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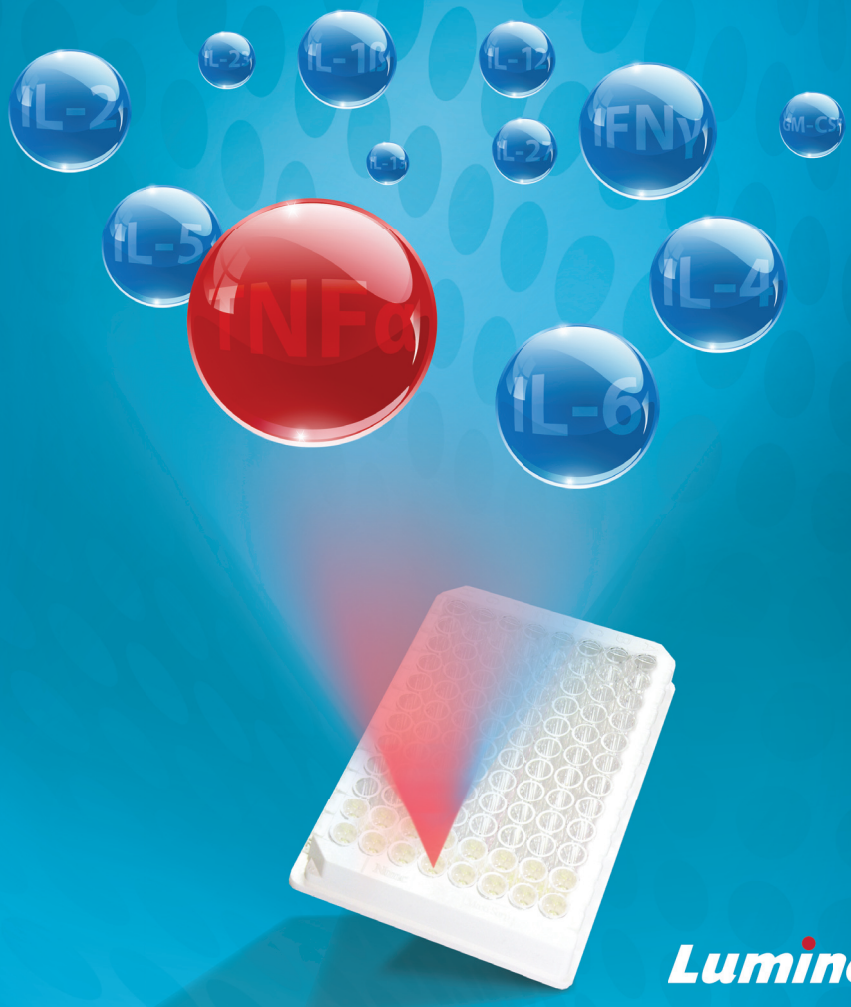
ProcartaPlex™ Analyst 1.0



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ProcartaPlex Analyst 1.0 Software

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System Requirements

The ProcartaPlex Analyst 1.0 software has been validated for use on PC computers running Windows 7 or higher, as well as for the use on Mac running OSX 10.9 or higher.

ProcartaPlex Analyst 1.0 Software License

You are granted a non-exclusive, non-transferable limited license without license fees to use this software to analyze and evaluate ProcartaPlex™ experiments using eBioscience products. You are not allowed to redistribute this software. This software contains third party components with additional licenses that apply to these components. A list of third party components can be found in the file components.txt in the directory "licenses" in the application directory. All referenced licenses can be found in that same directory.

Software Installation

The Software is complementary and can be downloaded from the website:

http://www.ebioscience.com/resources/procartaplex-analyst-1.0-software.htm

Luminex File Format

ProcartaPlex Analyst 1.0 is compatible only with comma separated value (*.csv) files generated by the Luminex system. The csv file is generated after the plate has been read on the Luminex Instrument and contains the MFI values of your standards and samples. Upload the original, not previously opened *.csv file, into ProcartaPlex Analyst 1.0. Here are two methods to generate a *.csv file from either a Bioplex or an xPonent system.

Bioplex Software 6.1

Under "File" select "Document Export Properties" => Select Output .csv format and click "Ok"

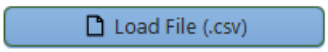
Under "File" select "Document Export" and "Save"

xPONENT Software + IS2.3 Software

The *.csv file is automatically saved in the Output folder on the desktop

ProcartaPlex Analyst 1.0 software was tested wit both xPONENT 4.2 and xPONENT 3.1 software versions.

Importing a CSV file

Click on Load File (.csv) 

Select the directory where the file is saved, highlight the .csv file and press the open button. Wells containing fluorescent (Fl) information will change their colors from dark grey to light grey. Wells with low bead count (>20) will be highlighted with an exclamation point.

Note: Each plate must be saved as a separate file. Import of files containing data of multiple plates can not be read by the software.

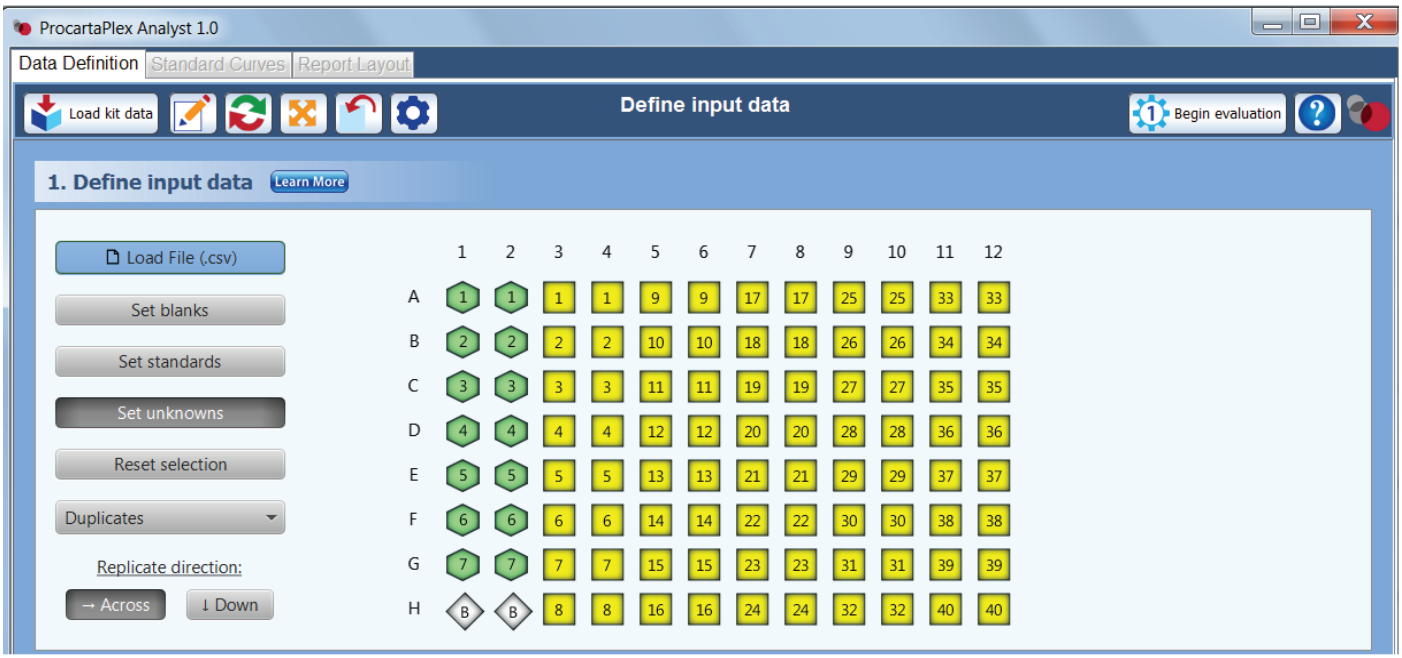
Tool Bar

Clicking on the "edit annotation"  button in the top left corner will bring up a pop up window to allow the creation of notes for the experiment in a notepad format. It allows the user to enter additional information, which can be viewed and printed in the final report. The notes or annotations can also be imported or exported as *.txt file. By selecting the "Restart ProcartaPlex Analyst" button  , the program will restart and bring you back to the previous page, but the *.csv file will not be removed. The "Reset" button  will reset the page to the original state and the previously loaded *.csv file will also be lost. The "Import Previous Evaluation"button  will reload the setting file (*.xml) created previously in the report section. The *.xml file saves your analysis along with all settings and statistics. Clicking the "Edit customized kits" button  in the top left corner will bring up a window to create customized kit data files. The kit data files will be used to set the starting dilution for the standard curve.

Defining the Plate Map

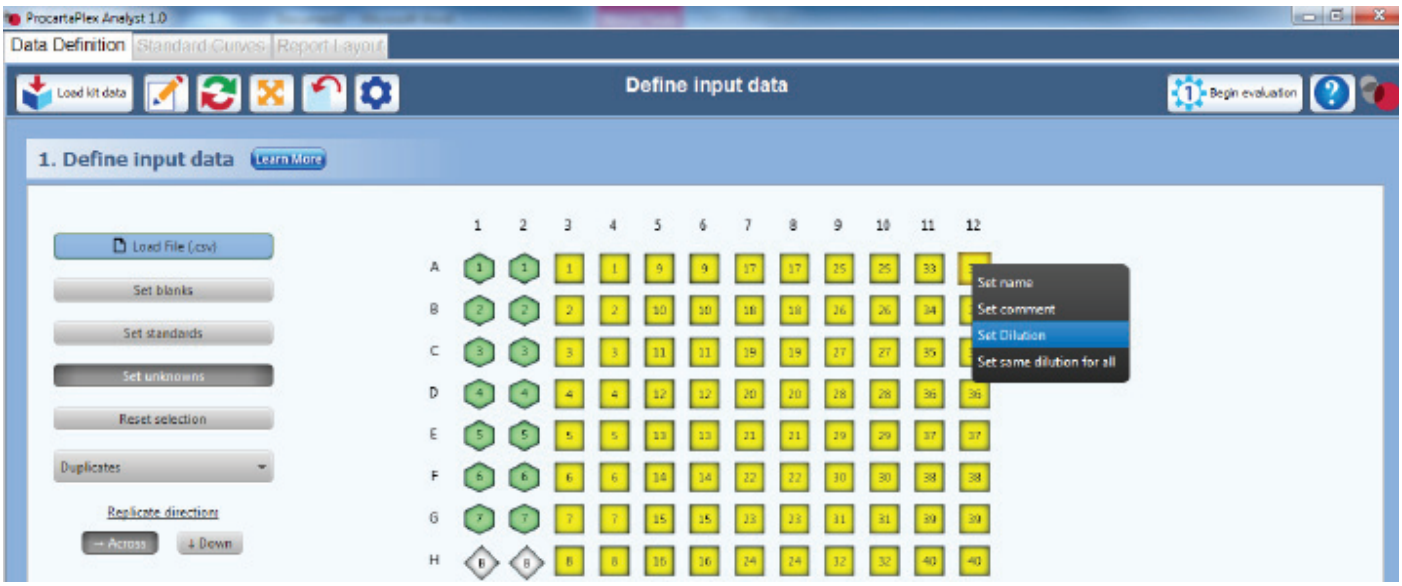
First specify the number of replicates (singlicates, duplicates, triplicates). Select the replicate direction by clicking on "Across" for replicate wells that are identical from left to right or select "Down" for replicate wells that are numbered from top to bottom, then left to right.

Select the "set blanks" button then click and drag a selection box over the blank wells. Select the "Set standards" button then click and drag a selection box over the standard wells. Please note that the maximum number of standards is 7 standard points. Select the "set unknowns" button then click and drag a selection box over the unknown wells. To clear the plate map, select the "Reset selection" button and click and highlight the wells to be reset.

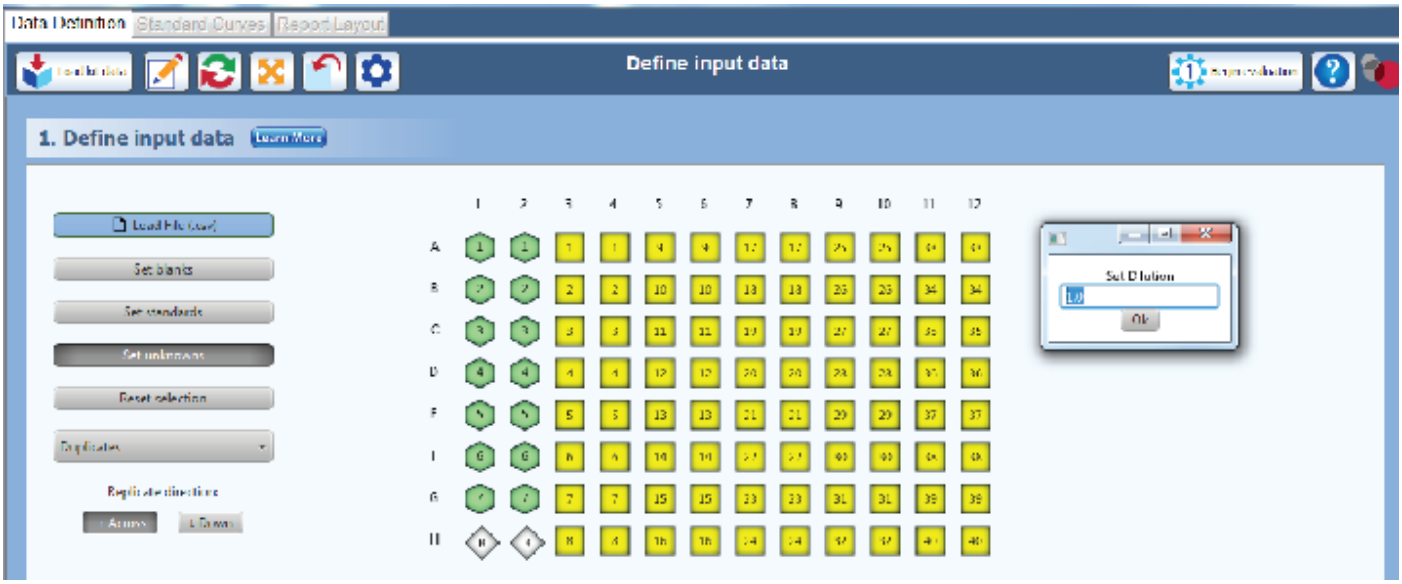


Set up of Sample Pre-dilution

First mark the sample that requires a higher dilution factor. After right mouse click a window with additional settings will appear. Choose the option "Set Dilution".

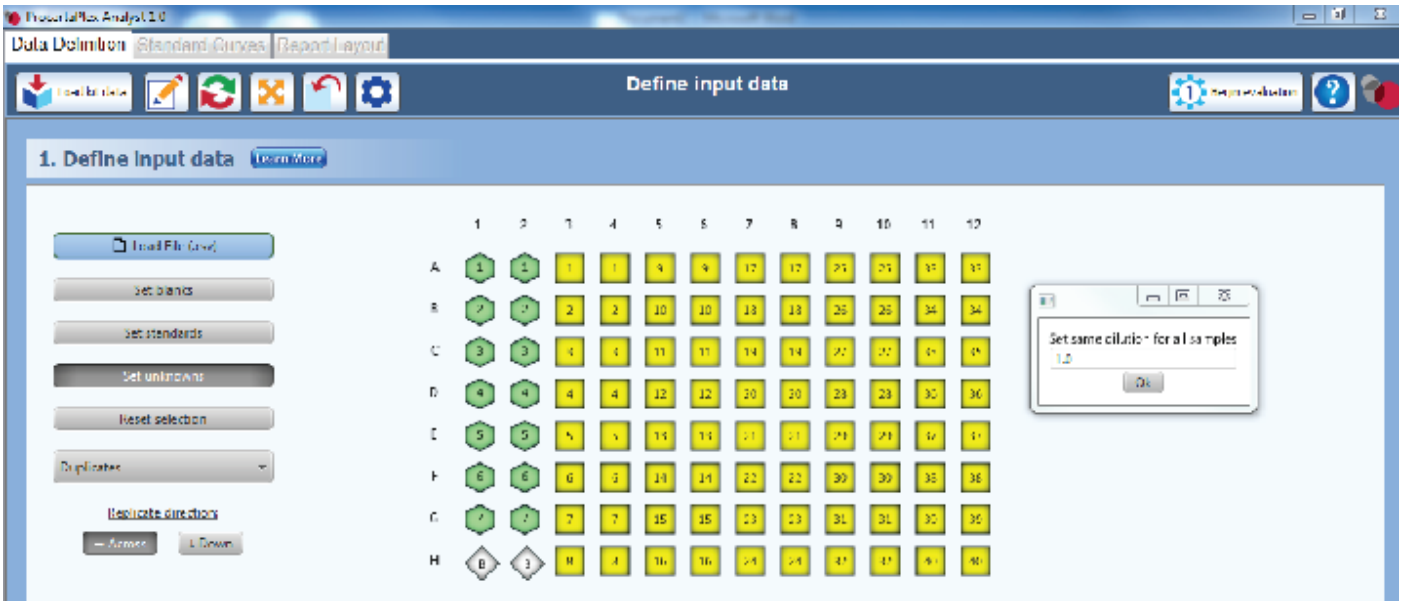


Please enter the recommended sample dilution into the filed “Set Dilution”.



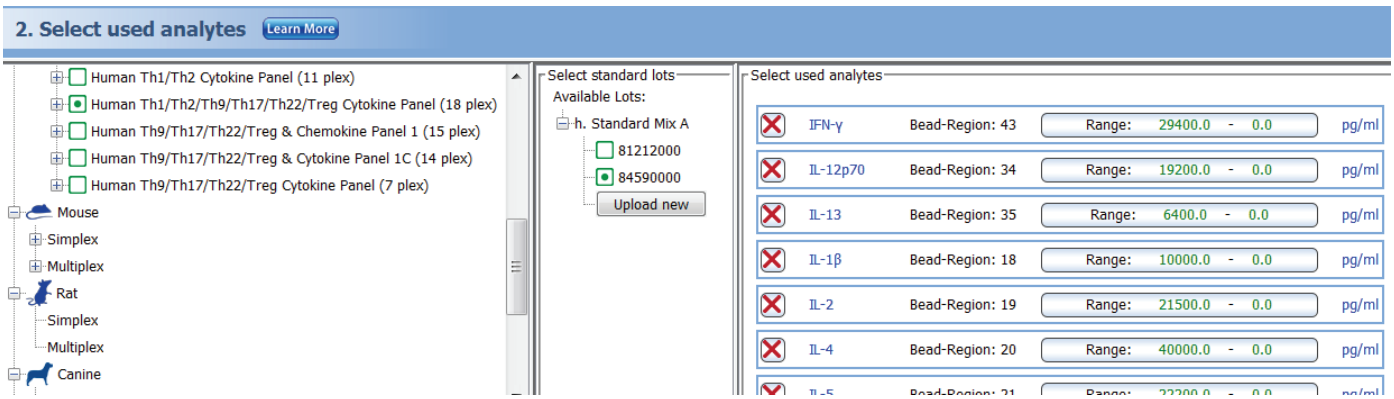
The Software provides an option to set up the same dilution factor for all samples. Therefore click on any of the samples defined in your Plate Map and click right mouse button. It appears the same window as above. Please choose the option “Set same dilution for all”.

Please enter the used sample dilution factor into the filed “Set same dilution for all samples”.



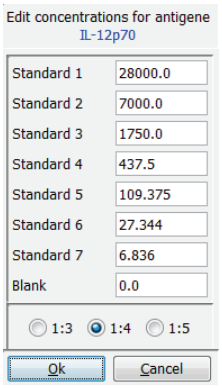
Select Used Analytes

Select the appropriate ProcartaPlex Kit Panel(s) or Simplex Kits that were used in your experiment by clicking the appropriate box in the bottom left window. Once the Multiplex Panels or combination of Simplex Kits click have been selected, the available analytes will then appear in the right half of the screen. Some of the analytes cannot be measured together, because they use the same bead set. The program will give a warning message if an improper combination is selected. Refer to the certificate of analysis to confirm the standard lots that were used during the experiment and select the appropriate Standard Mix.

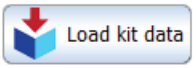


Select the standard lot(s) used in your assay and the software will load concentration and dilution information associated with this lot. To manually change the standard range for a certain analyte, click the box "Range: xxx – 0" 

A new window will appear, containing the default 1:4 dilution of the seven standard points. Enter in new values to manually change the standard.

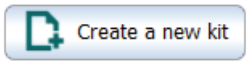


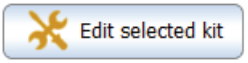
Import New Kit and Standard Lot Data

If your kit/lot is not listed in the software, the kit/lot data can be downloaded from http://www.ebioscience.com/resources/procartaplex-analyst-1.0-software.htm. Import downloaded kit data by using the "Load kit data" button 

in the top left corner. Import downloaded lot data by using "Upload New" button in the combo box "Available Lots" in the bottom middle window.

Creating Customized Kit Data

To create customized kit data, click on the "Edit customized kits"  button in the top left corner to bring up a window to create customized kit data files. Click "Create a new kit" 

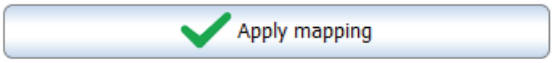
and enter a name for your customized kit. Select the desired analytes from the drop down menu and specify the bead population (see Certificate of Analysis). The standard panel associated with the selected analyte will be added automatically. If additional analytes are needed, select the "Edit Selected Kit" button 

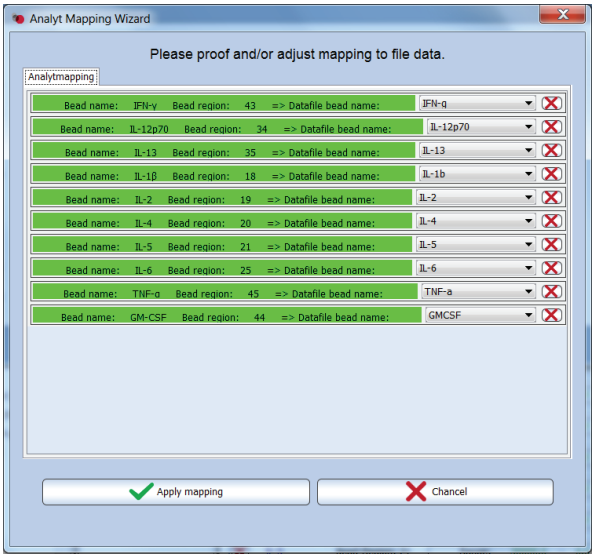
and then add or subtract analytes using the " + or -" buttons 

After adding or deleting the analytes, changes can be saved or discarded 

Begin Evaluation

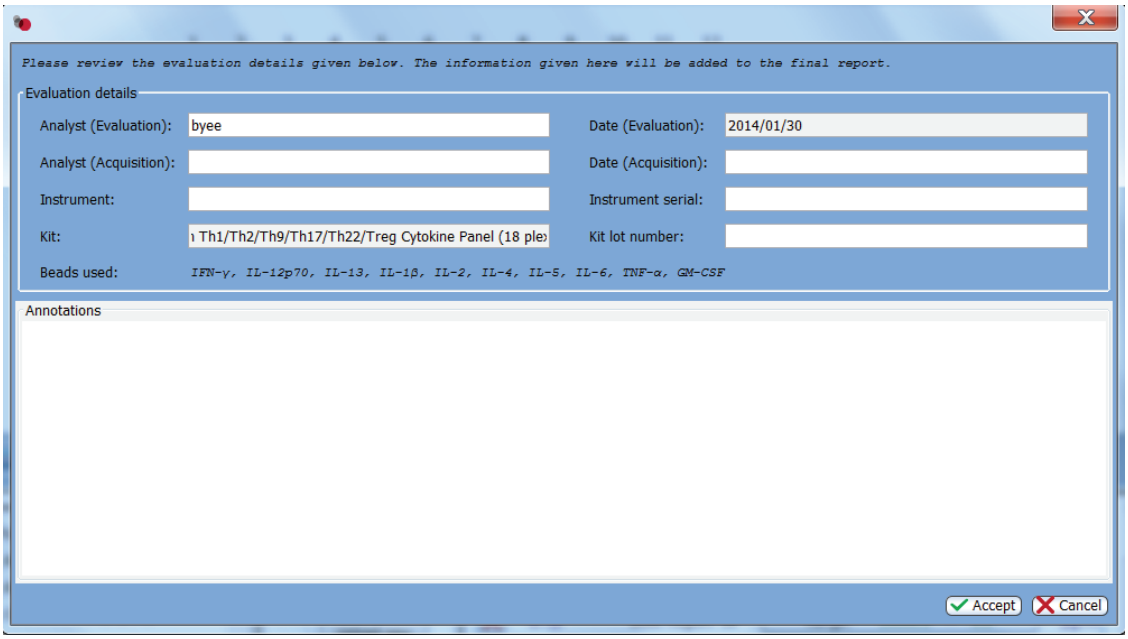
Select the "Begin evaluation" button 

to apply the bead map. An Analyst Mapping Wizard will appear and the software will match the analyte information from the loaded .csv file with the analytes of the selected kit data files. If an analyte is not properly mapped, the field will be highlighted in red. Select the correct analyte from the drop down menu and then click "Apply mapping" 



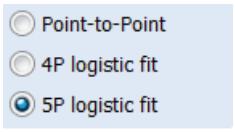
Evaluation Details

A window with evaluation details will appear once the analytes are mapped. Experimental information can be enterd in this window and can be printed with the final report.



Curve Fitting Analysis

The program offers three interpolation models:



By selecting **5P logistic (5PL) fit**, the software program will generate a curve using the 5 parameter equation

$$y = d + [(a - d)/(1 + (x/c)^b)g]$$

By selecting **4P logistic (4PL) fit**, the software generate a curve using the 4 parameter equation

$$y = d + [(a - d)/(1 + (x/c)^b)]$$

5PL/4PL: The parameter of the x-axis is concentration (in pg/ml), the parameter of the y-axis is given as MFI/MFI of blank * 100. The MFI (Median Fluorescence Intensity) of each standard point is blank-corrected by division of the Blank-MFI (B_B0 = MFI/MFI of Blank*100). The average curve fit of the mathematical model is stated in the top left of the screen (in %).

Point-to-Point: The program will interpolate between two adjacent points using a line (y=ax+b). The parameter of the x-axis is concentration (in pg/ml), the parameter of the y-axis is given as MFI. The MFI of each standard point is blank-corrected by subtraction of the Blank-MFI (MFI-MFI_Blank).

The software allows the user to change the axis labels from either a linear or logarithmic presentation. This can be done by selecting either “Linear axis” or “Logarithmic axis”.

The program automatically sets the Cut-off at 30% Bias. The Bias displays the variation from the ideal standard curve defined by the theoretical standard concentrations. The Cut-off defines the maximum acceptable Bias. However, if this value is not suitable for your particular application, adjust the Cut-off using the corresponding button on the right (see below).

The LLOQ (Lower Limit of Quantitation) and ULOQ (Upper Limit of Quantitation) are the highest and lowest standard curve points that can still be used for quantification. The calculation of LLOQ and ULOQ is based on the maximum acceptable Bias. Sample concentrations below LLOQ and above ULOQ, respectively, will later be marked red in the final report.

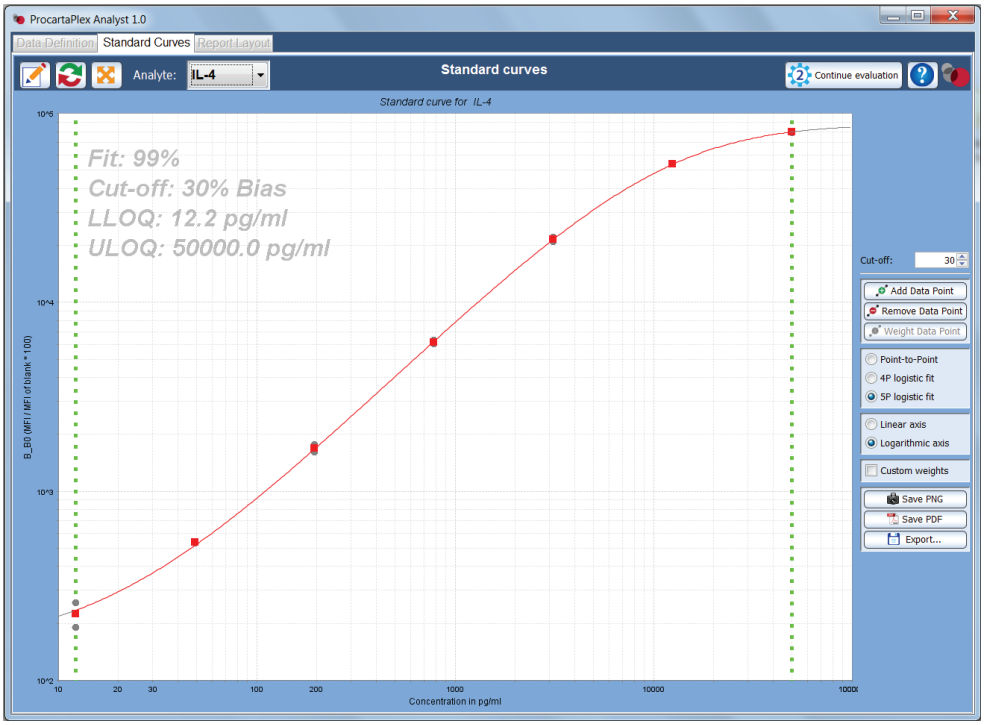
If you select a logistic curve fitting method (Logistic-5PL or Logistic-4PL), the software automatically weights the points in the curve. To change the weight for a certain standard point select “Custom weights”. Then click “Weight Data Point” and click on the data point you want to adjust. Enter a new value and press Ok. The software will automatically re-calculate the curve with the new weighting.

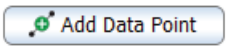
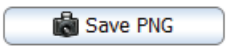
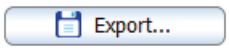
This new weighting has impact on the whole standard curve and will be applied to all analytes unless only one analyte is activated. The weighting will be added to the final report.

Note: The change of weighting of one standard point has an impact on the whole standard curve.

Standard Curve

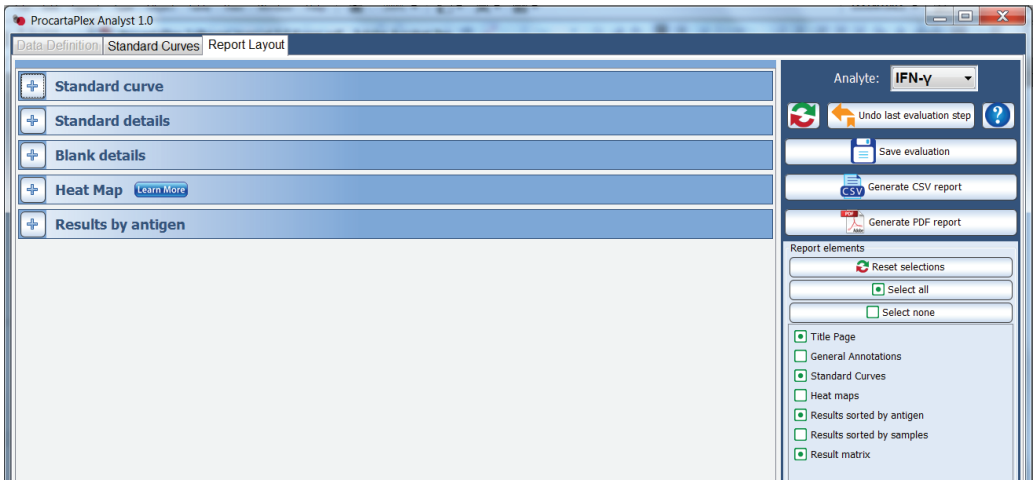
The standard curve for each analyte can be viewed on the standard curves tab. A standard curve created from a 7 point standard curve with the standards processed in duplicate is shown below.



To see the standard curves for the other analytes, select the desired analyte in the drop down menu at the top of the window. In the menu on the right side of the screen, data points from the standard curve can be removed by clicking on the "Remove Data Point"  button and then clicking on the desired data point to be removed. If the standard curve is created from the mean of duplicate or triplicate values, selecting the "Remove Data Point" button and then clicking the red square will remove the standard point from analysis and the replicates will be displayed as a blue dot. If the "Remove Data Point" button is selected and the outlier dot is selected, the dot will turn to a yellow triangle and that dot will not be included in the analysis. By selecting the "Add Data Point" button  and clicking on the blue excluded data point, the analysis will use the remaining point which is then reinserted in the standard curve. It is possible to save an image of the standard curve as a *.png file by selecting "Save PNG"  from the menu. The "Reset" button  will delete all changes for the standards. The "Restart" button will bring the analysis back to the start of the evaluation on Standard selection page, but all processing and calculations done so far will be lost. To save all settings (standard files used, curve fitting parameters, exclusion of data points from the analysis etc.) for later analysis, click the "Export" button  . A pop up menu will appear with the experiment details. To confirm, select "ok" then select the directory of the .xml file and save it. If all standard curve data is satisfactory press "Continue Evaluation" to proceed to the next page.

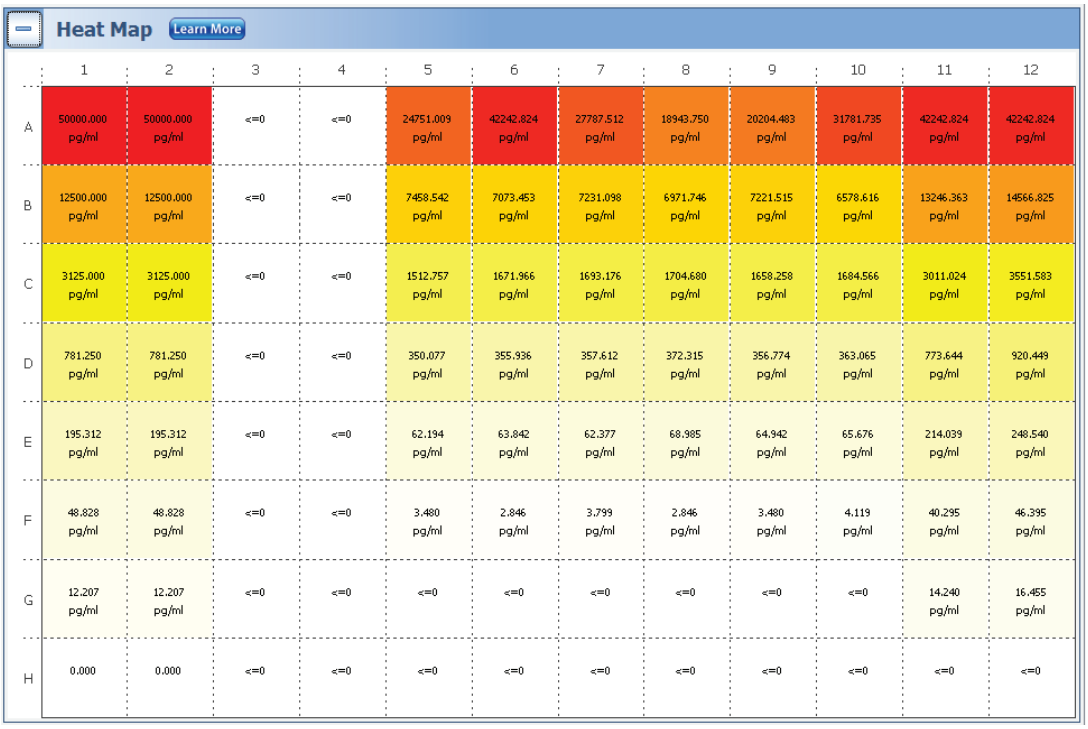
Report Layout

The results of each measured analyte for each analyzed sample are shown on this page. To preview a detailed description of each analyte, select the analyte in the upper right hand corner from the drop down menu next to the desired analyte.



Heat Map

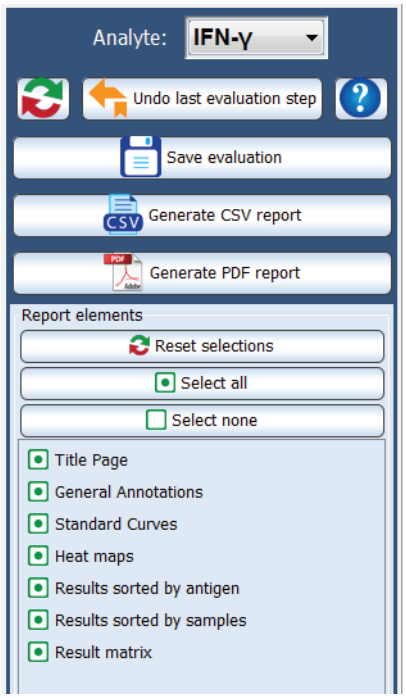
The heat map shows results in a 96 well plate format highlighting concentrations in distinct shades of color from yellow, orange and red. Lower values are represented by white/yellow color and higher values by orange/red color. Use the drop down menu at the top of the software to switch between the different analytes.

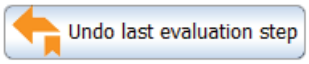


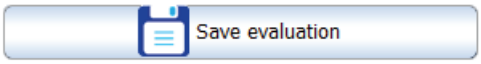
	1	2	3	4	5	6	7	8	9	10	11	12
A	50000.000 pg/ml	50000.000 pg/ml	<=0	<=0	24751.009 pg/ml	42242.824 pg/ml	27787.512 pg/ml	18943.750 pg/ml	20204.483 pg/ml	31781.735 pg/ml	42242.824 pg/ml	42242.824 pg/ml
B	12500.000 pg/ml	12500.000 pg/ml	<=0	<=0	7458.542 pg/ml	7073.453 pg/ml	7231.098 pg/ml	6971.746 pg/ml	7221.515 pg/ml	6578.616 pg/ml	13246.363 pg/ml	14566.825 pg/ml
C	3125.000 pg/ml	3125.000 pg/ml	<=0	<=0	1512.757 pg/ml	1671.966 pg/ml	1693.176 pg/ml	1704.680 pg/ml	1658.258 pg/ml	1684.566 pg/ml	3011.024 pg/ml	3551.583 pg/ml
D	781.250 pg/ml	781.250 pg/ml	<=0	<=0	350.077 pg/ml	355.936 pg/ml	357.612 pg/ml	372.315 pg/ml	356.774 pg/ml	363.065 pg/ml	773.644 pg/ml	920.449 pg/ml
E	195.312 pg/ml	195.312 pg/ml	<=0	<=0	62.194 pg/ml	63.842 pg/ml	62.377 pg/ml	68.985 pg/ml	64.942 pg/ml	65.676 pg/ml	214.039 pg/ml	248.540 pg/ml
F	48.828 pg/ml	48.828 pg/ml	<=0	<=0	3.480 pg/ml	2.846 pg/ml	3.799 pg/ml	2.846 pg/ml	3.480 pg/ml	4.119 pg/ml	40.295 pg/ml	46.395 pg/ml
G	12.207 pg/ml	12.207 pg/ml	<=0	<=0	<=0	<=0	<=0	<=0	<=0	<=0	14.240 pg/ml	16.455 pg/ml
H	0.000	0.000	<=0	<=0	<=0	<=0	<=0	<=0	<=0	<=0	<=0	<=0

Note: If a dilution factor is entered all samples data may be colored in red.

Report Tools

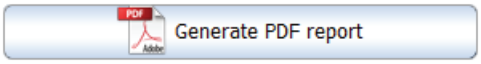


This page will enable the creation of a *.pdf, *.csv and *.xml file of your results. The "Undo last evaluation step" 

button will return the user back to the "Standard Curves" page. The "Restart"  button will return the user back to the start of the evaluation on Standard selection page, but all processing and calculations done so far will be lost. To save analysis (standard and sample files, settings, standard curves and calculation), click the "Save Evaluation" button 

in the right task bar. The pop up menu will provide experiment details. Confirm with "ok" select the directory of the *.xml file and save it.

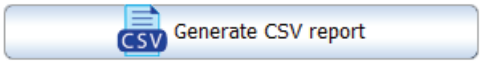
Generating a PDF file

The PDF Report contains the most extensive information. Customize your individual pdf report by selecting the individual parts that can be added. Select the contents of your report in the bottom right half of the screen by clicking the square checkboxes. After selecting the contents of your report, click the "Generate PDF Report button" 

Enter a file name and click "Save". The Standard Curves will be displayed in the report as well as the measured Fluorescence Intensity in a table together with the matching concentrations of the analytes. The Back calculation concentration and the Back calculation bias provide an additional control. The program back fits the standard values from the standard curve. Values highlighted in red lie outside your chosen cut-off range and should therefore be regarded as not significant. However, values indicated as <=0 or overflow, respectively, are marked red as well, because they are situated beyond the detection limits of the kit. Results calculated from duplicate (triplicate) determinations the following values are given in a table:

MFI 1, MFI 2, MFI 3:	Values of the measured Fluorescence Intensities
Back Calc Conc. in pg/ml (MFI):	The back calculated concentrations of the analyte
CV in % (Back Calc Conc.):	The coefficient of variation of the back calculated concentrations of the analyte
B_B0 (=MFI/MFI_Blank*100):	The MFI of each standard point is blank corrected by division of the Blank-MFI
Back Calc Conc. in pg/ml (Mean MFI):	see text above
Back Calculation Bias:	Difference between backcalculated and target (expected) concentration

Generating a *.csv file

Select the "Generate CSV Report" button 

to generate a *.csv file that can be later opened in a program such as Microsoft Excel. Select the report type (plate format or 384 well plate), tuple separator character, and text wrapper character followed by selecting a directory and file name. The *.csv file will generate a table with the experimental results, showing the concentration and mean fluorescence intensity of each analyte for each analyzed sample. The report will also provide the raw MFI data of each analyte in a 96 well plate format. Use Microsoft Excel to open and work with the *.csv-File. Check country settings (comma, semicolon) on your operating system and excel for a correct export of the *.csv. Save evaluation.

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