# Universal Antibody Titration Template with Integrated Stain Index Calculations for FCS Express 7

## Overview

The instructions below will guide users of the Universal Antibody Titration Template(s) in FCS Express 7. The template is designed to allow loading and analysis of data from any instrument for use with any parameter, including raw data spectral detector parameters from Cytek Aurora systems. When opening the layout, users will be presented with a "start-up script wizard" to select data files and parameters to use in the analysis which may also be dismissed for a manual set up by pressing cancel. The steps below outline manual setup and other details on layout usage. Please make sure to read the additional information in the Titration Layout Information page provided in the layout.

With the manual set up and instructions provided in this document, users will be instructed to load data into the data list, organize the data by titration points, change parameters on plots to adjust for parameter order difference, and replace gates to match gates to the correct parameters.

With both wizard and manual methods, stain index metrics for both classical stain index and the 84$^{th}$ percentile methods will automatically by graphed and regressed while providing the antibody amount to use at 90% binding. Data can be exported with "1 click" via the Batch actions.

The instructions below make use of a 7 point titration data set. Additional variations of the template with more or less titration points can be accessed at on the Staining Index Page of the De Novo Software website.

Please contact support@denovosoftware.com with any questions or for assistance using the template.

**Note – if you are using Raw mixed data files from the Cytek Auora, please make sure to update your FCS Express instrument specific settings to allow loading of the Raw detector channels. See the end of the document for information on updating Cytek Instrument Specific settings.*

## Manual Setup Instructions:

1.) Launch FCS Express and open the template .fey file from the **File** tab→**Open**.

2.) Add data files to the data list by clicking on the blue plus icon (*Figure 1 – red box*) or by dragging the data files into the data list.

3.) Ensure data files in the data list are ordered from the lowest to highest titration concentrations.

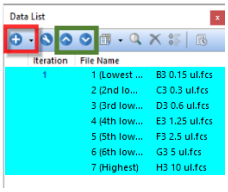



*Figure 1 - Order the data list with the lowest titration at the top and the highest at the bottom.*

**Note - If data files need to be reordered in the data list the up and down arrows can be used (Figure 1 - green box) or files can be dragged to the appropriate place in the list.*

4.) Multiple select all plots in the column of plots at the left by drawing a selection area around the plots (much like selecting multiple objects in PowerPoint) (*Figure 2– Green Border*).
*(Note: Steps 4 and 5 only required if correct parameters are not displayed on the plot)*

*Figure 2 - Completed Titration Layout. Green border; plots used to choose scatter parameters, orange border; plots used to choose negative and positive staining populations, blue border; plots displaying negative staining population and used only to determine 84th percentile calculations.*

5.) Click on the **Format** tab→**Overlays** icon. In the **Overlays** format window change the **X** and **Y parameter**s to desired parameters to display and gate for the "Scatter" population of interest (*Figure 3*).
**Notes – typing while the dropdown menu is open will facilitate selecting parameters.*

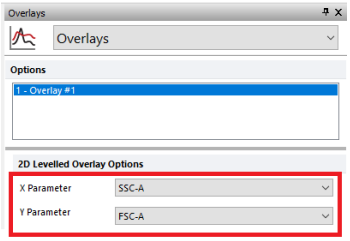



*Figure 3 - Formatting parameters for Scatter Plots in Overlays dialog.*

6.) Depending on your data files parameter order and range of data, the default Scatter gate may or may not appear in the column of plots on the left.
   a. If a Scatter gate <u>is not shown</u> on the plot:
      i. Select the **Gating** tab→choose the desired gate type→draw a new Scatter gate on the upper left plot (*Figure 2 – Plot 1*).
      ii. In the **Create New Gate** window select **Replace existing gate**→choose "**Scatter**" from the dropdown list→click **OK** (*Figure 4*).
   b. If a Scatter gate <u>is shown</u> on the plot continue to the next step.

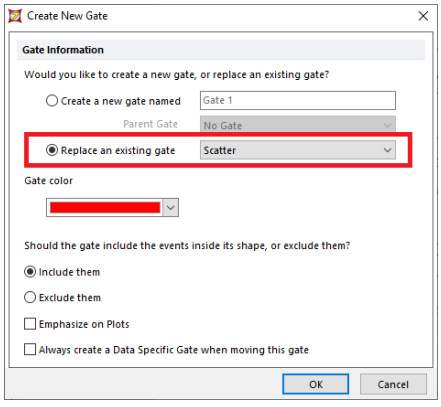



*Figure 4 - Replacing the Scatter gate.*

7.) Adjust "Scatter" gate as needed.

**Note* – Holding down the shift key while moving the gate will allow you to create Data Specific Gates for each data file if needed.

8.) Multiple select all plots in the middle and right columns of plots (Figure 2 – middle column orange border and right column blue border).

9.) In the **Overlays** format window (see step 5) change the **X parameter** to the channel for titration measurements to define negative and positive populations and change the **Y parameter** to the Side Scatter parameter used in step 5 (*example shown in Figure 5*).

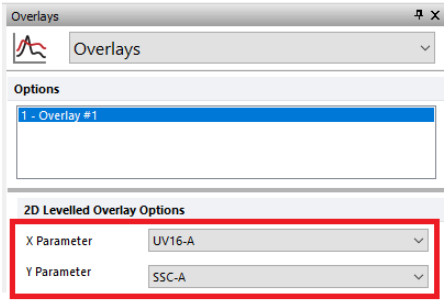



*Figure 5 - Formatting parameters for Staining Plots in Overlays dialog.*

10.) Repeat step 6 to view, replace, and adjust the "**Negative Staining**" and "**Positive Staining**" gates that are set up by default in the layout using the top plot from the middle column in the layout (*Figure 2 - Plot 2 and Figure 6*). Do not use the right column of plots which is reserved for 84th percentile calculations.

**Note – Holding down the shift key while moving the gate will allow you to create Data Specific Gates for each data file if needed.*

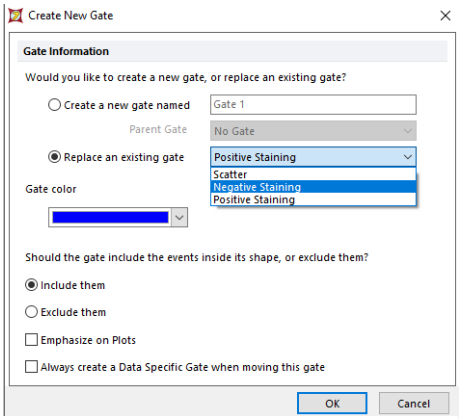
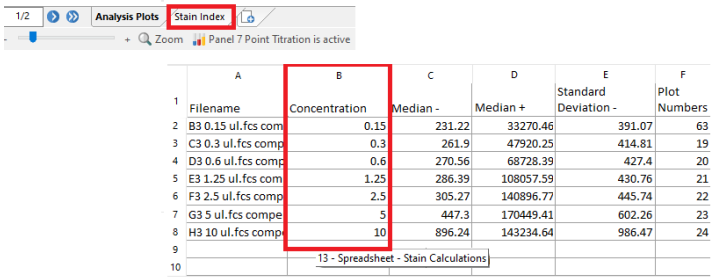



*Figure 6 - Replacing Negative and Positive Staining gates.*

11.) Update the **Concentration** in Column B in the spreadsheet to reflect experimental concentrations at the bottom of the "**Stain Index**" page in the template (*Figure 7*).

Data, plot, parameter, and gate setup is now complete (*As seen in Figure 2*). The statistic spreadsheets and regression plots located in the **Stain Index** page tab will automatically update as changes to plots/gates are made in the template.




| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Filename | Concentration | Median - | Median + | Standard Deviation - | Plot Numbers |
| 2 | B3 0.15 ul.fcs com | 0.15 | 231.22 | 33270.46 | 391.07 | 63 |
| 3 | C3 0.3 ul.fcs comp | 0.3 | 261.9 | 47920.25 | 414.81 | 19 |
| 4 | D3 0.6 ul.fcs comp | 0.6 | 270.56 | 68728.39 | 427.4 | 20 |
| 5 | E3 1.25 ul.fcs com | 1.25 | 286.39 | 108057.59 | 430.76 | 21 |
| 6 | F3 2.5 ul.fcs comp | 2.5 | 305.27 | 140896.77 | 445.74 | 22 |
| 7 | G3 5 ul.fcs compe | 5 | 447.3 | 170449.41 | 602.26 | 23 |
| 8 | H3 10 ul.fcs comp | 10 | 896.24 | 143234.64 | 986.47 | 24 |
| 9 | | | | | | |
| 10 | | | | | | |

*Figure 7 – Adjust the concentration column on theStain Index Page Spreadsheet as needed.*

## To quickly export results:

12.) Click on the **Batch** tab→**Batch Actions.** Double click on each action and confirm the location for exports to be saved for any actions selected is correct (*Figure 8*).

13.) Click on the **Batch** tab→**Run** to proceed with **Batch processing** which will export the results to PDF and save as a new layout.

14.) Select the spreadsheet reporting "Filename" in cell A1 at the bottom of the "**Stain Index**" page and choose the **Layout** tab now available at the top of the workspace (*Figure 9*).

15.) Click on the **Export** icon→define the location to be saved to/file name→click **Save**. This action will export the results of the FCS Express spreadsheet to Excel.

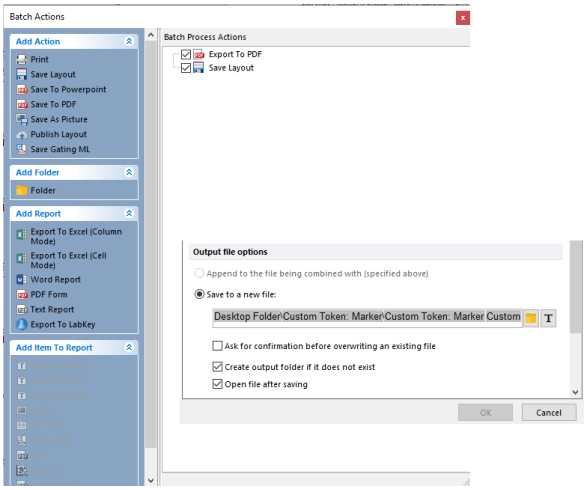



*Figure 8 - Batch Process Actions window and partial view of action window to display output file options.*

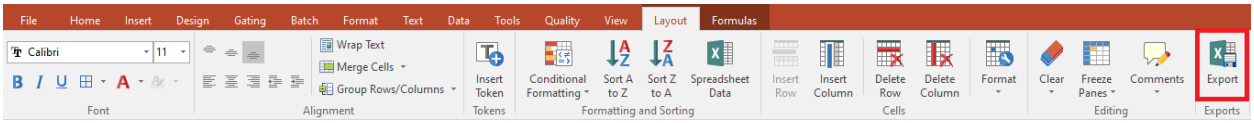



*Figure 9 - Layout tab and export icon to export spreadsheet from Stain Index page*

## Setting Up Cytek Instrument Specific Settings

If you are using Raw mixed data files from the Cytek Auora, please make sure to update your FCS Express instrument specific settings (*Figure 10*) to allow loading of the Raw detector channels as follows:

1. Click on the **File** tab→**Options** to open the FCS Express User Options.
2. Expand **Data Loading→FCS File Options→Instrument Specific Settings**.
3. Select **Cytek – SpectroFlo** from the listed instruments under **Instrument Specific Settings**.
4. Confirm that the checkbox next to **Delete fluorescence parameters after spectra merge is <u>unchecked</u>**.

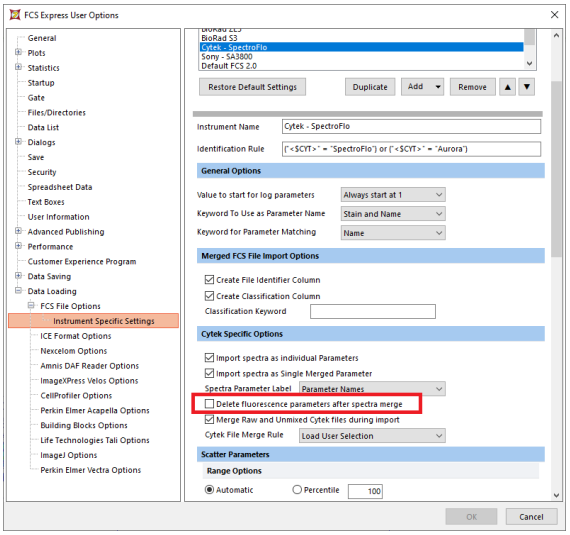



*Figure 10 - Adjusting Instrument Specific Settings for Cytek data files to allow loading of raw paramaters.*

Please reach out to support@denovosoftware.com with any questions.