BeadView Software Guide
Version 1

"Don’t just collect data...generate results."
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Introduction

BeadView™ software was specifically developed to standardize, analyze, and summarize MFI (Median Fluorescence Intensity) assay data measured by Luminex xMAP® 100 bead array platform. The software is compatible with the standard “output.csv” file generated by the Luminex 1.7 or IS 2.2 software. This file can be found in the “Batch” folder collected for each plate measured on the system. To load the file in BeadView, go to the File Menu, then Open, and choose “output.csv” in the appropriate Batch folder.

***Important Note*** When using BeadView with Luminex IS Software, set up the Luminex IS template for raw data collection only. It is not necessary to set up the reagent kit information. This will be entered in BeadView. See Appendix A for how to set up a “Raw Data Collection Only” template in the Luminex IS software.

Once the Output file has been loaded, the analysis generally falls into these steps:

1) Label well “Types” as Background (SB), Standard (S1, S2, S3...), Control (C1, C2, C3), or Unknown (?). The plate layout can be saved as a template for later use.
2) Identify replicates samples under the Group column in the Samples View.
3) Enter kit information such as standard serial dilutions and dynamic range in the Kit Setup View.
4) Calculate standard curves using “Best fit” in the Analysis View
5) Generate replicate report in the Replicate View.
6) Graph results using integrated excel graphing macro.

The “Best Fit” curve fitting routine is a time saving feature that will automatically present the best curve model (4PL, 5PL, 4PL log, 5PL log) for each different analyte.

BeadView does not support its own printing function. For optimal flexibility and customization, we recommend that all graphs and reports be printed from excel or other database software.

**HASP Device**

BeadView software operates in conjunction with a HASP key that was shipped with the original disk packaging. BeadView will not open unless this HASP key has been placed in the USB port of the computer. Additional HASP keys can be ordered from Upstate. Contact a representative for ordering information.
About this Guide

This user guide is broken down into three main sections:

1) Screen View Summary
2) Data Analysis Summary
3) Glossary of Functions

Section 1 provides you with an orientation of the software’s various interfaces and functions. Section 2 provides a step by step guide that demonstrates how to perform a basic plate and standard curve analysis. Section 3 is a glossary of functions that provides brief definitions of the various commands and tools.

Technical Support

General Tech Support:
800 548 7853 or techserv@upstate.com

BeadView Support:
800 233 3991 x 7679 or x 6155
Section 1 - Screen Overview

This section provides a brief summary of the different screen views in BeadView. The icons for each view can be found along the left hand side of the window.

Information View

When a Luminex “output.csv” file is opened with the BeadView Program, the information regarding the machine serial number, software version, and plate name will be displayed on the Information screen. Comments can be logged in by selecting New Comment.
**Plate View**

The Plate View displays **Well** and **Analyte** data in a 96-well format. When Well is selected, information regarding the well **Name**, **Type** (background, standards or unknown), **Group**, **Total Beads**, or **Dilution Multiplier** can be viewed or entered (this information can also be entered and viewed from the Samples screen). If the name of the sample well was entered into the Luminex software, this will automatically be imported into the **Name** field.

When Analyte is selected, the user can choose to display the **Raw MFI**, **%CV**, **Normalized MFI**, **Result (pg/ml)**, or **Bead Count** for each analyte in the assay.
To view information on different analytes, click the arrow for a pull down menu (Screen 4).

Different plate setup configurations can be saved as templates. Go to File > Export > Plate Information.
Analytes View

The Analytes view displays the Raw MFI, %CV bead size, Normalized MFI, Result (pg/ml), or Bead Count for all wells and all analytes on a single spreadsheet (Screen 5). Any portion of the sheet can be copied and pasted into another database or spreadsheet such as Excel®. In order to include the headings in the copied data, click the Show/Hide grid Headers Icon (See below). Values referred to as <HIGH> or <LOW> in the Result (pg/ml) are values that lie outside the calculable portion of the standard curve.
**Samples View**

The Sample view allows the user to enter and display information such as **Sample Name**, **Well Type** (background, standards or unknown), **Group Name**, and **Dilution Factor** (The same information can be found in a plate orientation in the Plate View). Guidance on how to define well Type can be found in the Data Analysis Summary (Section 2).

Sample **Name** and **Group** identifiers can be copied and pasted directly into BeadView from other database sources.
Kit Setup View

All information regarding the reagent kit can be viewed/entered in the Kit Setup View, including Kit Name, Lot #, Bead ID’s and recombinant standard serial dilutions. The right side of the screen has two tabs, Analytes and Standards. All Kit Setup information can be saved as a template. Go to File menu > Export > Kit Information.

The Standards tab allows the user to enter standard serial dilutions and define the “valid” portion of the standard curve.

Further information on entering standard serial dilution concentrations can be found in the Data Analysis Summary (Section 2).
**Diagnostics View**

A diagnostic routine has been integrated into BeadView. The diagnostics are optional and should be run after standard curves have been generated in the *Analysis View*. Click the **Run** tab on the right side of the screen to execute.
**Analysis View**

**AFTER** well types and standards have been defined, the *Analysis View* calculates the optimal solution for the standard curves that will generate the *Result* (pg/ml). If a *Kit Equation* is not defined, the software will present the “Best Fit” solution. To manually choose the equation employed by the analysis, click on the *Equation* command.

To automatically calculate the curve for all analytes, click on **Calculate All Analytes**. To calculate one curve at a time, click on “+”.

The *Analysis View* also allows the user to perform various functions on the curve itself. See **Glossary of Functions** for more information.

Curve images can be exported by a right click on the graph and then choose **Export Dialog**.
Replicates View

The Replicates view provides statistical information on replicate samples in the 96 well plate. There are four functions associated with the Replicates View. All data can be copied and pasted into other programs in a similar fashion as described in the Analytes View.

1) Generate Report – Generates a replicate report based on the Group identifier found in the Samples View. Samples with the same Group name are treated as replicates. If no Group identifiers have been inserted, then a report will not be generated.

2) "+" Show/Hide Details – Defines the option to show the individual replicate values in addition to the statistical values.

3) Fields – Defines what fields to show (MFI, pg/ml, %CV, Standard Deviation, Count, and Scale).

4) Graph – Exports the data to Excel® and executes a customized graphing wizard.
Section 2 – Analysis Summary

This section provides a step by step protocol on performing a standard curve analysis for conversion of MFI value to Results (pg/ml).

A plate can be laid out in any format. For this guide, examples are written under the assumption that plates will be laid out in the following format

1) For each plate analysis, open the associated “output.csv” file found in the batch folder generated by the Luminex software

2) Go to the Samples View
   - Enter sample names under the Name column (Sample names can be pasted in from another source). Sample Names are for identification purposes only. They are not necessary for standard curve analysis. If sample well names are entered into the Luminex software, they should automatically carry over into BeadView. Alternatively, they can be copied and pasted from another source.
   - Enter sample types into the Type column. Highlight the appropriate wells and then click on the related Type icon (background , standards or unknown ). If standards are run in duplicate or triplicate, define prior to clicking on . The standards will be placed in numerical format depending upon how many wells are highlighted and the replicate definition. S1 is always considered to be the lowest dilution. If the plate wells are organized from high to low dilution, highlight all the standard wells and click on the “Swap Cells Vertically” icon to change the standards from ascending order to descending order.
   - Enter duplicate sample names into the Group column. Replicates are recognized as all wells which have the identical Group name.
   - Optional: Save as Plate Template (see Glossary of Functions).
Sample View
3) Go to Kit Setup View

There are two tabs in the Kit Setup view: Analytes and Standards.

- **Analytes** displays the analytes and bead # ID’s that were entered into the Luminex program.
- Define Standard Dilutions:
  - What about the message screen that pops up asking if they want to enter standard information???
    - Under the Standards tab, enter the starting concentrations for each analyte under the column furthest to the right or the highest standard number.
    - Define the dilution value by clicking the down arrow on the dilution command $\times \frac{1}{[x]}$.
    - Highlight the relevant analyte rows and click on the dilution command $\times \frac{1}{[x]}$. All of the standard cells will be filled in automatically with the correct dilution value.
    - Double check that all of your standard dilution concentration values have been entered correctly.
- Choose “Best Fit” for all analytes under the Recommended Formula column (You will have the option to manually choose the curve fit in the Analysis View.
- Dynamic range can be defined in relative or absolute terms with respect to Concentration or MFI. If no range is defined, the default parameters will be used.
- Confirm that the correct units are defined.
- The kit setup can be saved as a template: File menu > Export > Kit Information.
4) Go to the Analysis View

- Click on the button to calculate standard curves for all analytes.
- Chart on the left will indicate the “Best Fit” equation used for each analyte, and graph on the right will display the standard curve.
- Calculated concentration values for each well can now be found under Result in the Plate View and Analyte View.
Glossary of Functions – Section 3

Change the Axis of the Graph.
Options for the axis are Log (y)/Log (x), Liner (y)/Log (x), Log (y)/Linear (x), and Linear (y)/Linear (x).
- From Analysis Screen, click on (Axis)
- Select axis type
Please note: The axis will be changed in the graphs for all of the analytes

Change the Equation.
This can be done for each individual analyte. The equation used to calculate a standard curve can be different for each analyte.
- From Analysis screen, click on desired analyte
- Select new equation, in this case 4PL
- Click on (Calculate selected analyte)
- New curve will appear

Copy/Paste into New Program.
The data can be copied from BeadView and pasted into a different program. This can be performed from Plate screen or Analyte screen.
- Click on (Hide grid headers)
- The chart titles will turn light blue
- Select the cells you wish to copy, click on (Copy)
- Paste into new program

Dilution Factors for Standards.
Under Kit Setup screen, available dilutions for the standard curve are:

Duplicates
This function enables sample types to automatically be entered in duplicates. Other options are no replicates or triplicates. This function is available under the Plate screen or the Samples screen.
Export Standard Curve
From *Analysis* screen, select the graph you would like to export.
- Hover the mouse over the graph
- Right click
- Select *Export Dialog*
- Under *Export*, select *JPG*
- Under *Export Destination*, select *File*
- Click on *Browse*
- Select file that the graph will be exported to
- Click on *Export*

Inactivate a Point on one of the Curves.
A point can be individually inactivated for one particular analyte, and is not applied to the same point for all of the analytes.
- From *Analysis* screen, select the analyte for which you would like to delete a point
- Click on 
  - (Reject Standard)
- Click on the point you wish to inactivate
  - Curve will disappear
  - X will appear over the inactivated point
- Click on 
  - (Calculate selected analyte)
- The curve will be recalculated
No Replicates. This function enables sample types to automatically be entered in singlicate. Other options are duplicate or triplicates. This function is available under the Plate screen or the Samples screen.

Reactivate a Point on One of the Curves. A point can be individually reactivated for one particular analyte, and is not applied to the same point for all of the analytes.

- From Analysis screen, click on the check  (Accept Standard)
- Click on the previously inactivated point
  - The X will disappear
- Click on (Calculate selected analyte)
- The curve will be recalculated

Replicate Cells Downward. This function is similar to a copy function, where a value in one cell will be replicated in all cells below it that are highlighted. The following is an example of this function used to replicate the 5000 pg/ml value for S7 among the analytes.

Replicate Cells Right. This function is similar to a copy function, where a value in one cell will be replicated in all cells to the right of it that are highlighted. The following is an example of this function used to replicate the sample types for the serial dilution.
Setting Standard Duplicates in Parallel Columns.

Data analysis can be performed with plates set up in different formats. For this example, the standards are plated in the following format:

| Background | background |
| Standard 1 | Standard 1 |
| Standard 2 | Standard 2 |
| Standard 3 | Standard 3 |
| Standard 4 | Standard 4 |
| Standard 5 | Standard 5 |
| Standard 6 | Standard 6 |
| Standard 7 | Standard 7 |

- Open Output file, go to Plate screen
- Select No Replicates, enter Background and S1-7
- Highlight first two columns, click on Replicate Cells Right
- Column will be replicated

Swap Cells Horizontally.
This function allows the order of highlighted rows to be reversed.

Swap Cells Vertically.
This function allows the order of highlighted columns to be reversed.

Templates.
There are two types of templates that can be used: 1) a Plate Template that will contain information regarding the location and number of replicates for the standards and unknown samples (sample names and dilution factors are optional for this template); 2) a Kit Template that will contain the analyte names, bead IDs, background subtraction, recommended formula and serial dilutions for the standard curve.

- Make Plate Template
  - Open Output File
  - In Sample view, fill in sample type
    - Optional: fill in sample name and dilution multiplier
  - Click on File, Export, Plate Information
  - Select Sample Types
    - Optional: If sample names and dilution factors were entered, select Sample Names and Dilution Factors as well
  - Click OK
  - Name the template, save
• Make Kit Template
  o In Kit Setup view, fill in **Background Subtraction** (under Analytes tab), **Recommended Formula** (under Standards tab) and serial dilutions (under Standards tab)
  o Click on File, Export, Kit Information
  o Name kit template, save

• Apply Plate and Kit Templates
  o Open output file in BeadView program
  o Click on File, Import, Plate Information
     ▪ Select plate template, Open
     ▪ Plate template will be applied to the data
  o Click on File, Import, Kit Information
     ▪ Select kit template
     ▪ Kit information, including Background Subtraction, Recommended Formula and serial dilutions for the standard curve will be applied.

Triplicates
This function enables sample types to automatically be entered in triplicate. Other options are no replicates or duplicate. This function is available under the Plate screen or the Samples screen.

Visualize the Location of the Unknown Samples on the Curve.
This can be used to display the well number, MFI and name of a data point.
• From Analysis screen, click on Unknown will appear as boxes on the curve
To find information regarding a sample, hover over the sample
Well number, MFI and name will appear on the bottom bar

Zoom
To zoom, follow the instructions below:

To undo zoom, right click and select *Undo Zoom*
Appendix A

Creating a “Data Collection Only” Template in Luminex IS 2.2/2.3

1) Go to the Create Template command in the template wizard. Choose “Data Collection Only” in the template type and then follow setup as normal.