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INTRODUCTION

Welcome to MILLIPLEX Analyst, the superior software solution for multiplex data analysis: full automation, accurate results, high throughput and simple user-interface.

MILLIPLEX Analyst Offers:

- State of the art graphical user interface built with .net technology
- Three simple wizard driven steps to analyze multiplex data
- Fully automated analysis including data reduction and concentration calculation
- Robust curve fitting and accurate detection limit determination
- Completely integrated visualization between original data, curve mapping and results
- Standardized reports with graphical presentation
- Supports both quantitative and qualitative analysis
- Supports background subtraction
- Normalization options for control analyte or control sample
- Supports quality control well with QC limit ranges
- Seamless integration with Luminex xPONENT with Watch-dog feature
- Customized data format supports multiplex data sets from other systems
- Integrated clustering capabilities ideal for downstream data analysis such as pathway and drug response
- 2D and 3D data presentations
- Curve potency analysis to ensure consistent results over different periods of time and across different systems
- Sample EC50 analysis

About this Guide

This user guide has three main sections:

1. Data Analysis
2. Screen Overview Detail
3. **Appendix**

**Section 1** provides a step by step guide that demonstrates how to do data analysis.

**Section 2** provides screen views of raw data view, platemap view and results view.

**Section 3** is an appendix of functions that provides definitions of the commands and features.
SECTION 1 – DATA ANALYSIS

1. Basic Analysis: Wizard

Step 1: Open File

1. Click on the Main Toolbar after MILLIPLEX Analyst is launched. Wizard step 1 dialog box will appear:

![Wizard Step 1 Dialog Box]

2. Select the csv files and click button to add them to the file list OR double click the file to add into the File Names list.

3. Select the csv files in the list and click button to remove the file.
4. If more than one file is to be analyzed, click or to adjust the plate order.

5. Click to open the csv files.

Note: All selected files will be opened in the program. The files will be shown in the order of A, B,C… plate alphabetically.

Step 2: Verify or Edit Platemap

Once a .csv file opens, the Platemap Editor will be displayed:

1. Verify the well layout.

2. Click to go to the next step.

Step 3: Analyze Data

After the platemap is defined in Step 2, MILLIPLEX Analyst will analyze the data automatically. Once Quick Start wizard steps are complete, the data will be saved and displayed as below:
See Result Data View for more information.

2. Quantitative Analysis

Quantitative analysis will include a standard curve for every analyte. Sample MFI intensities will be calibrated by the standard curve and measure the analyte concentration of each sample.

Quantitative platemap file includes the information for sample name, analyte name, concentration, standard well, QC well and blank well. Select Quantitative from the Analysis drop-down list in Quick Start Wizard Step 1.

2.1. Edit Sample ID Series

The default sample name is automatically generated by "Plate number + Well location _ G (Generated)".

To define the sample name, select the wells in Platemap Editor Window, or at Wizard Step 2, then click Edit Samples or select Edit Sample ID Series from the
Well Popup Menu to open the dialog box below:

1. Enter the **Prefix** of sample name or ID
2. Define the **Start Number** value
3. Enter **Replicate Number** value for replicate sample wells
4. Enter **Dilution Factor** if the sample has been diluted before running the experiment
5. Check **Name Vertically** if the sample name series runs vertically in the wells

**2.2. Edit Standard Curves**
**Step1:** Open **Edit Standard Curves** dialog box.

- Click at Wizard Step 2, Platemap editor, to open the **Edit Standard Curves** dialog box. From this dialog box, you can define the standard curve wells, group the wells to every dilution level, sort and list the standard curve wells in dilution order, set up starting concentration, and edit standard curves.

- In the Platemap Editor, move the cursor to the Standard Curve list on the left panel, click button to open a Standard Popup Menu to select Edit Standard Curve

**Step2:**

- Verify or redefine the standard curve well list in the left Standard Curve panel.

- Select the check boxes on the left of STD # well ID. If the check is in gray, it means the replicate well is the same as the one above.

**Step3:**

- Enter a value for highest concentration, dilution factor and how many decimal digits of calibrated sample concentration value.

- Check **Background Well** check box to indicate the standard curve wells include background wells.

- Check **Direction: Increasing** to define Standards curve well runs serial dilutions over the wells from the low to high; or Check **Decreasing** for from the high to low.

- Select **Apply To All Analytes** check box and click to apply the **Settings for all the analytes**.

- Check **Apply To All Analytes**, the standard curve set up for all analytes in the same experiment.

- Un check **Apply To All Analytes**, and check the analytes box individually, then click , the standard curve setting will only apply to those selected analytes. In this way, you can set up a standard curve separately for all the analytes and individually select analytes in the list,
and then click [Apply] to apply the settings for only selected analytes in the list.

2.3. **Edit Analytes**

Click [Edit Analytes] at Wizard Step 2, Platemap Editor Window, or select **Edit Analytes** from the Well Popup Menu to open the dialog box:

![Edit Analytes dialog box]

To change an Analyte ID, Control Type or Unit, follow the steps below:

1. Click on a **Current ID** field in the left list.
2. Select an **Analyte ID** from the list in right panel.

3. Select a **Control Type**.

4. Define the analyte **Unit**.

5. Click [QC Range] to verify the QC range.

6. Click **Save** to save the change in QC range and then click [OK] to end.

3. **Qualitative Analysis**

Qualitative analysis provides data in relative qualitative measurements in contrast to quantitative measurements that provide actual analyte concentration values. In qualitative analysis, different types of positive and negative controls are usually used in the analysis.

In MILLIPLEX Analyst V5.1, negative and positive controls for both sample and analyte are supported.

3.1. **Qualitative Analysis**

Set up qualitative analysis options at Quick Start Wizard Step 1.

Analysis Type:

- **Qualitative** --- Multiplex assay used to compare sample analytes against a qualitative reference using one or more controls or normalization factors.
Data Analysis:

- **Background Subtraction ---** Background well (BKG) Subtraction: Each standard, QC, control or sample wells will subtract background well and/or Zero concentration wells of standard curves. For Qualitative assays, each analyte intensity MFI(i) value will be adjusted by subtracting the corresponding analyte MFI(i) value in the background (BKG) well or wells.

  **Note:** If there are no background BKG wells pre-defined from the **Well Popup Menu** or from **Platemap Editor**, the following message will appear.

- **Neg-Ctrl Subtraction ---** Negative Control Analyte Subtraction: In each well, each analyte intensity MFI(i) value will be adjusted by subtracting the negative control analyte MFI(i).

  If there are no analytes pre-defined with **Control Type** as **Negative Control** from **Edit Analytes**, the following message will appear.

  **Note:** Background Subtraction (sample) and Negative Control Subtraction (analyte) cannot be used at the same time in a single
analysis process.

- **POS- Ctrl Analyte Global Normalization** --- Positive Control Analyte Global Normalization: In each well, the intensity MFI(i) of the positive control analyte MFI(i) will be normalized. Each analyte intensity MFI(i) value will be adjusted by multiplying the normalized positive control value. The normalization factor is derived by dividing the MFI(i) of the highest intensity positive control analyte on the plate by the MFI(i) of the positive control analyte.

If there are no pre-defined analytes with Control Type as Positive Control from Edit Analytes, the following message will show up.

![Normalization - Output5.csv](image1.png)

- **POS- Ctrl Analyte Normalization** --- Positive Control Analyte Normalization: Each analyte intensity MFI(i) in a well will be normalized to the intensity of the positive control analyte, MFI(i) in the same well.

If there are no analytes pre-defined with Control Type as Positive Control from Edit Analytes, the following message will appear.

![Normalization - Output5.csv](image2.png)

**Note:** Positive Control Analyte Global Normalization and Positive
Control (Analyte) Normalization cannot be used at the same time in a single analysis process.

- **POS-Ctrl Sample Normalization**-- Positive Control Sample POS (Well) Normalization: Each sample well normalized to Positive Control Sample Well. The analyte intensities MFI(i) of each sample will be normalized to the intensity MFI(i) of the corresponding analyte in the Positive Control Sample well(s).

If there are more than one Positive Control Sample well, you can pair these with sample wells and other non-Positive Control wells to form normalization group(s), Create Sample And Positive Pairs.

**Note:** POS is the keyword for positive control sample or positive control well, which can be defined in platemap editor window editor and the popup menu, Edit Positive Well ID Serial.

If there are no POS wells and POS-Sample well pair pre-defined from Edit Analytes, the following message will appear.

![Normalization - Output5.csv](image)

3.2. **Positive Control Sample Normalization**

**Step 1:** Select Qualitative Analysis

Select Qualitative analysis in Wizard Step 1 or from Menu list: Open|Open File|Qualitative to open file

**Step 2:** Define Positive Control POS well:
Right click on a positive control well, type in POS as well name at Wizard Step 2, Platemap Editor Window

Step 3: Create POS and Sample Well Normalization group pair

Hold down Ctrl key on the computer keyboard, select all sample wells within the POS normalization group, then right click on selected wells and click on Create Sample And Positive Well Pairs from the Well Popup Menu.

![Edit Sample ID Series](image)

Note: The sample wells will pair with the positive wells automatically by column if paired sample wells are not directly selected.

And the sample names will be "Sample name # Positive name" as shown below:

<table>
<thead>
<tr>
<th>POS1</th>
<th>POS2</th>
<th>POS3</th>
<th>POS4</th>
<th>POS5</th>
<th>POS6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>Sample1</td>
<td>Sample1</td>
<td>Sample1</td>
<td>Sample1</td>
<td>Sample1</td>
</tr>
<tr>
<td>Sample2</td>
<td>Sample2</td>
<td>Sample2</td>
<td>Sample2</td>
<td>Sample2</td>
<td>Sample2</td>
</tr>
<tr>
<td>Sample3</td>
<td>Sample3</td>
<td>Sample3</td>
<td>Sample3</td>
<td>Sample3</td>
<td>Sample3</td>
</tr>
<tr>
<td>Sample4</td>
<td>Sample4</td>
<td>Sample4</td>
<td>Sample4</td>
<td>Sample4</td>
<td>Sample4</td>
</tr>
<tr>
<td>Sample5</td>
<td>Sample5</td>
<td>Sample5</td>
<td>Sample5</td>
<td>Sample5</td>
<td>Sample5</td>
</tr>
</tbody>
</table>

3.3. Multiple Positive Control Sample Normalization

Select the wells first, and then select Edit Positive Well ID Series from the Well Popup Menu to open the dialog box as below:
1. Enter the **Prefix** with POS for POS well series

2. Define the **Start Number** of POS well series

3. Define the **Replicate Number** of POS wells

4. Check **Name Vertically** if the POS name series runs vertically in the wells

---

**4. Instrument Integration (Watch-dog)**

Watch-dog Process is the integration between MILLPLEX Analyst and the Luminex xPONENT instrument control software. The Watch-dog function provides users an interface to target file folders on the xPONENT computer to watch. After a run on the Luminex instrument has completed and xPONENT generates a .csv file into these watched file folders, MILLPLEX Analyst will automatically load the .csv file into the wizard process for analysis.

**Step 1: Open Watch-dog Process Panel**

Select **Analysis-Watch-dog** from main menu to open the **Watch-dog** process panel.
Step 2: Add Watch Folder

Click on the Watch-dog process Panel in Step 1, select a file folder on the Open file browser.

The selected file folder will be listed under the Watch Folder tab.
Note:

- MILLIPLEX Analyst supports up to 10 watch folders.
- All sub-directories or sub file folders under the watch folder will also be monitored by watch-dog function
- A watch folder and other sub file folders will not be monitored if one of its own sub folders is set as a watch folder.

Step 3: Watching

Click  on the toolbar of the Watch-dog process panel, the eye icon will begin blinking, this means the watch-dog process has started. The program will not monitor all watch folders and look for .csv files to be created.

The watch-dog function will automatically load a newly created .csv file in watched folders during the monitoring period, then use the predefined wizard settings or the protocol and Platemap editor will open as shown below:
Rest of the wizard steps will be the same as the regular analysis wizard. The following message will appear after a watched .csv file has been analyzed:

Click **Yes** to analyze the next .csv file in the watch queue. If there is no .csv files waiting in the queue, the program will continue monitoring the watch queue.

Click **No** to stop automatic loading and analyzing of watched .csv files.

**Note:** You need to select the appropriate analysis type before starting the Watch-dog.

### 5. Other Multiplex Data (Customized Format)

Customized Format is a feature used when the program cannot initially recognize a multiplex data file and for file formats that are not currently supported now. It allows the user to define the keywords in the data file, which will allow the file to be loaded and the data to be analyzed.
Note: Customized Format can be used with layouts that are similar to Luminex multiplex data files. Not all files types are supported and not all file types that are not initially recognized by the program can be utilized with the customized format function.

5.1. Create Customized Format

Step 1:

1. Open unsupported multiplex file at Quick Start Wizard Step 1 or from Menu list: Analysis|Customized Format to open Customized Format dialog box.

2. The Customized Format Dialog box has format creation in the left panel and opens the file in excel format in the right panel. This allows you to easily reference the file in order to create the format settings.

Format:

- **Predefined File**: Select a file saved in the folder of “****/VigeneTech/MILLIPLEX Analyst/Customized Format”

Data Block:
- **Detect Block:**
  - **By block mark:** Detect the data block by a mark.
  - **By all block keywords:** Detect the data block by each keyword of the data block.

- **Block Mark:** Only appears when you select **By block mark** in the Detect Block drop-down list. Input the keyword that indicates the beginning of the data block.

- **Data Keyword:** Input the keyword that indicates the beginning of the Median data.

- **Count Keyword:** Input the keyword that indicates the beginning of the Count data.

- **CV:** Input the keyword that indicates the beginning of the CV data.

- **Excluded Block:** Only appears when you select **By all block keywords** in the Detect Block drop-down list, used to identify data blocks to be excluded in the analysis.

**Data Block - Column Header**

- **Location:** The title of location column.

- **Sample:** The title of sample column.

- **Excluded Columns:** The keywords that indicate what column(s) information will be ignored. We recommend not changing this option.

- **Detect Block:** Whether to detect the header block.

- **Beginning Keyword:** The keyword that indicates the beginning of the header block.

- **End Keyword:** The keyword that indicates the end of the header block.

3. Click **Test**. A successful message will appear if the format created is correct.
4. Click OK

5.2. **Save Customized Format**

When the configuration is correct, click OK, input the file name in the Save dialog box to save a .format.xml file, which can be used for the same type of files for next same type file in the future.

![Save dialog box](image)

5.3. **Save as Default**

Click Save as Default to save a .format.xml file as the default predefined file. MILLIPLEX Analyst will check whether the default .format.xml will open the current file automatically.

5.4. **Open Customized Format Files**

Here are the steps to open customized data format file:

1. Wizard Step 1:

   Select file type as excel .xls file to open a Customized format file
2. **Customized format is Set as Default Format:**

   Program will open and run the file as Luminex .csv file.

3. **Customized Format is not set as Default Format:**

   The **Customized Format** dialog-box will be displayed and the raw data will be displayed in a table on the right of the dialog box.

   ![Customized Format Dialog Box]

   1) Open a file browser by click the **Predefined File** drop-down list to select a predefined .format.xml file.

   2) Click **Test** to check if selected protocol supports the opened file. A successful message will appear

   ![Test Successful Message]

   3) Click **OK** to open the file.
SECTION 2 - SCREEN OVERVIEW DETAIL

This section provides a summary of the screen views in MILLIPLEX Analyst for: Raw data view, Platemap view and Result view.

1. Raw Data View - File Open versus Quick Start

Once the Luminex data (*.csv) files are opened with MILLIPLEX Analyst, click at the bottom to view the Raw Data PlateView window.

There is a set of tabs at the bottom. Click on each tab to view the result details:

- **Median**: The middle value in the distribution of data.
- **Count:** The number of gated events that fall within the specified bead region. (Bead count)

- **Header Info:** Such as instrument settings, experiment protocol specifications .etc.

### 2. Platemap View

Click on toolbar (Wizard step 2) to open the **Platemap Editor**. The Platemap View displays the sample name, analyte name with the concentration, standard curves, QC, positive and blank wells.

There are quantitative platemap and qualitative Platemap Editor views.

#### 2.1. Quantitative Analysis Platemap

Quantitative Analysis Platemap Editor View contains: **Standard Curve List, Well Information, Well Layout Field** and **Standard Curve Toolbar**.

**Standard Curve List:** Lists all the Standard wells in order with well name and dilution.

**Well Information:** Displays the Standard curve concentration / sample ID and sample dilution.
Well Layout Field: Displays the plate number (Click to view each plate) and well layout.

Standard Curve Toolbar: Vertical list of useful tools to define the platemap.

To edit Standard Curves, Sample Names and Analytes, click corresponding buttons at the bottom.

2.2. Qualitative Analysis Platemap

Qualitative Analysis Platemap Editor View contains: Well Information and Well Layout Field.

Well Information: Displays the well name and dilution of sample, control and background.

Well Layout field: Displays the plate number (Click to view each plate) and well layout of each plate

3. Result Data View

3.1. General View

MILLIPLEX Analyst provides visual representation of your results data thru the following views: 3D Result, Bar Chart, Curve, Sample Detail Data, Sample
EC50, Clustering, Curve Potency and Report.

3.1.1. 3D Result View

MILLIPLEX Analyst provides a 3D view of your data, highlight the desired data, and then click on the Result Toolbar, the 3D View window will be displayed as below:

![3D Result View](image)

Left click in the window and hold down the left mouse button, to drag the mouse to rotate the 3D object.

- Press and hold down "Shift", move the mouse up or down to zoom in or zoom out.

- Press and hold down "Ctrl", then click on 3D object, drag the mouse to move.

**Note:** Only one 3D window can be viewed at a time, if another group of data is selected to view 3D, the last 3D view will be closed.

3.1.2. Result Data Bar Chart View

Highlight the data and click on the Result Toolbar, the Bar Chart View Window will be displayed:
If the data value user has selected is higher than **Data High Limit** set in **Reportable Range Option** or maxDC, Y-axis in bar chart will display a mark named "Max["'+Analyte Name+'"] or maxDC and an arrow will be displayed on the top of the bar.

**Note:** **Data High Limit** or **maxDC** (the lowest value) will be used as the high limit to report the data on Y-axis.

If the data value user selected is lower than **Data Low Limit** set in **Reportable Range Option** or minDC, Y-axis in bar chart will display a mark named "Min["'+Analyte Name+'"] or minDC and the bar will start from under
the Y-axis.

**Note:** Data Low Limit and minDC (the lowest value) will be used to as the low limit and displayed on Y-axis.

The selected samples or analytes will be listed at the bottom (can be defined in Option: View|BarChart Group By).

The bars are linked with the data in Result PlateView: There will be a frame on the selected data and the analyte or sample that is currently viewed will be highlighted in the bar chart.

### 3.1.3. Curve View

Highlight one or more analytes in the Concentration Tab, then click in the Toolbar. The Curve(s) will be displayed:
Click on one analyte in the result PlateView to highlight its curve. There will be a black frame on the selected area of data in Result PlateView.

Definitions of curve fitting

There is a curve icon for each analyte and has the following definitions:

- Status: Good. The curve has not exceeded any of the QC thresholds
- Status: Warning. The curve has at least one threshold value outside of the expected range, but does not exceed twice the threshold value.
- Status: Poor. The curve has exceeded at least twice the threshold values.
- The curve fitting uses best fitting

3.1.4. Sample Detail Data View

Highlight one or a group of sample data and click on the Result Toolbar to display single sample data as below:
Sample detail data view shows each sample and displays sample mapping to the standard curve.

Click on CV, curve or R2 column to link the analyte; click one of the other columns to link the sample.

Definitions of curve fitting

There is a curve icon for each analyte and has the following meaning:

- --- Status: Good. The curve has not exceeded any of the QC thresholds
- --- Status: Warning. The curve has at least one QC value outside of the expected range, but does not exceed twice the threshold value.
- --- Status: Poor. The curve has exceeded at least twice the QC threshold.
- --- The curve fitting uses best fitting

### 3.1.5. Clustering Analysis

Clustering analysis is based on characterization of the data in order to separate data sets into different clusters or groups.

MILLIPLEX Analyst can classify MFI intensity data and the result
concentration data.

Click on the Plate Toolbar or Result Toolbar to open the Clustering Window for clustering analysis.

There are three sections: Toolbar, Analyte & Sample List, and Result & Data.

**Analyte & Sample List**: List all the analytes and samples of the selected data file.

**Result & Data**: Display data table with 2D data heatmap, and the clustering results data table and clustering heatmap.

**Note**: The data in Result & Data is linked to the data in PlateView, click either of them and the other will be highlighted.

**Data Heatmap**: Display the intensity MFI data or analyte concentration data of sample with the color map to illustrate the relationship of the values.
**Clustering**: Display the hierarchical clustering data. Clustering is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar (in some sense or another) to each other than to those in other clusters.

**Note**: After the clustering, click to generate the clustering summary report.

### 3.1.6. Report View

Select a report type from the drop down list of on the Main Toolbar to display all types of result data.

MILLPLEX Analyst exports report in Excel, PDF, and Word format.

There are five types of reports: **Detail Report**, **Bar Chart Report**, **Curve Report**, **Report by Analyte** and **Summary Report**. Here are some report samples:

**Note**: Qualitative analysis only has Summary Report.
MILLIPLEX Analyst provides various views of the result data in Quantitative and Qualitative Analysis.

3.2. Quantitative Analysis

The quantitative analysis result data is displayed in the Result PlateView window as shown below:
There are **Concentration**, **CV %**, **EC50**, **QC Report** and **Summary** tabs at the low left side of Result **PlateView** window. Click the tabs to switch to different result view.

### 3.2.1. QC Report View

Click **QC Report** tab at the left bottom of Result PlateView window.

The data table will be displayed as below:

![QC Report Table](image)

The report includes the following sections:
• **Low Bead Count**: List the sample name and Analyte ID with bead counts below the range defined in Reportable Range Options: Reportable Range|Low Bead Count.

• **High Bead CV(%)**: List the sample and Analyte ID that Bead CV exceeded the range defined in Reportable Range Options: Reportable Range|High Bead CV.

• **Hook Effect**: List the analyte ID that has a standard curve with a hook effect, indicating that the last standard value is smaller than the second to last standard value.

• **Bad Curve**: List the analyte ID when it’s standard curve exceeds curve fitting quality threshold.

• **Out of QC Range**: Values highlighted in RED indicate the data is above the upper limit; In BLACK indicate the data falls below the low limit; In BLUE indicate the data is in the range; And in GREEN indicate the data cannot be found in the QC range file.

### 3.2.2. Summary Result

Click the Summary tab at the left bottom of Result PlateView window.

Summary report table view will be displayed:

<table>
<thead>
<tr>
<th>File Name</th>
<th>E:\02-Millplex\Milliplexdata\output\output.csv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Count</td>
<td>1</td>
</tr>
<tr>
<td>Total Samples</td>
<td>30</td>
</tr>
<tr>
<td>Total Analytes</td>
<td>5</td>
</tr>
<tr>
<td>Low Bead Counts</td>
<td>0</td>
</tr>
<tr>
<td>High Bead CV(%)</td>
<td>80</td>
</tr>
<tr>
<td>Hook Effect Curves</td>
<td>0</td>
</tr>
<tr>
<td>Bad Curve</td>
<td>0</td>
</tr>
<tr>
<td>Out of QC Range</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Curve Summary

<table>
<thead>
<tr>
<th>Curve</th>
<th>Chi</th>
<th>CV</th>
<th>R²</th>
<th>MinDC</th>
<th>MaxDC</th>
<th>QC1</th>
<th>QC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>4.73%</td>
<td>0.23%</td>
<td>1</td>
<td>0.0017</td>
<td>13573</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>24</td>
<td>10.07%</td>
<td>0.45%</td>
<td>0.999</td>
<td>0.022</td>
<td>8995</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>25</td>
<td>7.19%</td>
<td>0.42%</td>
<td>0.999</td>
<td>0.040</td>
<td>11800</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>26</td>
<td>5.45%</td>
<td>0.43%</td>
<td>1</td>
<td>0.36</td>
<td>27567</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>30</td>
<td>2.21%</td>
<td>0.26%</td>
<td>1</td>
<td>0.030</td>
<td>44178</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
The summary report view includes:

1. **File Names**: The file and file path.
2. **Plate Count**: The plate count of the file.
3. **Total Samples**: The count of samples in all plates, include the standard wells.
4. **Total Analytes**: The count of analytes in all plates.
5. **Low Bead Counts**: The count of samples that is below the range set in the Reportable Range Options.
6. **High Bead CV(%)**: The count of samples that exceed the range set in the Reportable Range Options.
7. **Hook Effect Curves**: The total number of hook effect curves.
8. **Bad Curves**: The total number of bad curves.
9. **Out of QC Range**: The total number of samples that fall outside of QC Range.
10. **Curve Summary**: List of curve fitting parameters of all analyte standard curves.

### 3.2.3. Sample EC50

Click at the bottom of the Result PlateView window.

Sample EC50 report table is shown as blow.

<table>
<thead>
<tr>
<th>Sample</th>
<th>12</th>
<th>24</th>
<th>25</th>
<th>28</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A5)</td>
<td>0.38</td>
<td>0.32</td>
<td>0.09</td>
<td>0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

EC50 is the concentration of an analyte that induces an effect that is 50% of the maximum and the value is the EC50 point on the analyte standard curve.

**Note**: This view appears only if there is at least one Sample curve defined. Reference [Edit Sample Curves](#) to define a sample dilution curve.

### 3.2.4. Curve Potency View

Potency refers to a comparison of the required quantities of two substances, (such as drugs). For example, a standard (reference) drug and the amount of a
newer test drug that is needed to produce the same defined effect. The necessary quantity of the reference drug is divided by the necessary quantity of the test drug to arrive at the ratio of potency.

Parallelism is to determine if the two substances have a similar biological response.

To view Curve Potency:

1. Click on the Toolbar after quantitative analysis to open the Curve Potency dialog box.

   **Note:** If you open the Curve Potency dialog box by selecting View-Curve Potency from the Application Menu, you can select any saved .mfd file as Curve 1.

2. Choose the analytes as **Curve 1** on the left list field.

3. Click tab on the top toolbar, select a file directory or [Loaded Files] from the drop-down list, a result data files(*.mfd), or the curves in current analyzed file that are displayed under the drop-down list.

   **Note:** Curve 2 must follow all the principles as below:

   - Curve 2 must contain at least one analyte in Curve 1.
   - Each curve in Curve 2 cannot be a bad curve (The curve has exceeded at least twice the QC threshold).
• Curve 1 and Curve 2 can have different Standard Curve Fitting Model.
• Only supports 3 types of Standard Curve Fitting Model: Four-parameter curve fitting (log scale), Five-parameter curve fitting (linear scale), Five-parameter curve fitting (log scale).

4. Double click one of the items displayed in the list or select one and click to add them to Curve 2 field.

5. Click , the potency result data and curves are displayed blow.
Potency Curve:

- Green curve is Curve 1.
- Cyan curve is Curve 2, which is used as the reference curve for curve potency analysis on Curve 1.
- Yellow curve is the Potency Curve. The Potency Curve fits the Curve 1 data into Curve 2 using Curve 1 fitting parameters except for the IC50 value.

**Potency List:** displays potency data of all analytes.

- Potency: when the potency value is 1, the reference curve and the Potency Curve are identical. For example, if Potency >1, the potency curve is to the left of Curve 1, this means Curve 1 will need a lower amount of analytes to have the same effect as the reference.

- Parallelism: In the range of 0%-100%. The higher the value is the more reliable the potency is.

**Potency Data:** Displays the selected potency detail data comparing the green and yellow curves.

6. Click one data item, the corresponding curve graph is displayed on the left field.

7. Select the data check boxes and click to save potency summary report (*.xls) or click to montage the report results.
3.3. Qualitative Analysis

The qualitative analysis result data is displayed in the Result PlateView window as shown below:

![Result PlateView](image)

There are Normalized MFI, CV %, QC Report, Summary tabs. Click the tabs to switch to different result view.

3.3.1. Summary Result

Click Summary tab at the left bottom of Results View window. Summary report table will be displayed:

<table>
<thead>
<tr>
<th>File Name</th>
<th>E:\Multiplex\20220929_Mouse_Cytoplasm_HKSC CMv1.1-Multiplex\Output\Output5.png</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Count</td>
<td>1</td>
</tr>
<tr>
<td>Total Samples</td>
<td>48</td>
</tr>
<tr>
<td>Total Analytes</td>
<td>5</td>
</tr>
<tr>
<td>Low Bead Counts</td>
<td>0</td>
</tr>
<tr>
<td>High Replicate CV (%)</td>
<td>0</td>
</tr>
</tbody>
</table>

The summary includes File Name, Plate Count, Total Samples, Total Analytes, Low Bead Counts, High Replicate CV (%).
 SECTION 3 – APPENDIX

1. Use Large Icons

MILLIPLEX Analyst provides large icons feature to display Main Toolbar, Plate Toolbar and Result Toolbar.

Click on the Main Toolbar to switch normal sized icons on the Toolbar to large sized icons.

Click on the Main Toolbar to switch large sized icons to normal sized icons.

2. Open File

Select Application Menu: File|Open file|Quantitative... to open the following dialog box:
1. Select the csv files and click button to add them to the file list or double click the files to add.

2. Select the csv files in the list and click button to remove the file if needed.

3. Select one file and click or to adjust the plate order.

4. Click .

3. Flag Data

Flagging or marking out a data point will remove it from the analysis and it will be flagged like . Unflagging it will allow the data point to be included in the analysis it will be flagged like . Flagging can be done manually, or the software may automatically flag a data point that appears to be an outlier.

Flag data:

- Select one or a group of data, right click the data and select Flag from the popup menu, or
- Click on the Plate Toolbar or Result Toolbar, then left mouse click on the data.

Un-flag data:

- Select one or a group of data, right click the data and select Un-Flag from the popup menu

Cancel flag:

- Click on the Plate toolbar or Result toolbar, then right mouse click on the data.

4. View Multiple Plates

If multiple plates are opened, the plate number of each plate will be displayed on the top left of the Results PlateView as shown below:
Note: The plate number of current viewing is highlighted.

<table>
<thead>
<tr>
<th>Plates:</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>IL-2</td>
<td>IL-1ra</td>
<td>IL-4</td>
<td>IL-6</td>
<td>EGF</td>
</tr>
<tr>
<td>(A3,B3) QC1</td>
<td>2.47</td>
<td>20.69</td>
<td>&lt;0.04</td>
<td>222</td>
<td>54.11</td>
</tr>
<tr>
<td>(C3,D3) QC2</td>
<td>7.54</td>
<td>10.05</td>
<td>&lt;0.04</td>
<td>10.76</td>
<td>31.89</td>
</tr>
<tr>
<td>(E3) TE3</td>
<td>1.06</td>
<td>5.10</td>
<td>&lt;0.04</td>
<td>14.29</td>
<td>39.28</td>
</tr>
<tr>
<td>(F3) TF3</td>
<td>0.99</td>
<td>5.28</td>
<td>&lt;0.04</td>
<td>14.56</td>
<td>34.58</td>
</tr>
<tr>
<td>(G3) TG3</td>
<td>2.32</td>
<td>2.32</td>
<td>&lt;0.04</td>
<td>21.78</td>
<td>43.36</td>
</tr>
</tbody>
</table>

Use and button (Or and ) to select the plate when more than five files are opened, then click the plate number to view the plate data.

5. Add or Remove Standard Curves

- **Add Standards**

Click and select the well in Platemap Editor window, then click on the Standard Curve Toolbar to add the well to the standard curve list on the left.

- **Select a single well:** Click on the well on the right of the panel
- **Select multiple wells:** Hold down "Ctrl" button on the keyboard to move multiple standard wells on the panel.

- **Remove Standards**

Click and select the Standard well from the list in the Platemap Editor, then click on the Standard Toolbar to remove.

6. Edit Sample Curves

Select the sample wells which have the same name, right click the selected well (s) and select Edit Sample Curves from the well popup menu to open the dialog box as below:
Follow the steps below to edit Sample Curves:

1. Click the drop down list of the Name column and select one item.

2. Select Varied Category.

3. Enter Highest Value of sample concentration and dilution Serial Factor.

4. Click OK.

7. Regroup To Standard Curves

Click above the Standard Curve list in the Platemap Editor window or right click on the standard curve list, select Regroup To Standard Curves from the popup menu to add the selected well to another standard well as replicate wells.
8. Move Standard Curves

Click \[\text{Paste with Replicate}\] on the Standard Curve list or right click on the standard in Standard Curve list to show Standard Popup Menu. Select \text{Move Standard Curve} | \text{Move to STD1 at 1A1} for example.

![Standard Curve List and Popup Menu]

9. Paste with Replicate

In the Standard curve wells, you can paste the selected standard curves (standard name and standard well information) from the clipboard multiple times.

In the Sample wells, you can paste the selected samples (only the sample name) from the clipboard multiple times.

Steps:

1. Select one or multiple standard curve wells or sample wells you want to copy.

2. Copy them by selecting \text{Copy} from the right-click popup menu.

3. Right click the first well to paste and then select \text{Paste with Replicate} from the popup menu.

4. Type the number of times you want to paste and double click it.
10. Change Curve Fitting Model

You can select the curve fitting model from Option dialog box before performing an analysis. You can also change the curve fitting model at the result Curve View window.

Right click on an analyte (one row) in the curve detail list table in Curve View window, select a curve fitting model from the pop up list.

Note: The model with a check and bracket is the current curve fitting model.

11. Multiple Standard Curves Group Analysis

To analyze several assay groups in a single plate, please follow the steps:

1. Set up all the standard curves and sample wells

2. Flag all the standard and sample wells by un-checking in the **Well Information** and save to report.

3. Open Platemap Editor again, un-flag one group to perform analysis on while excluding the other groups.

4. Repeat until all the groups have been analyzed.
5. Define the **Dilution Fold** if the sample is diluted.

6. Check **Name Vertically** to define the sample names vertically.

**12. QC Range Editor**

Select **Analysis|QC Range...** to open the file browser to load in a Quantitative QC Range (*.qcn or *.pdf) file. Then QC Range Editor will pop up as below:

**Note:** MILLPLEX Analyst only supports the specified .pdf format converted from the QC template. Please refer to \C:\VigeneTech\MILLIPLEX Analyst\Limit\MILLIPLEX Analyst QC Template File Guide.doc.

---

### Change analyte:

1. Select an analyte in the list.

2. Change the Name, Lower limit, Upper limit and Unit.

3. Click **Save** to save.

### Add analyte:

1. Define the Name, Lower limit, Upper limit and Unit for the new analyte.
2. Click [Add] to add the new analyte to the list.

3. Click [Save] to save.

Or just select a QC range data outside MILLIPLEX Analyst, and click [Paste] to add.

**Remove analyte:**

1. Select an analyte in the list.

2. Click [Remove] to remove the selected analyte from the list.

3. Click [Save] to save.

### 13. Save and Load Protocol

The protocol (*protocol.xml*) file contains the platemap and option settings.

- **Save Protocol**

  Click on the Main Toolbar or select **File|Save File|Protocol...** to save the protocol file.

- **Load Protocol**

  The saved protocol file can be loaded in both the Quantitative Analysis and Qualitative Analysis at Quick Start Wizard step 1. After a protocol has been loaded, you can proceed with the Quick Start process to analyze the file.

### 14. Save and Load Status

The status (*mpsx*) file contains the csv file, the manually flagged information, platemap, option settings and protocol.

- **Save Status**
Select **File/Save Status *.mpsx...** to save the status (*.mpsx) file.

- **Load Status**

Select **File/Load Status *.mpsx...** to load in the status (*.mpsx) file.

And the raw data with saved manually flag information will be displayed in Raw Data PlateView.

### 15. File Format

<table>
<thead>
<tr>
<th>Data Type</th>
<th>File Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result data (include EC50)</td>
<td>.mfd</td>
</tr>
<tr>
<td>Platemap</td>
<td>-platemap.mfd</td>
</tr>
<tr>
<td>Protocol</td>
<td>.protocol.xml</td>
</tr>
<tr>
<td>Status File</td>
<td>.mpsx</td>
</tr>
<tr>
<td>Clustering summary</td>
<td>.xls</td>
</tr>
<tr>
<td>Clustering report</td>
<td>.xls, .pdf, .doc</td>
</tr>
<tr>
<td>Potency summary report</td>
<td>.xls</td>
</tr>
<tr>
<td>Customized format setting file</td>
<td>.format.xml</td>
</tr>
<tr>
<td>QC range file</td>
<td>.qcn</td>
</tr>
<tr>
<td>Sample Curve Report (EC50)</td>
<td>.SampleCurve.xls, .SampleCurve.pdf, .SampleCurve.doc</td>
</tr>
</tbody>
</table>
Contacting EMD Millipore

For any technical support, product information and MILLIPLEX Analyst Standard Version and Advanced Version, please contact Millipore customer support and technical support.

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Telephone      1-866-441-8400 (Toll Free USA)
                1-636-441-8400 (Outside USA)
Fax             1-636-441-8050

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