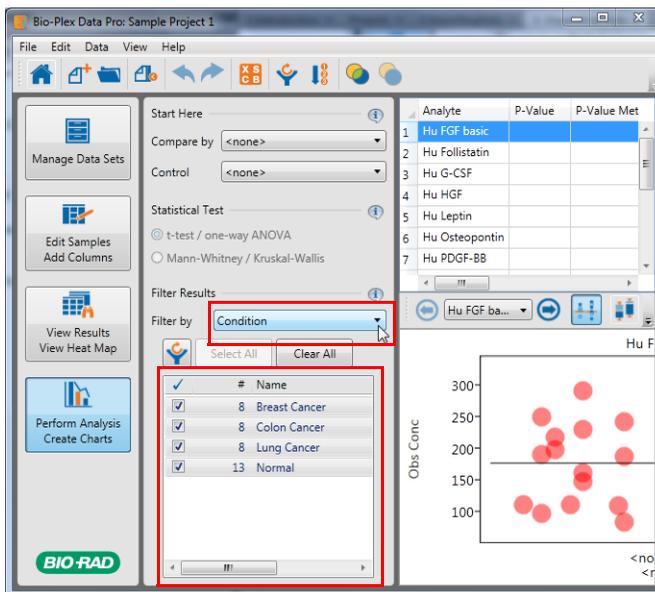
Filtering Data in the Perform Analysis/ Create Charts View

The filter tool allows you to filter your data in the same way that you filter your data in the Results tables.

To filter data in the Perform Analysis/Create Charts view:

1. Click “Filter by” and choose the column you want to use to filter your data.

The list of samples is displayed in the Filter section.

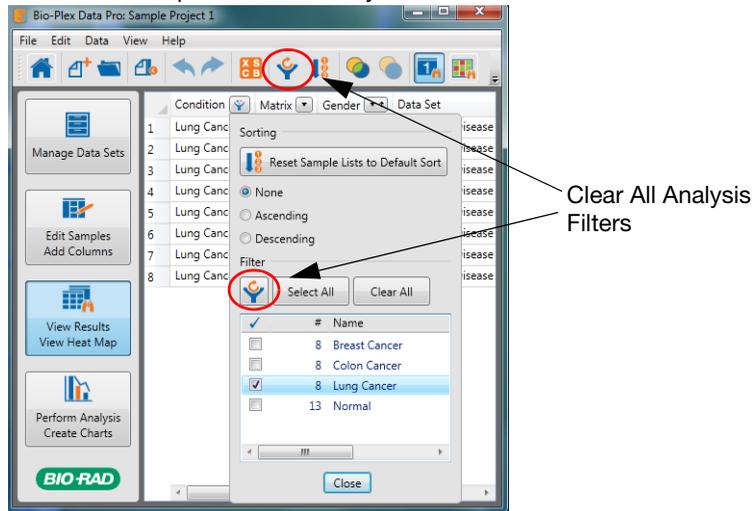


2. Select the checkboxes of the samples you want to include, and clear the checkboxes of the samples you want to exclude.

Removing Filters

To clear filtering:

- To clear filtering on a column, click the button in the column header to display the drop-down list and select Select All.
- To clear all filters on all columns in the table, click Clear All Analysis Filters. There are two places where you can do this. You will find it in the drop-down list for any column or in the toolbar.



Adding a Column

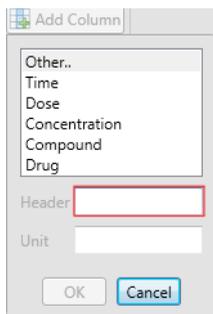
In addition to the predefined columns, you can add up to ten custom columns to the Sample or Results tables. When a custom column is added to a table, you must specify its attributes.

A column that is added to the Sample table is also added to the Results table and vice versa. Columns are added to the left of existing columns. You can reposition a custom column by clicking the column heading and dragging it to a new position in the table, or by using the Customize Sample Table dialog box.

The column order you create in the Sample table also applies to the Results table; similarly, the column order you create in the Results table also applies to the Sample table. Note, however, that the Results table with multiple analytes has fixed columns.

To add a column to the Sample table:

1. Click Add Column in the toolbar to open the drop-down list.



2. Select a column from the list or select Other and type a name for the column in the Header box. You can select from the following predefined column headings:
 - Time (numeric units only: select seconds, minutes, hours, days, or months)
 - Dose (numeric)
 - Concentration (numeric)
 - Compound (text)
 - Drug (text)The type of data that is valid for each column appears in parentheses.
3. (Optional) Type a unit in the Unit field.
4. Click OK. The column is added to both the Sample and Results tables.

Hiding a Column

Any column can be hidden from view. Columns you hide in the Sample table are hidden in the Results table and the reverse also is true. Hiding columns enables you to exclude data from being exported. Only data visible in the table are exported.

To hide a column in the Sample table:

1. In the Edit Samples/Add Columns view, click the Customize Sample Table in the toolbar.
2. In the Customize Sample Table dialog box, clear the Visible checkbox for each column you do not want to display.
3. Click OK.

To hide a column in the Results table:

1. In the View Results/View Heat Map view, click Customize Result Table in the toolbar.
2. In the Customize Result Table dialog box, clear the Visible checkbox for each column you do not want to display.
3. Click OK.

Sorting Data

You can sort any number and combination of columns of data in ascending or descending order. You apply a sort to the first column, and the rows of data are resorted based on the order you select. If you then apply a sort on a second column, the first sort persists, and the second sort is applied to the sorted data. You can always return your data to its original unsorted state.

All tables in the application can be sorted by clicking on the column header. In addition, you can sort the Sample and Results tables by clicking on the triangle in the column header.

For more information on sorting, see the following:

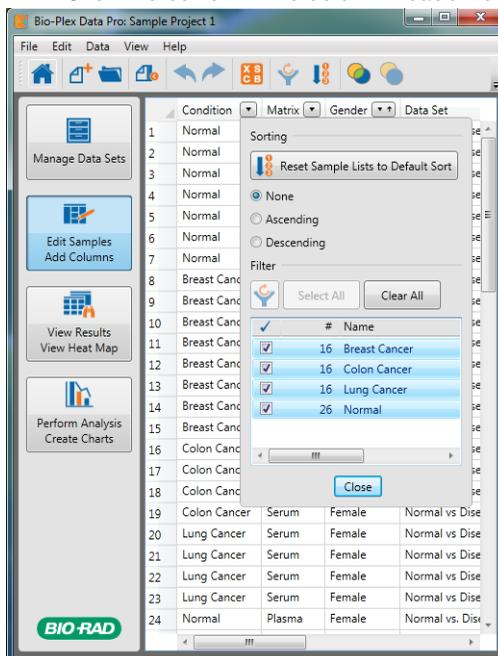
- Defining a Sort in the Sample or Results Tables on page 30
- Basic Sorting in Bio-Plex Data Pro Software on page 34

Defining a Sort in the Sample or Results Tables

Sorted data in the Sample or Results tables persist between sessions.

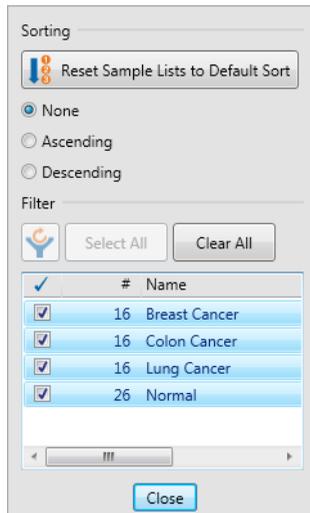
To define a sort in the Sample or Results tables:

1. Click the button in the column header to display a drop-down list.



Note: The icon that appears on the button varies depending on whether the data are sorted, filtered, or neither. See Sorting and Filtering Icons on page 33 for a description of the icons.

2. In the Sorting section, select Ascending or Descending to sort the data in the column.



After a sort is applied to a column, an arrow appears in the column either pointed up (ascending sort) or pointed down (descending sort). A black arrow indicates the first sort, also known as the primary sort. All subsequent sorts are indicated with a grey arrow.

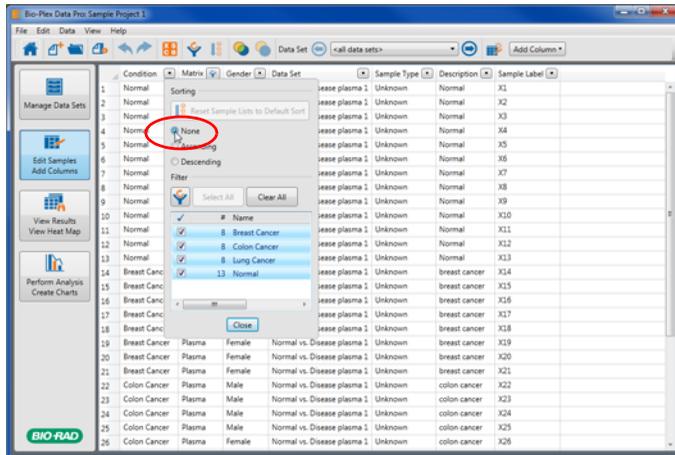
TIP: You can sort a single column by clicking the column header. Clicking multiple times cycles through ascending sort, descending sort, and no sort. When you click on a column header, any existing sort settings on the table are cleared.

You can clear sorting on a single data column, or you can clear sorting on all table columns at one time.

Clearing Data Sorting

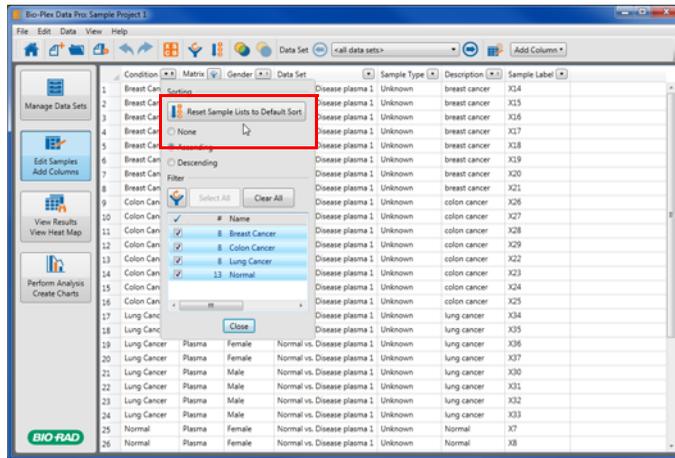
To clear sorting on one column:

- To clear sorting on the column, click the button in the column header to display the drop-down list, and select None. When you clear sorting on one column, the sorting on any other column is retained. The data will resort based on the remaining sort settings.



To clear sorting on all columns:

- To clear sorting on all columns in the table, click Reset to Default Sort in the drop-down list for any column or click the Reset to Default Sort button in the toolbar.



SORTING AND FILTERING ICONS

For the Sample and Results tables, a button on each column displays a triangle by default. The icons on the button change depending on the combination of sorting and filtering applied on the column.



Column is not sorted or filtered. This is the default.



Column is filtered only.



Column is sorted only. The arrow pointing up indicates ascending order. An arrow pointing down indicates descending order.



Column is both sorted and filtered. In this example, the down-pointing arrow indicates descending order.

Basic Sorting in Bio-Plex Data Pro Software

This section describes how to sort Bio-Plex Data Pro data if you are not using the sort tool available in the Sample and Results tables. Sorting that is done without the sort tool — that is, basic sorting — does not persist between sessions. The data return to their pre-sorted order.

For information on sorting in the Sample or Results table, see Defining a Sort in the Sample or Results Tables on page 30.

To sort data:

- Click the column header once to sort the data in ascending order. A triangle appears in the column header indicating an ascending sort is applied on the data.
- Click the column header a second time to sort the data in descending order. The triangle in the column heading is now inverted, indicating a descending sort is applied on the data.
- Click the column header a third time to return the data to its unsorted state. The triangle disappears, indicating the data are unsorted.

TIP: To sort on multiple columns, hold the SHIFT key down and click on the column header. You can sort on as many columns as you like.

Copying Data in Tables

Copying a Data Row

When you copy a data set row to another application, only the selected rows are copied. The column heading is not copied.

To copy a row from the data set table:

1. Select the rows you want to copy.
2. Right-click and click Copy in the context menu.
3. Right-click and click Paste in the application of your choice.

Copying the Data Set Table

You can copy the data set table to the clipboard and paste it into another application. Doing so copies the entire table, including the column headings.

To copy the data set table to the clipboard:

1. In the data set table, right-click to open the context menu, and then click Copy Table to Clipboard.
2. Paste the table into the application of your choice.

Viewing Data Values with Full Precision

Values in the data tables are rounded off. You can see the data values with full precision by copying the data to another application; for example, Microsoft Excel or Notepad. This is useful for verifying the software's calculations.

To view the data with full precision:

1. In the data set table, right-click to open the context menu, and then click Copy Table to Clipboard Full Precision.
2. Paste the table into the application of your choice.

Note: Check to see that the application is displaying the values with the level of precision you desire. For example, the default in Microsoft Excel is to display two decimal places. Use Format > Cells to change the level of precision.

Copying Plots and Graphs

You can copy a plot or graph to the clipboard and paste it into an application.

To copy a plot or graph:

1. In the plot or graph, right-click to open the context menu, and then click Copy Plot to Clipboard.
2. Paste the table into the application of your choice.

Exporting Plots and Graphs

Plots and graphs can also be exported as Portable Network Graphics (.png), JPEG, bitmap, or TIFF files. These exported files are of higher quality than the ones produced using the copy and paste tools.

To export a plot or graph:

1. In the plot or graph, right-click to open the context menu, and then click Export Plot.
2. In the Export Plot dialog box, enter the name of the file.
3. Select the type of file in which you want to save the plot or graph.
4. Click Save.

5 Viewing the Data

There are several different ways you can view your results in Bio-Plex Data Pro™ software. You can look at the results for one analyte at a time. You can view all the analytes at once and compare the results. You can view the results graphically in a heat map. In addition, you can merge multiple rows of data for the same sample to simplify your view of the data.

Viewing Results

In the View Results/View Heat Map view, the Results table lists data by individual samples from every RBX file imported into the project. It presents a view of the aggregates of all the results across plates.

	Condition	Gender	Data Set	Sample Type	Description	Well Locations	Sample Label	Obs Conc
1	Normal	Male	CDG kit1	Unknown	Normal1	D3, D4	X1	74.51
2	Normal	Male	CDG kit1	Unknown	Normal2	E3, E4	X2	69.67
3	Normal	Male	CDG kit1	Unknown	Normal3	F3, F4	X3	41.67
4	Normal	Male	CDG kit1	Unknown	Normal4	G3, G4	X4	113.02
5	Normal	Male	CDG kit1	Unknown	Normal5	H3, H4	X5	61.45
6	Normal	Male	CDG kit1	Unknown	Normal6	A5, A6	X6	97.93
7	Normal	Male	CDG kit1	Unknown	Normal7	B5, B6	X7	39.45
8	Normal	Male	CDG kit1	Unknown	Normal8	C5, C6	X8	85.68
9	Normal	Male	CDG kit1	Unknown	Normal9	D5, D6	X9	89.07
10	Normal	Male	CDG kit1	Unknown	Normal10	E5, E6	X10	86.66
11	Normal	Male	CDG kit1	Unknown	Normal11	F5, F6	X11	97.93
12	Normal	Female	CDG kit1	Unknown	Normal12	G5, G6	X12	94.72
13	Normal	Female	CDG kit1	Unknown	Normal13	H5, H6	X13	90.97
14	Normal	Female	CDG kit1	Unknown	Normal14	A7, A8	X14	77.13
15	Normal	Female	CDG kit1	Unknown	Normal15	B7, B8	X15	67.18
16	Normal	Female	CDG kit1	Unknown	Normal16	C7, C8	X16	96.34
17	Normal	Female	CDG kit1	Unknown	Normal17	D7, D8	X17	70.22
18	Normal	Female	CDG kit1	Unknown	Normal18	E7, E8	X18	66.90
19	Normal	Female	CDG kit1	Unknown	Normal19	F7, F8	X19	98.16
20	Normal	Female	CDG kit1	Unknown	Normal20	G7, G8	X20	85.68
21	Normal	Female	CDG kit1	Unknown	Normal21	H7, H8	X21	97.48
22	Normal	Female	CDG kit1	Unknown	Normal22	A9, A10	X22	87.87
23	Normal	Female	CDG kit1	Unknown	Normal23	B9, B10	X23	57.57
24	Normal	Female	CDG kit1	Unknown	Normal24	C9, C10	X24	33.96
25	Ovarian Cancer	Female	CDG kit1	Unknown	OC1	D9, D10	X25	57.57

Use this table to review the results of the experiment you imported. You can specify how you want to view the results by filtering or by adding custom columns. Columns and the order you select in the Results table persist until you change them.

You can also select different analytes and switch from the table to a heat map view. Heat maps help you quickly identify the intensity readings for an analyte.

The following additional buttons appear in the toolbar. Each option affects the data shown in the Results table.



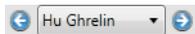
Results Table (Single Analyte)



Heat Map Result View



Multi Analyte Table



Analyte drop-down list with left and right arrows



Fluorescent intensity of the selected analyte



Observed concentration of the selected analyte



Observed concentration that falls within the “usable” range of the standard curve

	The normalized ratio, fold expression, or gene expression value calculated in Bio-Plex Manager™ software
	Coefficient of variation
	Standard deviation over the mean of a group of replicates
	Customize Result Table
	Add Column

The Results table displays the following columns by default. You can add columns by clicking Add Column in the toolbar.

Sample ID — you can edit this field.

Data Set — one source data set; if the sample belongs to multiple data sets, the sample is listed multiple times.

Sample Type — Unknown, Control, Standard, or Blank.

Description — displays information that was entered in the sample description in the originating Bio-Plex Manager results file. You can edit this field.

Well Locations — the well on the plate where the experiment was run; for example, A1, B1 or C1, C2, C3).

Sample Label — name of the sample; for example, S1–Sn, X1–Xn, and C1–Cn. Samples with the same data set and sample label are merged.

Obs Conc — observed concentration (custom column).

Conc %CV — concentration of the coefficient of variation (custom column).

View Results for All Analytes

You can view the results for all analytes by clicking Multi Analyte Table in the toolbar. Columns are displayed with headings and subheadings. You can sort data in the columns, but you cannot change the order of the columns. (See [Sorting Data](#) on page 29.) You can customize the table by showing and hiding columns. (See [Hiding a Column](#) on page 28.) Filters applied elsewhere in the application are preserved here, but you cannot apply a filter in the Multi Analyte table.

This table is useful for copying data to spreadsheet software for further analysis.

Sample Properties				Hu sEGFR1			Hu FGF basic			Hu Follistatin			
Data Set	Sample Type	Description	Condition	Gender	Matrix	Obs Conc	Conc %CV	Obs Conc	Conc %CV	Obs Conc	Conc %CV	Obs Co	
1	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	43984.51	2.18	98.44	1.08	249.32	6.31	47
2	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	33893.54	2.77	74.83	5.70	212.43	7.80	34
3	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	36944.59	8.26	82.80	12.61	452.60	0.367	25
4	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	65858.22	5.74	99.19	6.40	208.44	0	45
5	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	39541.12	1.21	95.03	3.98	180.76	8.26	22
6	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Male	Plasma	6405.89	9.12	81.98	8.52	361.14	4.13	34
7	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	17175.74	7.76	160.29	1.57	435.00	5.34	136
8	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	18766.03	1.88	218.40	4.32	801.93	0.525	833
9	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	17254.34	2.71	147.31	2.66	489.02	1.36	732
10	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	22744.77	0.307	109.75	1.37	367.30	2.79	83
11	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	23048.23	0.741	110.11	6.39	413.29	1.81	777
12	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	25281.72	0.301	197.69	7.11	517.25	4.50	229
13	Normal vs. Disease plasma 1	Unknown	Normal	Normal	Female	Plasma	17222.89	0.387	208.04	5.35	735.31	2.17	1387
14	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Male	Plasma	13571.29	0.253	176.49	1.36	959.23	4.25	562
15	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Male	Plasma	10478.72	3.61	241.70	7.32	514.30	5.34	724
16	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Male	Plasma	12154.68	6.90	322.20	0.380	893.28	3.98	472
17	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Male	Plasma	12231.59	1.23	298.67	3.07	903.46	4.22	759
18	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Female	Plasma	19902.92	3.31	242.19	7.30	357.87	3.73	803
19	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Female	Plasma	19109.11	0.389	187.64	0.414	553.75	2.71	165
20	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Female	Plasma	39135.26	9.46	162.36	0.256	418.57	5.55	188
21	Normal vs. Disease plasma 1	Unknown	colon cancer	Colon Cancer	Female	Plasma	16507.52	2.63	211.43	4.35	447.91	5.56	491
22	Normal vs. Disease plasma 1	Unknown	lung cancer	Lung Cancer	Male	Plasma	33450.73	1.92	123.89	5.36	544.32	2.75	132
23	Normal vs. Disease plasma 1	Unknown	lung cancer	Lung Cancer	Male	Plasma	15133.49	1.46	105.81	6.78	473.74	3.86	1886

Merging Data

Samples may be run several times for one of the following reasons:

- Dilution requirements may be very different for different assays.
- Bead regions for the assays may overlap.
- Assays from different kits cannot be mixed together for other reasons.

The outcome of this is that results for a particular sample appear in several data sets. When the data files are imported, the sample is listed multiple times in the Sample and Results tables. (The tables in the Perform Analysis/Create Charts view are generally unaffected depending on your experimental setup.) The “Merge samples run on different panels/assays” command allows you to combine the rows of data so that each sample appears only once in the table. Merging data reduces the complexity of the data.

Data can be merged based on a matching Sample ID, Description, or Sample Label, or a combination of these fields. You can also merge the data using custom fields. Another use for merged data is to average the data in the grouped samples.

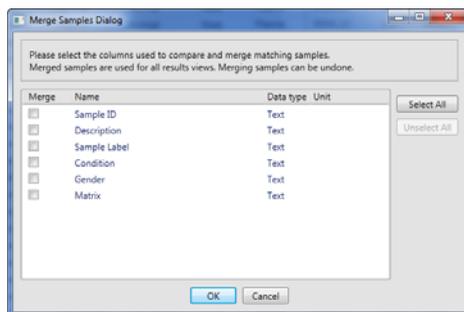
You can always unmerge merged data. Merging data does not change the original data.

To merge results data:

1. In the Results table, choose “Merge samples run on different panels/assays” in the toolbar.



2. In the Merge Samples Dialog dialog box, select the columns to match and merge samples.



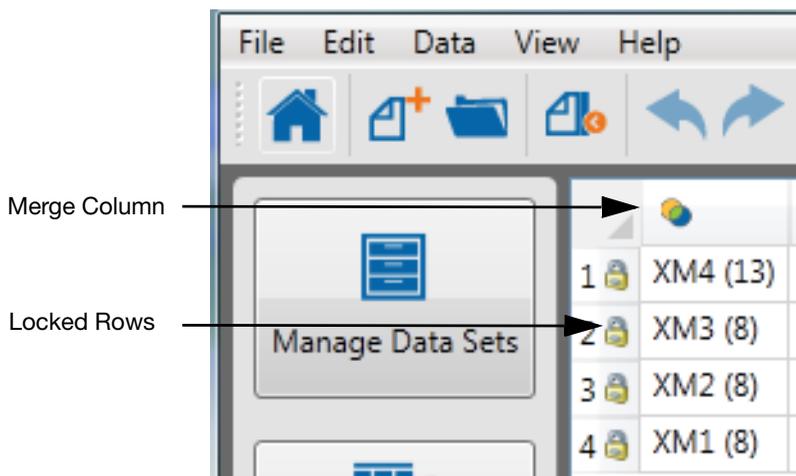
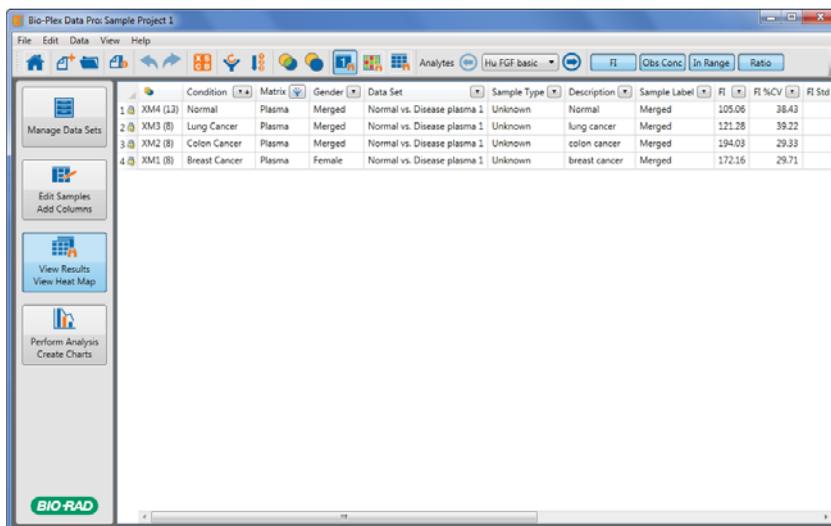
Records with matching names in the selected column are merged appropriately. When data are merged, a column is added to the left side of the table (Merge column), and this column is used to sort the table in ascending order.

To unmerge results data:

- Select Undo Merge Samples in the toolbar. In the Results table, all merged data are separated.

Viewing Merged Data in the Results Table

In the Results table, the Merge column displays the number of rows that have been merged for each group. The lock in the first column indicates that the data in these rows cannot be edited.



In a merged table, Observed Concentration (Obs Conc) and Concentration of the coefficient of variation (Conc %CV) columns report average values for merged rows.

The multiple analyte view shows all the analytes and observed concentration merged values for each analyte.

Viewing Merged Data in the Sample Table

Merged data in the Sample table are displayed as individual rows. The rows are sorted so that all merged rows are grouped next to each other. You can edit the merged data in the Sample table; for instance, if you mislabeled a sample. The data will be remerged dynamically based on your edits.

When you import data into a merged project, the new data are merged automatically.

	Condition	Matrix	Gender	Data Set
1	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
2	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
3	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
4	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
5	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
6	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
7	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
8	XM1 Breast Cancer	Plasma	Female	Normal vs. Disease p
9	XM2 Colon Cancer	Plasma	Male	Normal vs. Disease p
10	XM2 Colon Cancer	Plasma	Male	Normal vs. Disease p
11	XM2 Colon Cancer	Plasma	Male	Normal vs. Disease p
12	XM2 Colon Cancer	Plasma	Male	Normal vs. Disease p
13	XM2 Colon Cancer	Plasma	Female	Normal vs. Disease p
14	XM2 Colon Cancer	Plasma	Female	Normal vs. Disease p
15	XM2 Colon Cancer	Plasma	Female	Normal vs. Disease p
16	XM2 Colon Cancer	Plasma	Female	Normal vs. Disease p
17	XM3 Lung Cancer	Plasma	Male	Normal vs. Disease p
18	XM3 Lung Cancer	Plasma	Male	Normal vs. Disease p
19	XM3 Lung Cancer	Plasma	Male	Normal vs. Disease p
20	XM3 Lung Cancer	Plasma	Male	Normal vs. Disease p
21	XM3 Lung Cancer	Plasma	Female	Normal vs. Disease p
22	XM3 Lung Cancer	Plasma	Female	Normal vs. Disease p
23	XM3 Lung Cancer	Plasma	Female	Normal vs. Disease p
24	XM3 Lung Cancer	Plasma	Female	Normal vs. Disease p

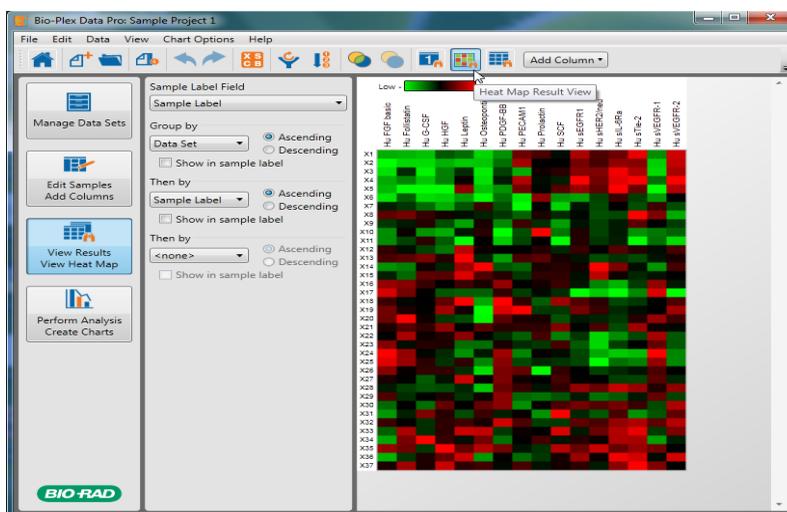
Heat Map Result View

A heat map is a graphical representation of data that enables you to quickly identify the intensity readings and to find patterns in the data. Each column in the heat map corresponds to an analyte, each row to a sample, and each cell to a data point. The cells are color coded according to their median or midpoint compared to other samples for that analyte.

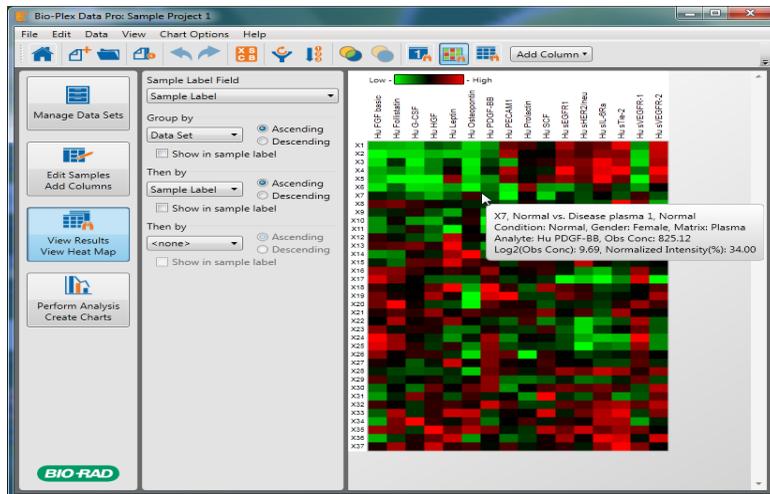
You can copy heat maps and tables to the clipboard and paste them into the application of your choice. (See Copying Plots and Graphs on page 35.)

To switch from table to heat map view:

- Click Heat Map Result View in the toolbar. The Results table data appear as a heat map.



Hovering over a cell in the heat map displays a description of that cell's contents.



Commands to the left of the heat map control the order of the samples and the labels used in the heat map. The Chart Options menu allows you to change the manner in which the intensities are calculated and displayed.

CHART OPTIONS MENU COMMANDS

From the Chart Options menu, you can access the following commands:

Frame Cells — a toggle that turns on or off contrasting lines framing each cell.

Sample Label Field — opens a drop-down list of the types of data that can appear as the sample label to the immediate left of the heat map.

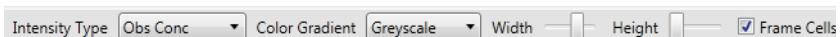
Intensity Type — opens a drop-down list of the types of data that can be displayed.

Data Transform — opens a drop-down list of the ways the data can be calculated.

Color Gradient — lists colors and color combinations you can select to change the appearance of the heat map.

TOOLBAR COMMANDS

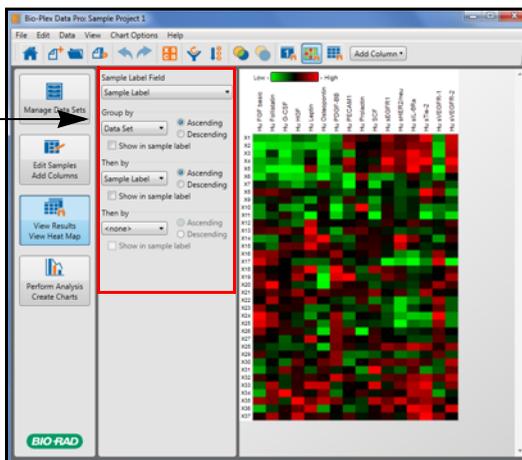
Intensity Type, Color Gradient, and Frame Cells controls also appear in the toolbar. In addition, Width and Height controls make it easy to resize the heat map.



HEAT MAP SETTINGS

The pane to the left of the heat map controls the labeling and sorting of the samples.

Use the controls
to label the heat map
and to sort samples

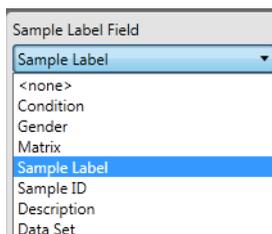


LABELING THE HEAT MAP

The Sample Label Field drop-down list displays the columns in the Results table. You can label the heat map with the values of one of these columns.

To specify a sample label in the heat map:

- Click Sample Label Field drop-down list and select one of the choices in the list.



If <none> is selected, no sample label appears next to the heat map.

GROUPING DATA IN THE HEAT MAP

You can specify how data in the heat map are grouped. You can group your data by any column in the Results table.

Note: The “Group by” drop-down list includes all visible predefined and custom columns in the Results table. Hidden columns do not appear in the list.

Using the controls to the left of the heat map, you can apply up to three levels of sorting to the heat map data. The primary sort is set using the “Group by” control. The second and third-level sorts are applied using the “Then by” controls. Each subsequent sort is done within the constraints of the previous sorts. For each sort level, you can also select whether the data appear in ascending or descending order.

To sort the heat map data:

1. Click “Group by” and select a column from the list.
2. Select Ascending or Descending.
3. If you want the sort option to appear in the sample label, click the “Show in sample label” checkbox.
4. If you want to apply another level of sorting, click “Then by” and select another column from the list. Follow the procedure in Step 2 and Step 3.

Adding a Column

You can add columns to the Results and Sample tables. See Adding a Column on page 27.

6 Viewing the Results and Basic Statistics

In the Perform Analysis/Create Charts view, you can view the graphs and tables that present your results in biologically relevant groupings.

Proper annotation of your samples is a prerequisite for using this view. At a minimum, the “Compare by” drop-down list should include column headings that contain the biological replicate groups you want to compare and display on the x-axis of your graphs. Subpopulations of samples can be analyzed by excluding samples from the view with the sample Filter drop-down list. Changing the column in the “Compare by” or “Filter by” drop-down lists dynamically updates all views.

The column selected in the “Control by” drop-down list is the biological replicate group that represents the control for your experiment. By choosing a control group, several calculations are automatically generated, including a pair-wise P-value calculation (either a t-test or Mann-Whitney, depending on which test is selected) and a log-scale fold change between the control and each of the experimental groups.

Statistical analysis is useful for identifying analytes that display differences between the groups under observation. Selecting the Perform Analysis/Create Charts view displays a table of statistical information for all analytes. This view enables visualization and discovery of statistically significant differences (P-value and fold change) among sample groups within each analyte in the project.

Analysis Workflow

The statistical analysis workflow comprises the following actions:

- Choose the variable for comparison. In order to do this, you must first annotate the data.
- (Optional) Choose the biological replicate group that represents a control for your experiment (if one exists) to do pair-wise comparisons between the control and all other groups.
- Select the statistical test to apply to the data.
- Use the filter to exclude data or refine group selection. If the desired samples are not available in any of the Filter options, add a new custom column in the Sample table or edit the annotation of an existing column (Filter).
- For each analyte, visually inspect the differences between the groups.

Analysis Workspace

Analysis configuration

The screenshot shows the Bio-Plex Data Pro software interface. The top menu bar includes File, Edit, Data, View, and Help. Below the menu bar are several tool icons and tabs for 'Obs Conc', 'In Range', and 'Ratio'. The main workspace is divided into three panes:

- Analysis configuration (left pane):** Contains controls for 'Compare by' (set to 'Condition'), 'Statistical Test' (set to 'Mann-Whitney / Kruskal-Wallis'), and 'Filter by' (set to 'Condition'). There are also buttons for 'Select All' and 'Clear All'.
- Analyte table (top right pane):** A table with columns: Analyte, P-Value, P-Value Method, Fold Change, Avg, Std Dev, %CV, and # Valid Samples. The first row is highlighted in blue.
- Analyte charts and tables (bottom right pane):** A scatter plot titled 'Hu FGF basic' showing data points for four conditions: Breast Cancer, Colon Cancer, Lung Cancer, and Normal. The y-axis ranges from 50 to 250.

There are three panes in the workspace:

- Analysis configuration — the series of controls on the left most side of this view that define the specifics of your desired analysis.
- Analyte table — table at the top of this view. It shows all analytes in the selected samples with descriptive statistics for each analyte.

- Analyte charts and tables — charts and tables that provide the results to the analysis you have configured using the controls in the analysis configuration pane.

Analysis Configuration Pane

The area on the left of the Perform Analysis/Create Tables view is called the analysis configuration pane. It has the following controls to filter your data, indicate how data are compared, and specify which statistical test to apply for analysis.

Compare by — specifies the variable used to group the data for presentation in the charts and to apply statistical tests. The list displays the columns in the Results table.

Control — specifies the control group for your analysis. The list displays the groups for the variable selected in the “Compare by” drop-down list.

Statistical Test — specifies the statistical test applied.

- If the data are normally distributed, choose t-test/one-way ANOVA.
- If the data are not normally distributed or you are unsure how the data are distributed, choose Mann Whitney/Kruskall Wallis. This is the default.

Filter by — includes or excludes groups from your analysis. By default, it displays the same column heading selected in the Compare field. You can change this setting.

Clear All Analysis Filters — selects and includes all items that were cleared with any filter setting.

Note: This feature removes any filters that were set in any table in the product. Any filters you set in any of the other views will be cleared.

Select All — selects all items in the list, therefore including them in the analysis.

Clear All — clears all items in the list, therefore excluding them from the analysis.

Analyte Table

The analyte table is the table at the top of this view. It contains a list of all analytes in the selected samples. Each row in the table contains the analyte name and descriptive statistics for the population of samples for this analyte.

ANALYTE TABLE TOOLBAR COMMANDS



In the main toolbar you can select one of the following measurements for the analyte table. If all toolbar commands are not visible, click the triangle on the right side of the toolbar to view hidden commands.

Fluorescent intensity (FI) — a relative measurement of fluorescent intensity based on the median, a robust statistical measure

Observed concentration — observed concentration of the analyte, calculated from its fluorescent intensity and the standard curve

Observed concentration within range — observed concentration that falls within the range of standard concentrations, given a specified recovery range

Ratio — normalized ratio, fold expression, or gene expression value calculated in Bio-Plex Manager™ software

	Analyte	P-Value	P-Value Method	Fold Change	Avg	Std Dev	%CV	# Valid Samples	Total Samples	# Outliers	# Groups
1	Hu FGF basic	0.00101	one-way ANOVA	0.885	142.31	60.17	42.28	37	37	0	4
2	Hu Follistatin	0.048	one-way ANOVA	0.592	270.19	104.75	38.77	37	37	0	4
3	Hu G-CSF	0.244	one-way ANOVA	1.66	510.18	700.90	137.38	37	37	0	4
4	Hu HGF	2.25e-05	one-way ANOVA	1.07	362.82	144.27	39.76	37	37	0	4
5	Hu Leptin	0.303	one-way ANOVA	0.709	4017.72	2789.96	69.44	37	37	0	4
6	Hu Osteopontin	0.028	one-way ANOVA	0.620	10540.93	3967.89	37.64	37	37	0	4
7	Hu PDGF-BB	0.00023	one-way ANOVA	2.70	3380.85	3006.64	88.93	37	37	0	4
8	Hu PECAM1	0.714	one-way ANOVA	0.303	699.84	264.99	37.86	37	37	0	4
9	Hu Prolactin	0.244	one-way ANOVA	1.43	967.24	1311.37	135.58	37	37	0	4
10	Hu SCF	0.00992	one-way ANOVA	0.649	422.13	138.26	32.75	37	37	0	4
11	Hu sEGFR1	0.145	one-way ANOVA	0.541	3090.53	1275.83	41.28	37	37	0	4
12	Hu sHER2/neu	0.070	one-way ANOVA	0.517	5007.05	1469.38	29.35	37	37	0	4
13	Hu sIL-6Ra	0.00066	one-way ANOVA	0.740	7527.82	2504.95	33.28	37	37	0	4

Interpreting P-Value Statistics

Bio-Plex Data Pro™ software automatically calculates P-values. You can quickly sort the analytes by their P-values and examine the analytes with the lowest P-values in order to visually see the differences between the samples groups. Bio-Plex Data Pro uses P-values to discriminate between sample groups.

The smaller the P-value, the greater the probability that two or more groups of samples have significantly different analyte measurements. Typically, a P-value of less than 0.05 (5%) is considered significant. Note that when a project contains many analytes, some of them may have a low P-value by chance, even if the samples from different groups do not truly differ. Therefore, a low P-value is not itself an indicator of a good separation between sample groups. Analytes with low P-values may have a large overlap of concentrations between the sample groups. However, a large P-value is generally an indicator that significant differences do *not* exist.

Statistical tests require that two or more groups be identified, and they require that each group have more than one sample. If the Statistical Test options are disabled, then your groups do not meet these requirements. Select a different choice from the “Compare by” drop-down list or edit sample attributes to identify more than one group with the required minimum number of samples.

If samples are grouped by the “Compare by” column, the P-value column displays the P-value and the P-value Method column displays the method used to calculate the P-value.

Bio-Plex Data Pro uses different algorithms to calculate the P-values depending on the number of sample groups per analyte and the user’s preference for one test or another. All P-values are *two-tailed* probabilities. Bio-Plex Data Pro does not make any assumptions about the direction of difference between two or more groups.

The following describes which statistical method is used to calculate the P-value. For data in a normal distribution, select t-test/One-Way ANOVA. The following logic is used to determine which test to apply:

- If there are two groups, a t-test is applied.
- If there are three or more groups, then the One-Way ANOVA test is applied.

For data that are not in a normal distribution, or if you are uncertain, select Mann-Whitney/Kruskal-Wallis. The following logic is used to determine which test to apply:

- If there are two groups, the Mann-Whitney test is applied.
- If there are three or more groups, then the Kruskal-Wallis test is applied.

Bio-Plex Data Pro does not assume that data are in a normal distribution and uses the Mann-Whitney or Kruskal-Wallis test by default.

Understanding Fold Change Statistics

Fold change is calculated for each analyte. Samples are grouped by the field specified in the “Compare by” list. The mean is calculated for each of the groups. The fold change is calculated by dividing the highest mean value by the lowest mean value, and then calculating the base 2 logarithm of the result.

Note: The values used in statistical calculations and plots depend on the measure type (FI, Obs Conc [observed concentration], In Range [concentration in range], or Ratio) selected in the main toolbar. All statistics — including P-values, fold change values, and descriptive statistics — incorporate input only from valid sample values. If a sample value is out of range or below range, it is not included.

Analyte Charts and Tables

The analyte charts and tables pane displays the following detailed information about the analyte selected in the analyte table. (These views can be accessed from the toolbar in the analyte charts and tables pane.)



Scatter Plot — displays the data for the selected analyte as a scatter plot. The x-axis in this plot does not represent a value. Dots are distributed in each group to facilitate visualization of each sample.



Box and Whisker Plot — displays the data for the selected analyte as a box and whisker plot.



Bar Graph — displays the data for the selected analytes as a bar graph with standard error bars.



Summary Table — displays descriptive statistics for each sample group in a separate row.



Export to Statistics Software — displays the data grouped as defined in the configuration pane in a format that is convenient for export to statistical software. Each column displays the data for one group.



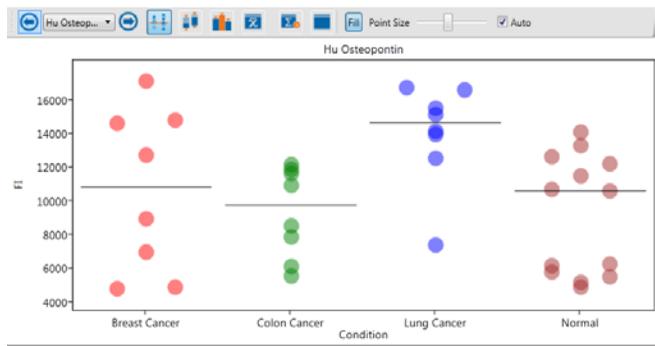
Results by Sample — displays the data used to create the plots and other tables along with descriptive data for the samples of the selected analyte.

The following are additional options available on the toolbar:

- **Analytes** — drop-down list of analytes in the data set.
- **Fill** — toggle between displaying the dots in the scatter plot as outlines or filled in.
- **Point Size** — changes the size of the dots in the scatter plot.
- **Auto** — when selected, controls the band width and margin of the scatter plot, bar and whisker plot, and bar graph.
- **Band Width** — when Auto is not selected, it controls how close the dots are to one another in the scatter plot. For the bar and whisker plot and bar graph, it controls the width of the bands.
- **Margin** — when Auto is not selected, it controls the distance between the bands in the bar and whisker plot and in the bar graph. In the scatter plot, it controls the distance between the sets of dots.
- **Legend** — when selected, a legend appears identifying the analytes in the bar graph.

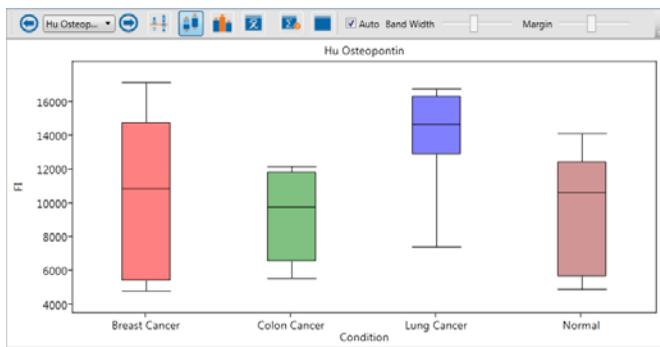
Scatter Plot and Box and Whisker Plot

The scatter plot and box and whisker plot display each group of samples as a category (band) along the horizontal axis. The scatter plot displays each sample as a point. Points within the same group are artificially spread out along the horizontal axis in order to minimize overlapping samples.



A box and whisker plot is a quick way of graphically examining the distribution of points in each group. This plot is particularly useful for comparing distributions between several groups or sets of data.

The box and whisker plot displays a box from the first quartile to the third quartile. The bottom and top horizontal bars are the minimum and maximum sample values, respectively. The horizontal bar within the box represents the median sample value.



When the Auto checkbox is selected, the plot displays all groups inside the view port and maximizes the use of the view port. When Auto is not selected, the Band Width (the width of the individual sample categories) and the Margin (the width between sample categories) can be adjusted manually.

Hovering over either plot displays detailed information about the object under the cursor.

Note: Plots display only samples with valid values.

Bar Graph

Bar graphs make it possible to view the graphs of different analytes simultaneously. You can choose to display selected analytes and filter out others. Doing so makes it easier to find analytes that have different expressions across different groups. For example, you can compare one drug to another to look for a different expression between the drugs. You can view the results for more than one analyte.

The first analyte in the table is selected by default. You can select other analytes.

To select an analyte:

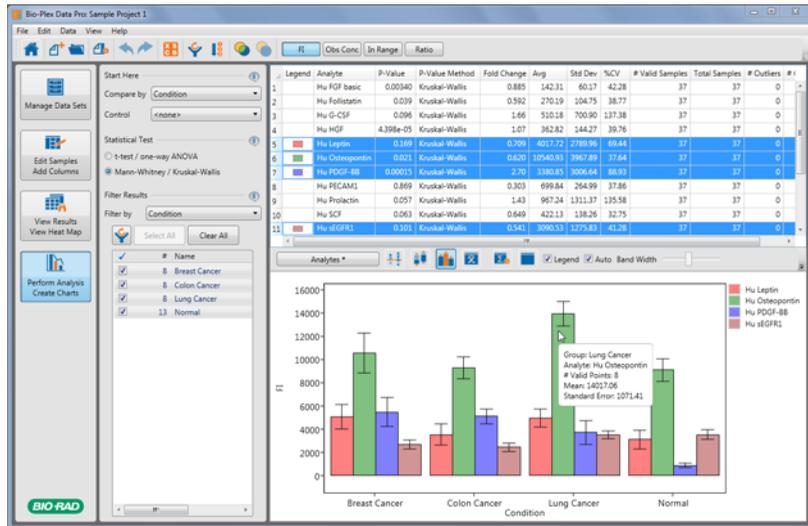
- In the analyte table, click anywhere in the row to select a single analyte. To select additional analytes, press CTRL and select additional rows.
- In the analyte charts and tables pane, select one or more analytes in the Analytes drop-down list.

Analyte Charts and Tables

When you select the Bar Graph icon, a Legend column appears in the analyte table. Each selected analyte corresponds to a color. These colors match the colors of the bars in the bar graph. You can also display a legend in the analyte charts and tables pane by clicking the Legend checkbox in the toolbar.

When you hover over the bar, an annotation appears with information about the analyte including the mean value for the analyte, the P-value, fold change, and the statistical method used.

On each bar, an error bar plots one standard error from the mean.



Summary Table

The Summary Table displays descriptive statistics for each sample group. Each group is displayed as a row in the table. If you select a Control group, you will see the following statistics:

- **P-Value** — the P-value is the result of comparing the selected group with the control.
- **Fold Change** — the fold change is the base 2 logarithm of the ratio between the control group and group being compared. The minus sign indicates the down regulation of the group compared to the control. No sign indicates the up regulation of the group compared to the control.

For example, if the mean of the control group is 1 and the mean of the group being compared is 1, then the fold change is 0. If the mean of the group being compared is 2, then the fold change is 1. The following table shows the calculated fold change for different control groups and compare groups.

Mean Value of Control Group	Mean Value of Compare Group	Fold Change
1	1	0
1	2	1
1	4	2
2	1	-1
4	1	-2

Condition	# Samples	# Valid Samples	P-Value	P-Value Method	Fold Change	Mean	Std Dev	%CV	Standard Error	Min	Q1	Me
1 Normal	13	13	---	---	---	3093.81	2980.32	96.33	826.59	179.50	581.63	233
2 Breast Cancer	8	8	0.082	Mann-Whitney	0.709	5055.69	2958.24	58.51	1045.89	1316.75	2200.50	447
3 Colon Cancer	8	8	0.562	Mann-Whitney	0.190	3529.25	2650.83	75.11	937.21	696.00	1362.06	247
4 Lung Cancer	8	8	0.070	Mann-Whitney	0.684	4969.59	2213.98	44.55	782.76	1632.75	3412.88	477

Export to Statistics Software

This table displays each group as a column and includes only the sample values. Unlike the Results by Sample table, it includes only samples with valid values. This table makes it possible to copy data to third-party software applications that support this format for sample groups

	Normal	Breast Cancer	Colon Cancer	Lung Cancer
1	1088.50	6914.25	6539.00	3765.75
2	333.00	8459.00	1071.25	1632.75
3	2364.75	4843.25	696.00	4893.50
4	3978.00	1316.75	4639.50	7999.25
5	6588.00	9326.75	2370.00	3295.25
6	614.75	4106.50	8102.00	5672.50
7	1472.50	1661.50	2581.75	7977.00
8	3313.00	3817.50	2234.50	4520.75
9	548.50			
10	2923.75			
11	179.50			
12	7657.50			
13	9157.75			

Results by Sample

This table displays all the samples for the current analyte in rows. If a “Compare by” column is selected, the table includes this column to display the sample annotation for the group. In addition, this table includes the sample value for the currently selected measure type.

Sample Label	Data Set	Description	Condition	FI
1 X14	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	6914.25
2 X15	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	8459.00
3 X16	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	4843.25
4 X17	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	1316.75
5 X18	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	9326.75
6 X19	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	4106.50
7 X20	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	1661.50
8 X21	Normal vs. Disease plasma 1	breast cancer	Breast Cancer	3817.50
9 X22	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	6539.00
10 X23	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	1071.25
11 X24	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	696.00
12 X25	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	4639.50
13 X26	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	2370.00
14 X27	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	8102.00
15 X28	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	2581.75
16 X29	Normal vs. Disease plasma 1	colon cancer	Colon Cancer	2234.50
17 X30	Normal vs. Disease plasma 1	lung cancer	Lung Cancer	3765.75

A Error Messages

As you view your results in the Perform Analysis/Create Charts view using different measurements, you may see errors. The following table describes the conditions in Bio-Plex Manager™ that may cause the error.

Measurement	Bio-Plex Data Pro™ Error Message	Error Condition
FI	Acquisition Error	No data present or not run ^a
	Not Run	No Bio-Plex Manager data for that analyte
Obs Conc	Acquisition Error	No data present or not run
	Cannot Calculate	No data present or not run for other reasons
	Not Run	No Bio-Plex Manager data for that analyte
	OOR>	OOR>
	OOR<	OOR<
Conc in Range	Acquisition Error	No data present or not run
	Cannot Calculate	No data present or not run for other reasons
	Not Run	No Bio-Plex Manager data for that analyte
	OOR>	OOR>

Measurement	Bio-Plex Data Pro™ Error Message	Error Condition
	OOR<	OOR<
Norm Ratio	Acquisition Error	No data present or not run
	Not Run	No Bio-Plex Manager data for that analyte
Ratio	Acquisition Error	No data present or not run
	Not Run	No Bio-Plex Manager data for that analyte

- a. Not run means the sample is not run for any analytes and any replicates. All measurements are error not set.

Glossary

Analyte

The substance being measured, identified, quantitated, or otherwise analyzed in an experiment. In the Bio-Plex® system, analytes are molecules that are being quantitated.

Attribute

Custom field relevant to customer research, entered manually or copied in from another list (Excel, for example).

Concentration

The amount of substance present in a sample, usually expressed in weight per volume.

Data set

The sample and analyte information available after a run has been performed and analyzed. A data set is roughly equivalent to a plate and is imported via a file import; for example, from Bio-Plex Manager™ software via an RBX file.

FI

Fluorescent intensity. A relative measurement of fluorescent intensity based on the median, a robust statistical measure.

Heat map

Graphical representation of peak expression data. Each column of a heat map represents a spectrum, each row represents a peak cluster, and each cell represents a specific peak that is color coded to reflect its expression level. Displays relationships between samples and analytes.

LLOQ

Lower limit of quantitation.

LOD

Limit of detection.

Outlier

Sample concentration derived from FI below the background value. Sample concentration that meets a user-defined criteria, including %CV, concentration above or below a certain range.

P-value

Statistical representation of the chance that the relationship being observed is observed by pure chance. For example, a P-value of 0.01 means that there is a 1% chance that the relationship between the independent variable and the dependent variable established by the model does not actually exist.

Plate

Assay plate. Bio-Plex assay plates have 96 or 384 wells. The plate contains assay data.

Sample

A solution of analyte standards or unknowns that, when added to an assay, will bind to the microspheres in that assay and be identified and quantitated by the array reader.

UOLQ

Upper limit of quantitation.

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