

Setup for Z'-LYTE® Assay on Molecular Devices FilterMax™ F5 Reader with SoftMax® Pro 6.1 Software

The Molecular Devices FilterMax F5 Multi-Mode Plate Reader was tested for compatibility with Life Technologies' Z'-LYTE® assays using the Z'-LYTE® Tyr6 kit (PV4122) against JAK2 JH1/JH2 kinase. The following document is intended to demonstrate setup of this instrument.

For more detailed information and technical support of Life Technologies assays, please call 1-800-955-6288 and enter extension 40266 or email drugdiscoverytech@lifetech.com.

For more detailed information and technical support of Molecular Devices instruments or software, please contact Molecular Devices at 1-800-635-5577 or www.moleculardevices.com.

Setup Guide on the Molecular Devices FilterMax™ F5 Multi-Mode Microplate Reader

A. Recommended Optics

P/N	Filter information
6590-0048	FilterMax™ Fluorescence Excitation Filter 405 nm (bandwidth 30 nm)
6590-0056	FilterMax™ Fluorescence Excitation Filter 465 nm (bandwidth 35 nm)
6590-0057	FilterMax™ Fluorescence Excitation Filter 535 nm (bandwidth 25 nm)

Note: Ex 405/30 and Em 465/35 filters do not ship with the F5. Em 535/25 does ship with F5 as part of default Emission Slide 1, but because it is not recommended to make changes to the default slide configuration, users may wish to order a separate 535-nm filter.

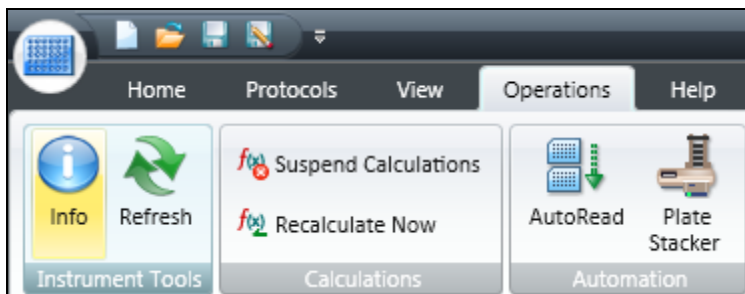
Users will need to obtain additional filter slides in which they can install the above filters:

P/N	Slide information
5008588	FilterMax™ Custom Fluorescence Excitation Slide (#21) Empty slider can be configured for all Methods except FP.
5008589	FilterMax™ Custom Fluorescence Emission Slide (#21) Empty slider can be configured for all Methods except FP.

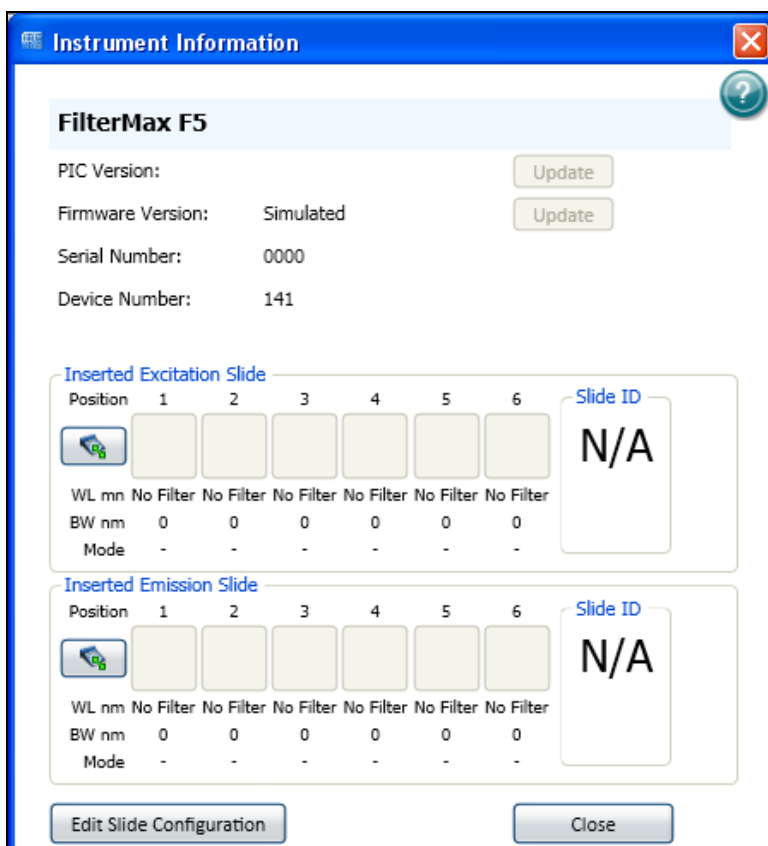
B. Instrument Setup:

Configuration of filter slides in SoftMax® Pro software

1. Open SoftMax® Pro software. In the Operations tab, click "Info":

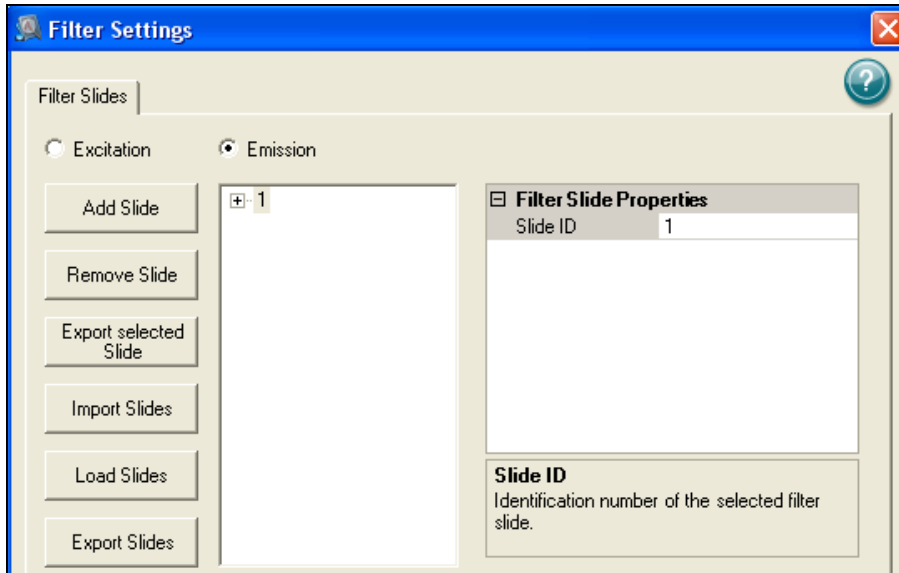


This opens the Instrument Information window:

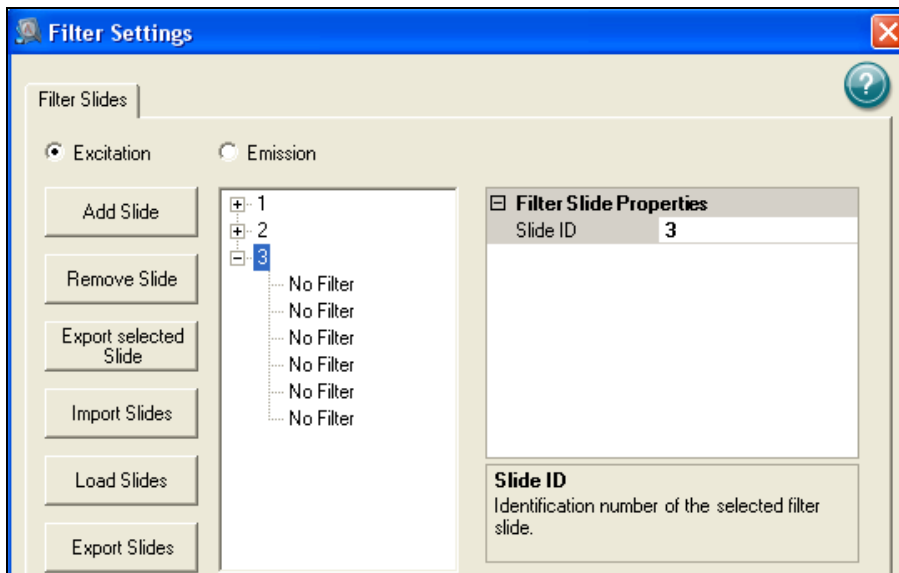


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- Click "Edit Slide Configuration". This opens the Filter Settings window:

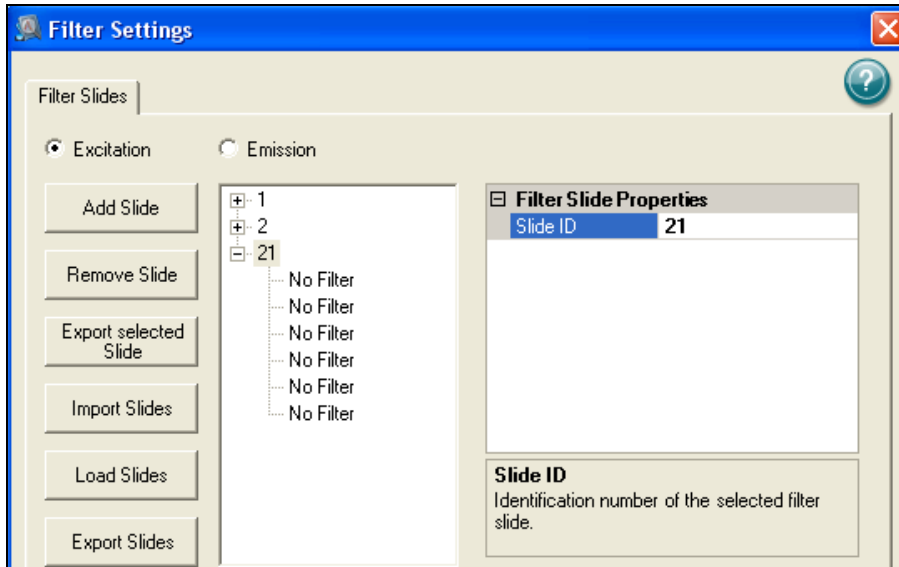


- Click the radio button next to "Excitation" and click "Add Slide".

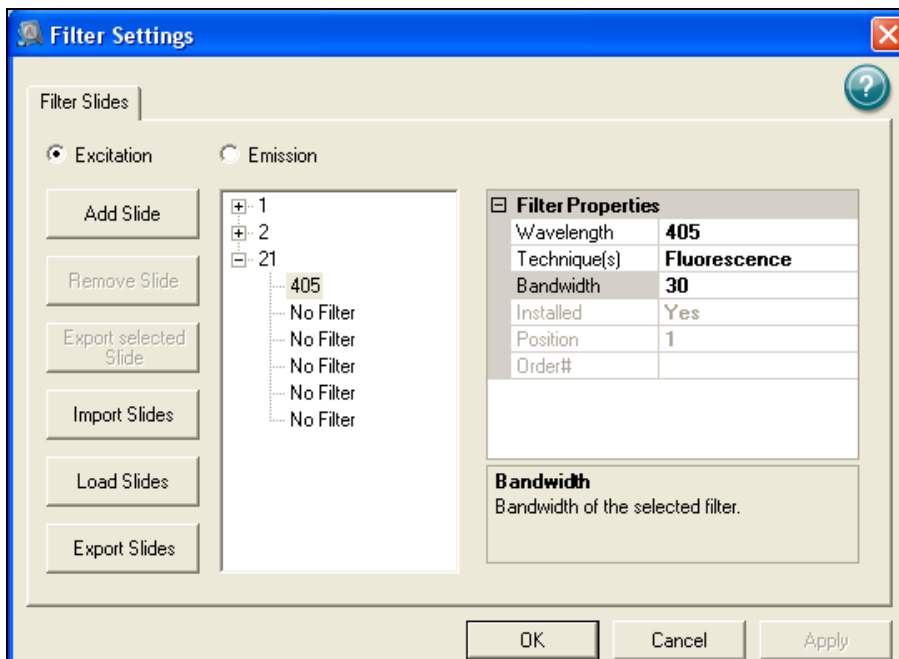


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4. Under Filter Slide Properties, type "21" next to Slide ID.

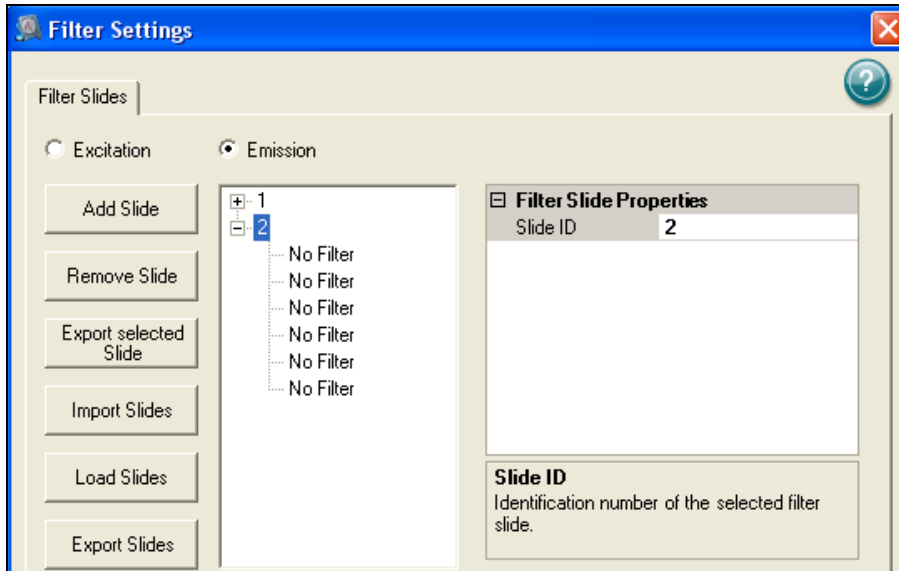


5. In the list below the slide number 21, select the position where the excitation filter is installed and enter the appropriate values under "Filter Properties". Click Apply to save.

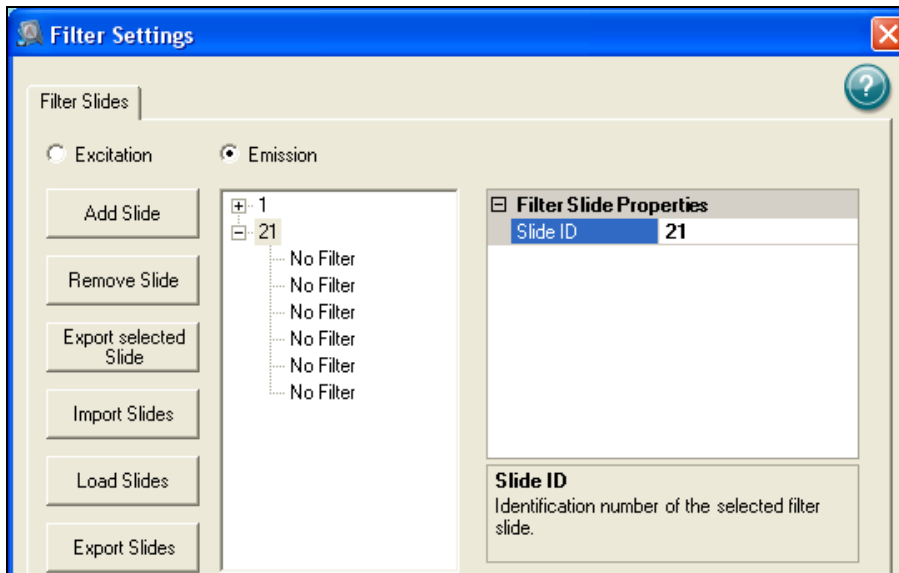


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- Click the radio button next to "Emission" and click "Add Slide"

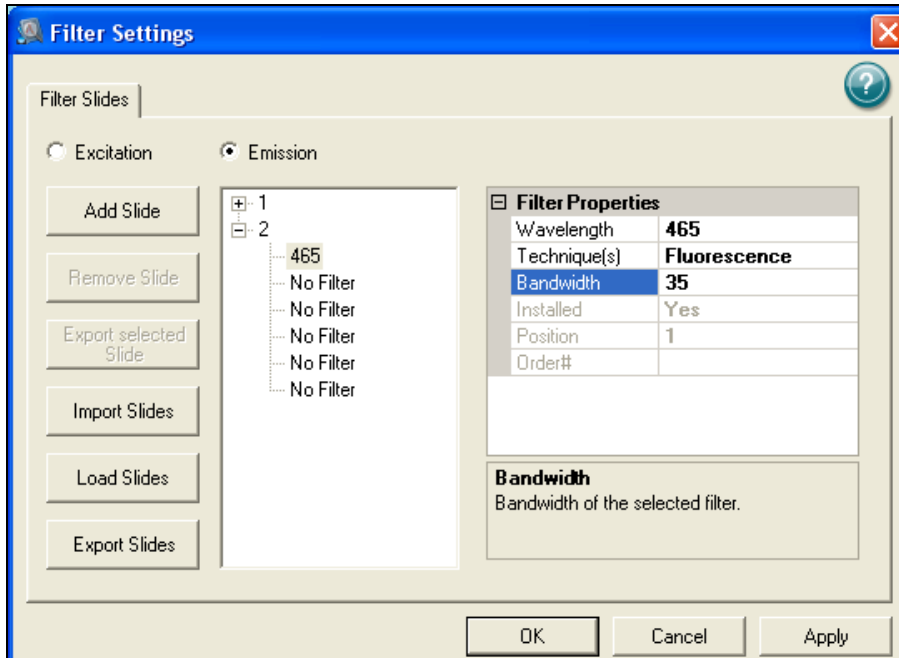


- Under Filter Slide Properties, type "21" next to Slide ID.



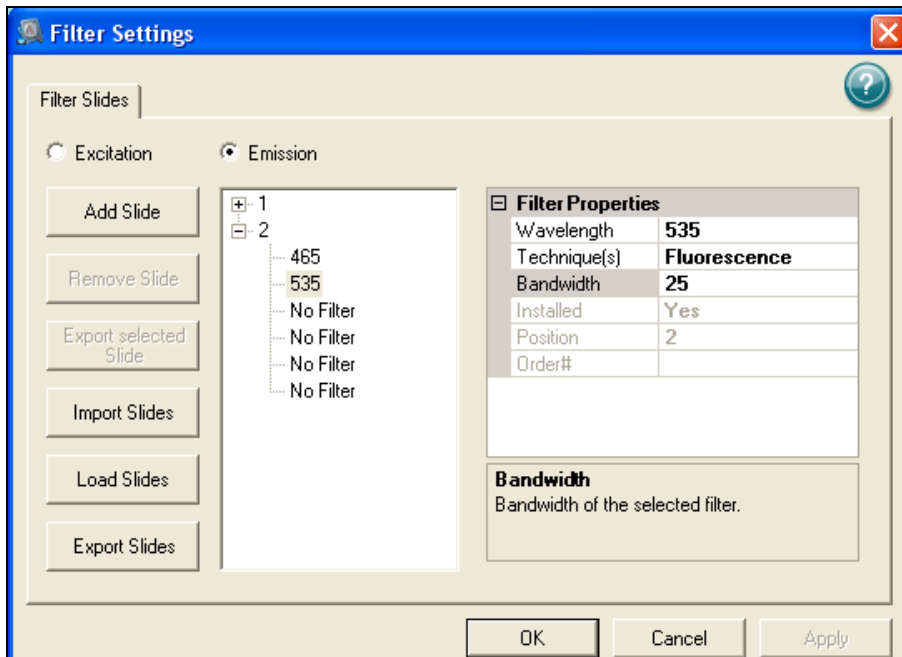
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8. In the list below the slide number 21, select the positions where the emission filters are installed and enter the appropriate values under "Filter Properties". Click Apply to save.



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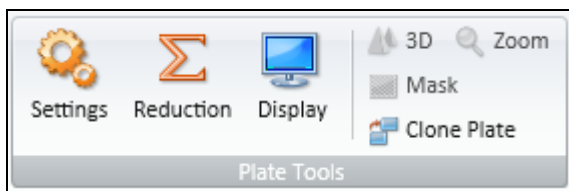
9. Repeat for second emission filter.



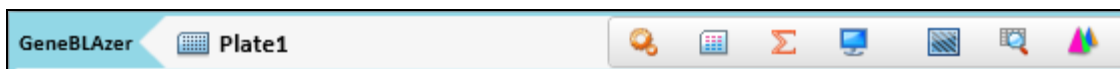
10. When finished, click Okay to exit the configuration, and then click Close to exit Instrument Information.

Instrument settings

11. Click on the microplate icon in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen or in the plate section header.

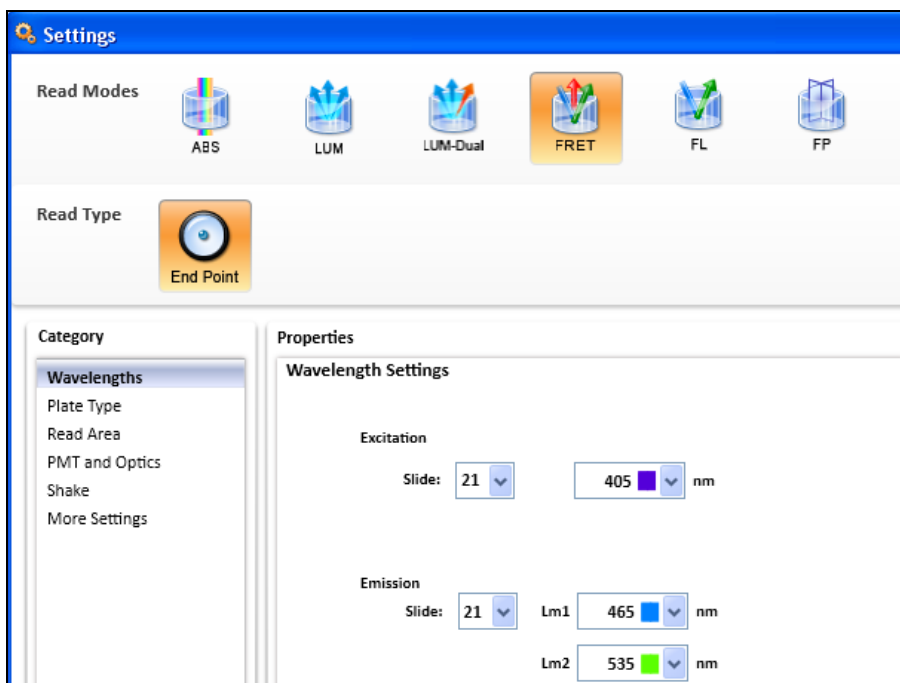


Or

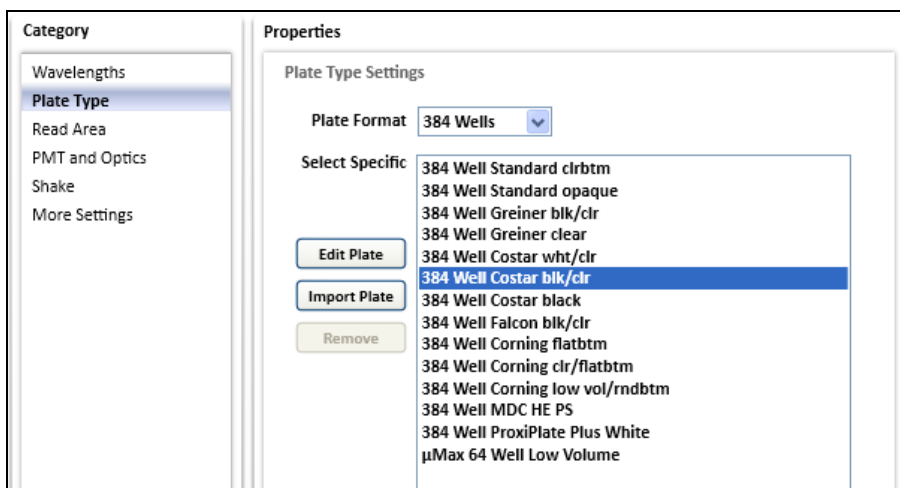


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12. Select the FRET read mode and End Point read type. Select the appropriate excitation and emission slides, and select the wavelengths as shown below.



13. Choose the desired plate type, using the upper dropdown menu to choose plate format (96 or 384 wells) and the "Select Specific" menu to choose the specific plate type.



Have a question?

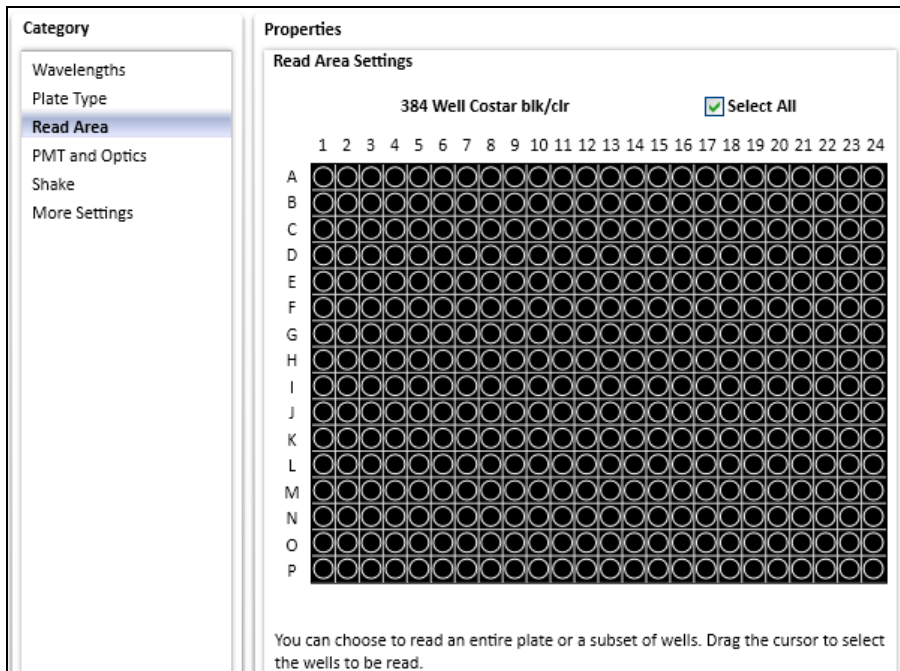
NA: 800-955-6288 or INTL: 760-603-7200 ext. 40266

Contact our Technical Support Team

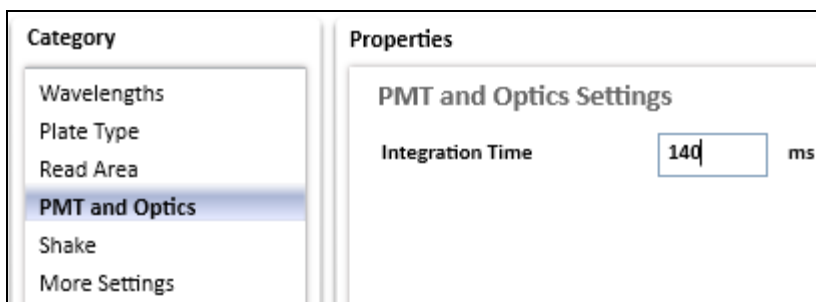
Email: drugdiscoverytech@lifetech.com

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14. Now choose the area of the plate to read.



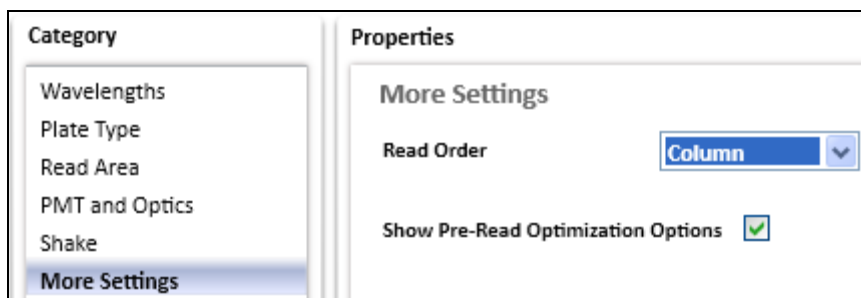
15. In the PMT and Optics Settings enter the desired integration time. Shorter integration times enable faster reading, while longer integration times enable better performance.



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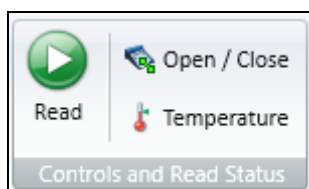
16. In "More Settings", choose the read order corresponding to how the assay plate is set up. If the entire plate is to be read, choose "Row". If entire rows of a partial plate are to be read, choose "Row"; if entire columns of a partial plate are to be read, choose "Column".

Check the box "Show Pre-Read Optimization Options" to enable the Microplate Optimization and Read Height Adjustment options upon initiation of the plate read.



Click OK to close the Settings window.

17. To read the plate, click the green "Read" button at the top of the screen.

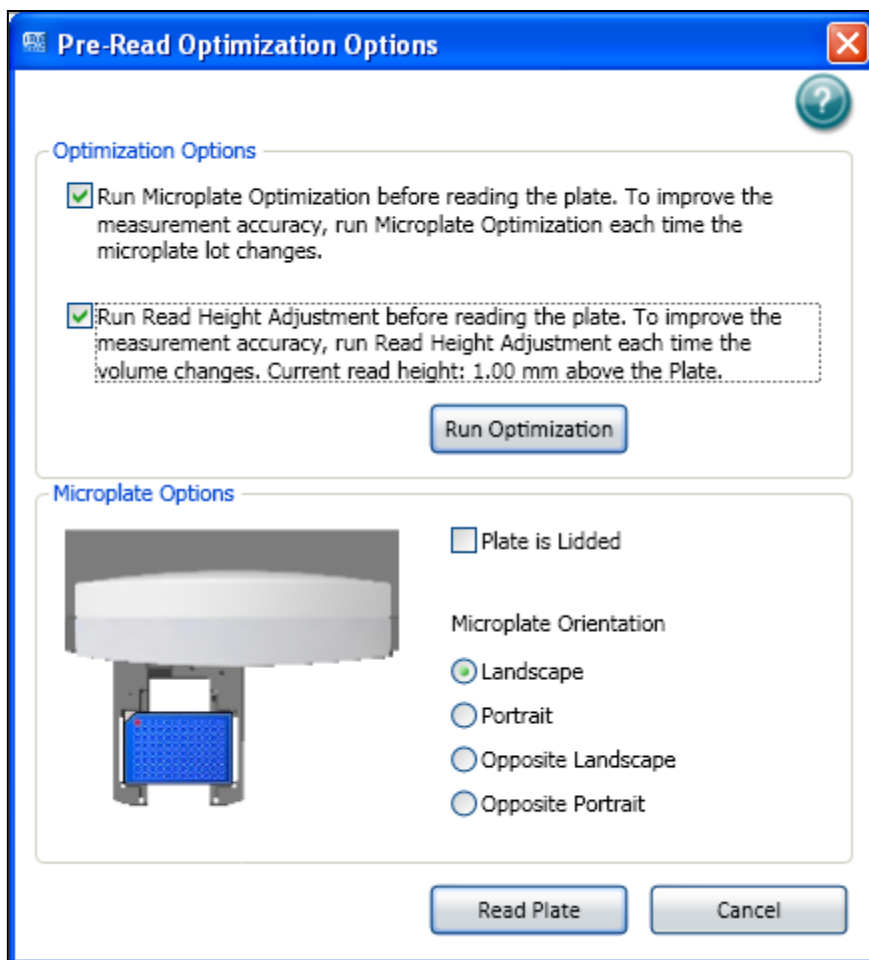


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18. If selected, pre-read optimization options will appear.

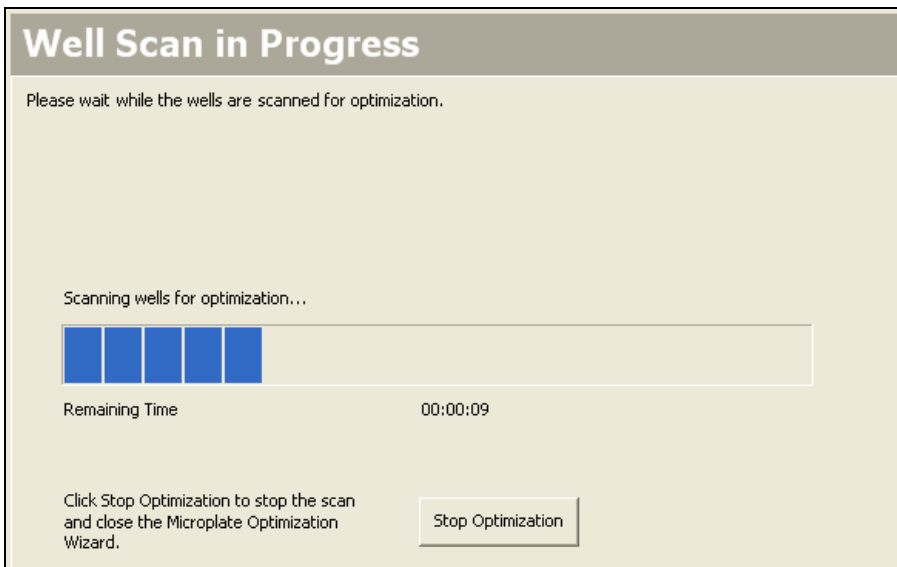
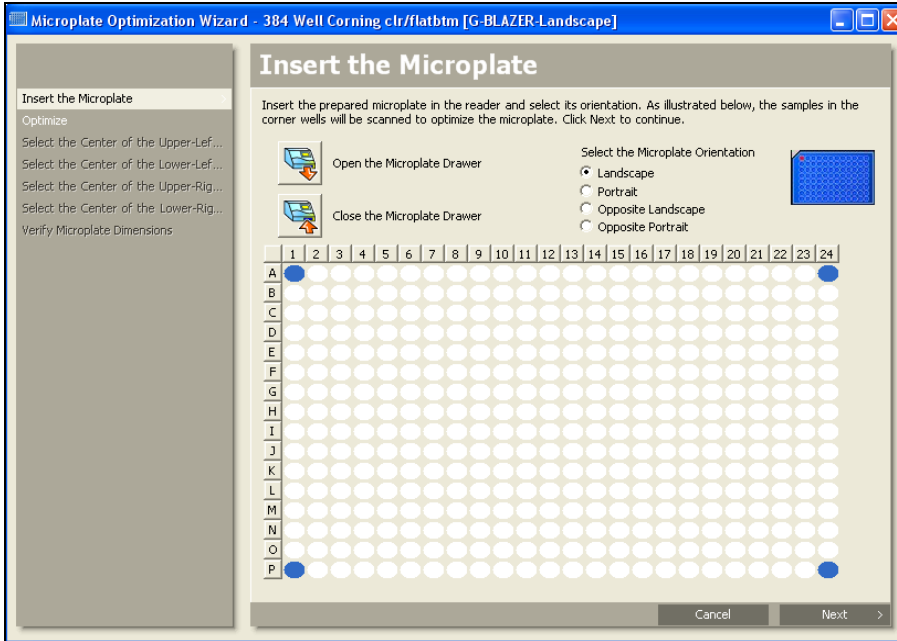
- Microplate Optimization scans the four corner wells of the plate and adjusts the microplate dimensions if necessary to improve accuracy. It requires that all four corners of the microplate contain detectable fluorescent material (i.e. positive control samples).
- Read Height Adjustment determines the height above the plate at which the best signal is detected. It can be performed using any well in the plate with a relatively strong fluorescent signal (i.e. positive control sample).
- If the plate is lidded, check the box. Make sure that the selected microplate orientation matches the orientation of the actual assay plate.

Click "Run Optimization" to proceed. Alternatively, if no optimization is desired, leave the boxes unchecked and click "Read Plate".



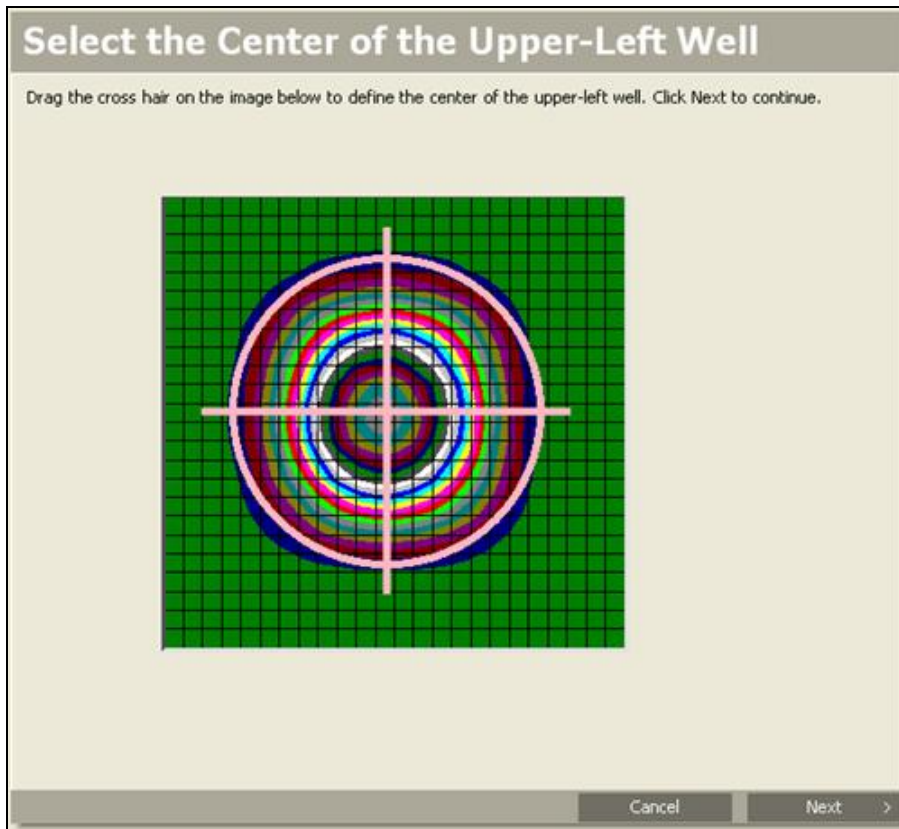
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19. If optimization was selected, a wizard will pop up. Follow the steps outlined in the wizard.



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20. Center the pink target over the image of the scanned well. Click "Next" and repeat for the remaining three wells. This adjusts the microplate definition to match the actual plate.



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21. Click "Save" to save the modified plate dimensions with the Microplate Name as shown. This optimized microplate type will be available in the Settings for future use.

Verify Microplate Dimensions

Verify the dimensions of the microplate. You can edit the values in the fields or return to a well step to redefine its center. Type a name for the microplate definition in the Microplate Name field. Click Save to save the microplate definition.

<input checked="" type="checkbox"/> Microplate Dimensions	
Bottom-row y offset (mm)	8.99
Column spacing (mm)	4.5
Left-column x offset (mm)	12.12
Right-column x offset (mm)	12.12
Row spacing (mm)	4.5
Top-row y offset (mm)	8.99

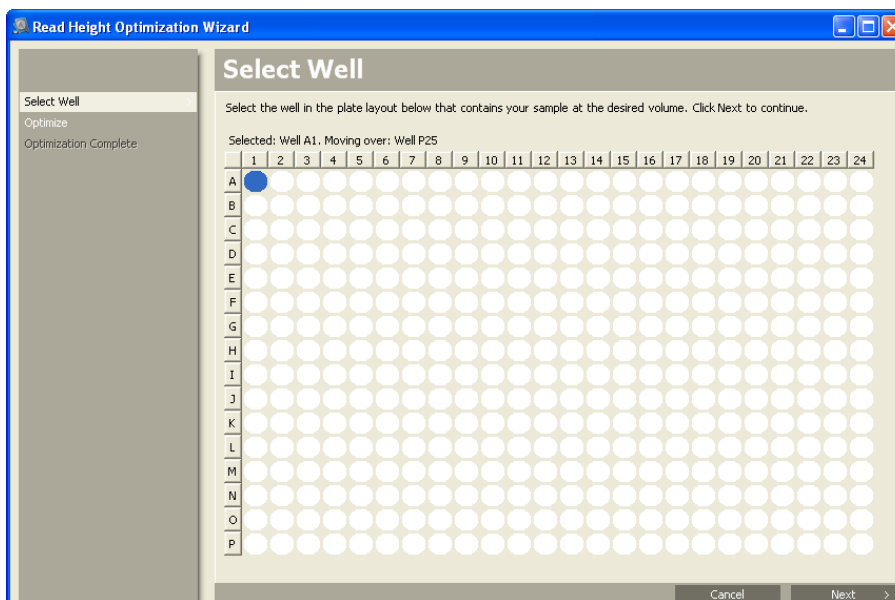
<input checked="" type="checkbox"/> Microplate Name	
Microplate Name	384 Well Corning clr/flatbtm [G-BLAZER-Landscape]

Bottom-row y offset (mm)
The distance in millimeters from the lower edge of the microplate to the horizontal center of the bottom row.

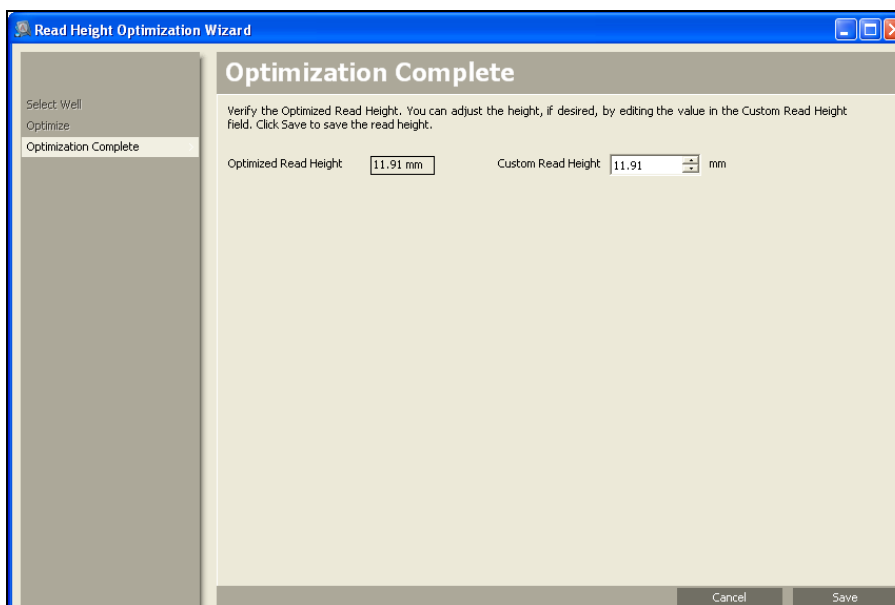
Cancel < Back Save

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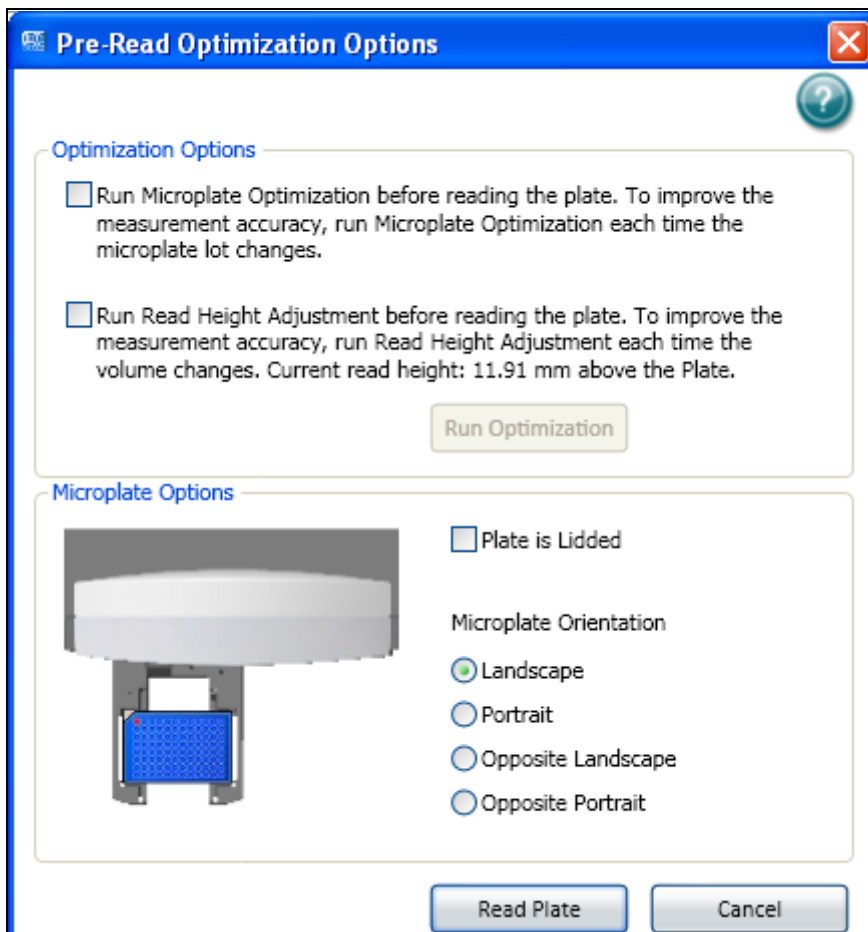
22. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control.



Click "Next"



23. After optimization is complete, click "Read Plate" to proceed.

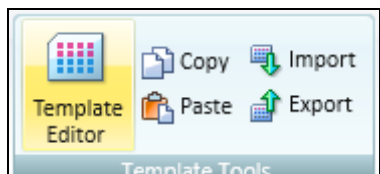


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24. After the plate is read, data will appear in the plate section:

		Plate1																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	5e5	5e5	5e5	1e6	1e6	1e6	9e6	9e6	9e6	1e7	1e7	9e6	7e6	4e6	4e6	3e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	2e6	2e6	2e6	3e6	3e6	2e6	3e6	3e6	3e6	4e6	4e6	2e5	2e6	2e6	2e5	2e5	2e5	1e5	
B	5e5	5e5	5e5	1e6	1e6	1e6	1e7	9e6	9e6	1e7	1e7	9e6	7e6	5e6	4e6	4e6	4e6	7e5	1e6	1e6	2e6	5e5	5e5	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	2e6	2e6	2e6	2e6	2e6	2e6	3e6	4e6	3e6	4e6	4e6	2e5	2e6	2e6	3e5	2e5	2e5	2e5	
C	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	9e6	1e7	1e7	8e6	5e6	4e6	4e6	4e6	8e5	1e6	1e6	6e5	5e5	6e5	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	3e6	2e6	2e6	3e6	3e6	2e6	3e6	3e6	4e6	4e6	4e6	3e5	2e6	2e6	2e5	2e5	2e5	2e5	
D	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	7e6	5e6	4e6	4e6	4e6	7e5	1e6	1e6	5e5	5e5	6e5	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	3e6	2e6	2e6	3e6	3e6	3e6	3e6	3e6	3e6	4e6	4e6	2e5	2e6	2e6	2e5	2e5	2e5	2e5	
E	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	5e6	4e6	5e6	4e6	7e5	1e6	1e6	6e5	5e5	6e5	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	2e6	2e6	3e6	3e6	3e6	2e6	3e6	3e6	4e6	4e6	4e6	2e5	2e6	2e6	2e5	2e5	2e5	2e5	
F	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	5e6	4e6	6e6	4e6	7e5	1e6	1e6	5e5	6e5	5e5	3e6	
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G	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	5e6	4e6	4e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	5e5	
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H	4e5	5e5	8e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	5e6	4e6	4e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	6e5	
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I	5e5	5e5	5e5	1e6	1e6	3e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	4e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	6e5	
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J	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	5e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	5e5	
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K	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	6e6	5e6	5e6	4e6	7e5	1e6	1e6	5e5	6e5	5e5	5e5	
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L	5e5	5e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	5e6	4e6	7e5	1e6	1e6	5e5	5e5	6e5	5e5	
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M	4e5	4e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	5e6	4e6	4e6	1e6	1e6	5e5	5e5	5e5	5e5	
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N	4e5	4e5	8e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	5e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	5e5	
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O	4e5	7e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	9e6	6e6	5e6	4e6	4e6	7e5	1e6	1e6	5e5	5e5	5e5	6e5	
	1e5	1e5	1e5	2e6	2e6	2e6	3e6	3e6	3e6	3e6	3e6	3e6	4e6	4e6	4e6	4e6	4e6	2e5	2e6	2e6	2e5	2e5	2e5	2e5	
P	4e5	7e5	5e5	1e6	1e6	1e6	1e7	1e7	1e7	1e7	1e7	1e7	8e6	5e6	4e6	4e6	4e6	6e5	1e6	1e6	5e5	5e5	1e6	5e5	
	1e5	1e5	1e5	2e6	2e6	2e6	3e6	2e6	3e6	3e6	2e6	2e6	3e6	3e6	4e6	4e6	4e6	2e5	2e6	2e6	2e5	2e5	2e5	2e5	

25. To set up template for data analysis, click on Template Editor icon in the Template Tools section of the top toolbar, or on the plate section header:

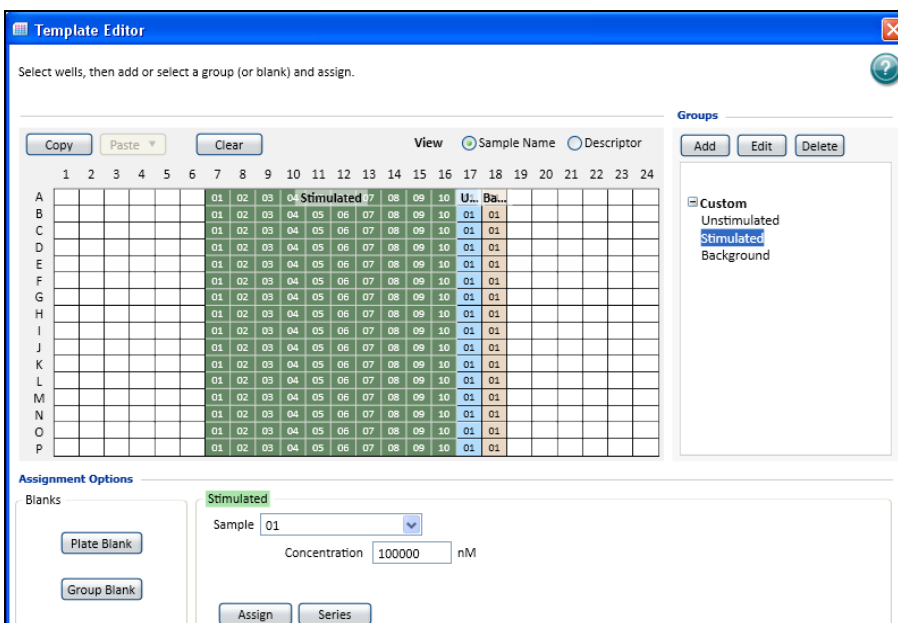


Or



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26. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.



27. When wells are assigned to template groups, data will populate group tables where analysis can be done:

Expt1				
Stimulated				
Sample	Concentration nM	AvgRatio	SDratio	
01	100000.000	5.77	0.145	
02	25000.000	6.05	0.358	
03	6250.000	6.05	0.304	
04	1562.500	5.85	0.558	
05	390.625	6.10	0.482	
06	97.656	6.24	0.736	
07	24.414	3.79	0.263	
08	6.104	1.93	0.162	
09	1.526	1.51	0.158	
10	0.381	1.34	0.152	

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C. Results

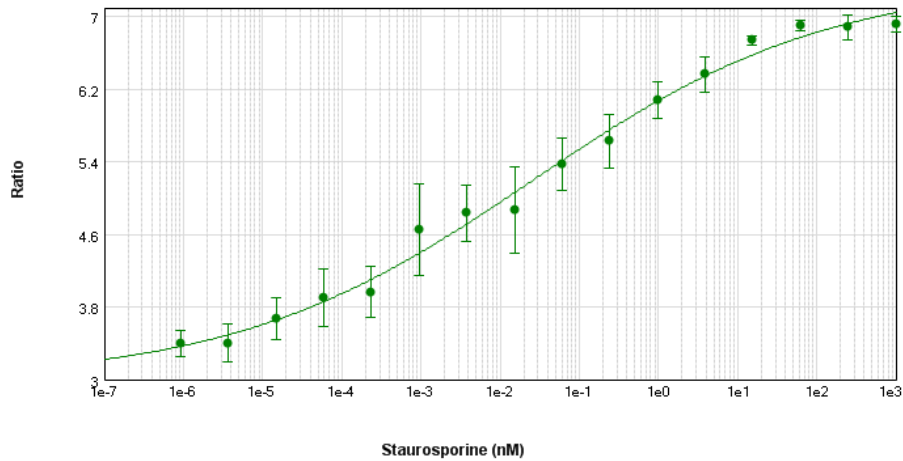


Figure 1: Z'-LYTE® Assay. JAK1 JH1/JH2 Dose-Response Curve read on the Molecular Devices FilterMax™ Multi-Mode Plate Reader. Z' = 0.77.