Setup for GeneBLAzer® Assay on Molecular Devices FilterMax™ F5 Microplate Reader with SoftMax® Pro 6.1 Software

Molecular Devices' FilterMax™ F5 Multi-Mode Microplate Reader was tested for compatibility with Life Technologies' GeneBLAzer® assays. 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.

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 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.

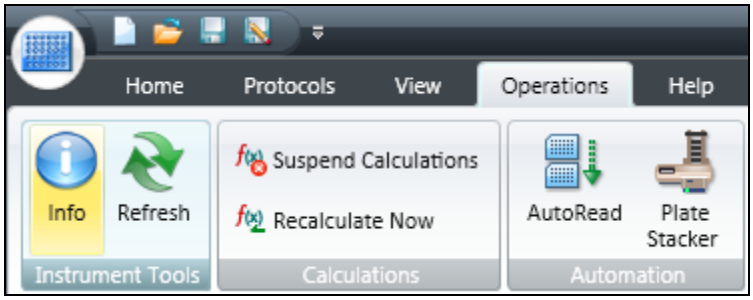
Users will need to obtain additional filter slides in which they can install the above filters:

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.

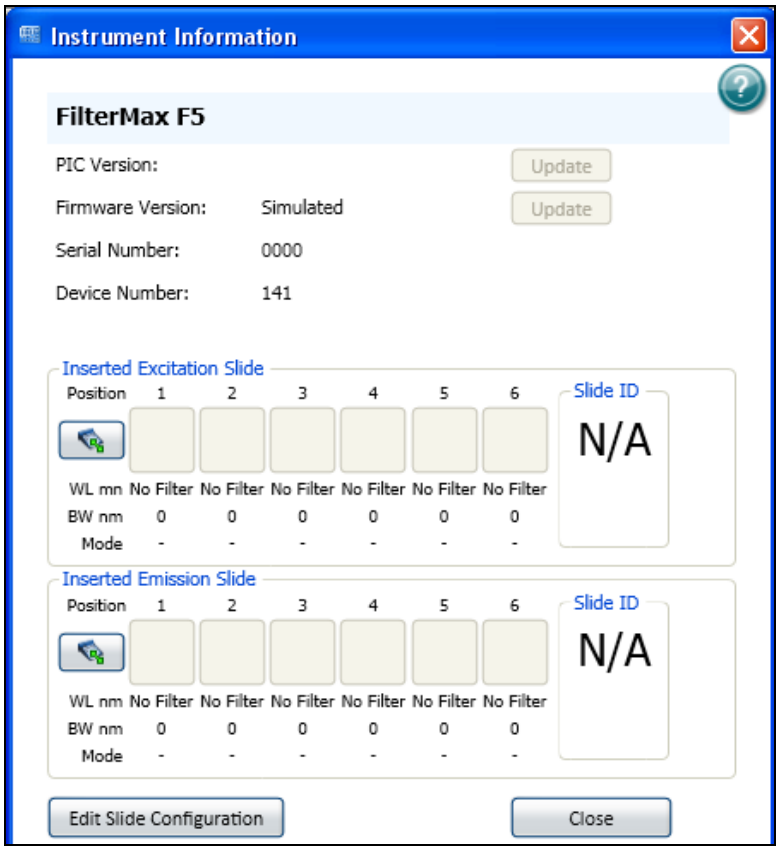
B. Instrument Setup:

Configuration of filter slides in SoftMax® Pro software

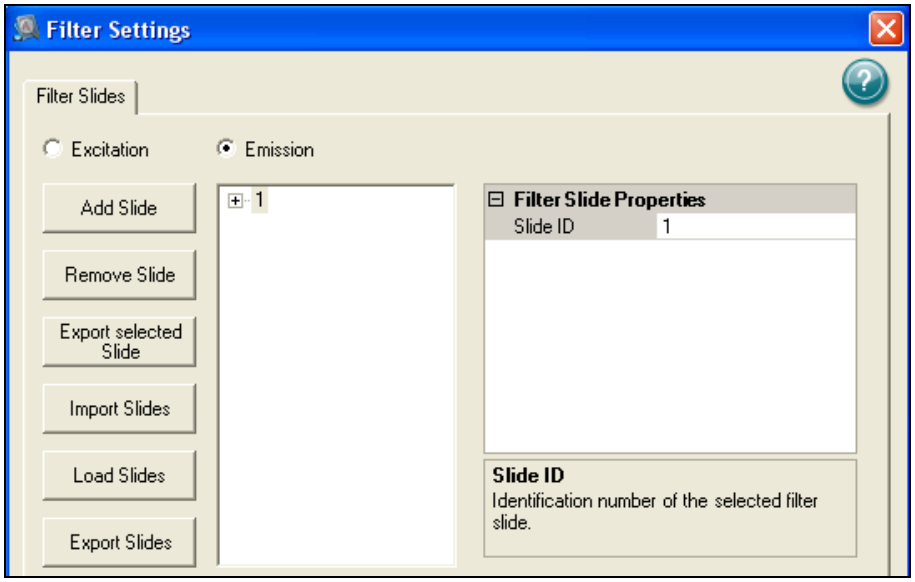
1. Open SoftMax® Pro software. In the Operations tab, click "Info":



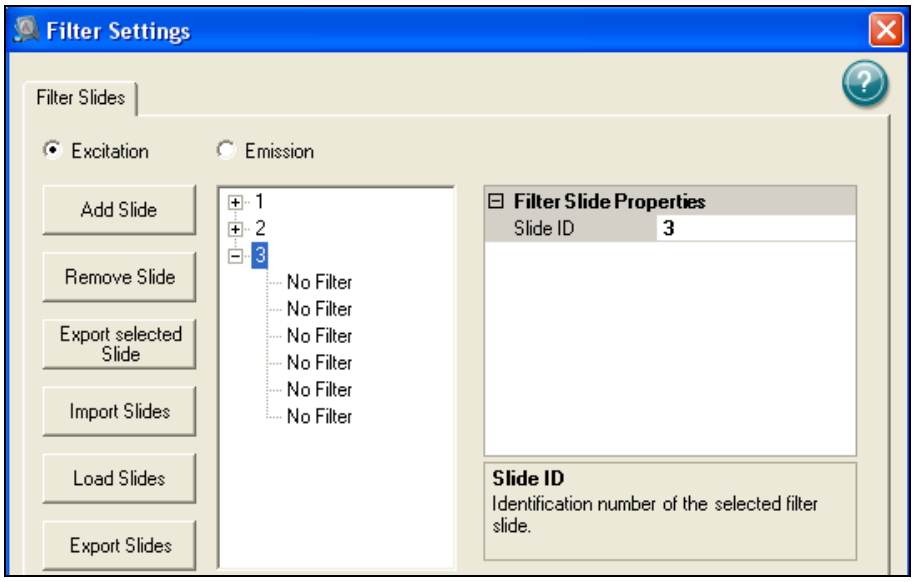
This opens the Instrument Information window:



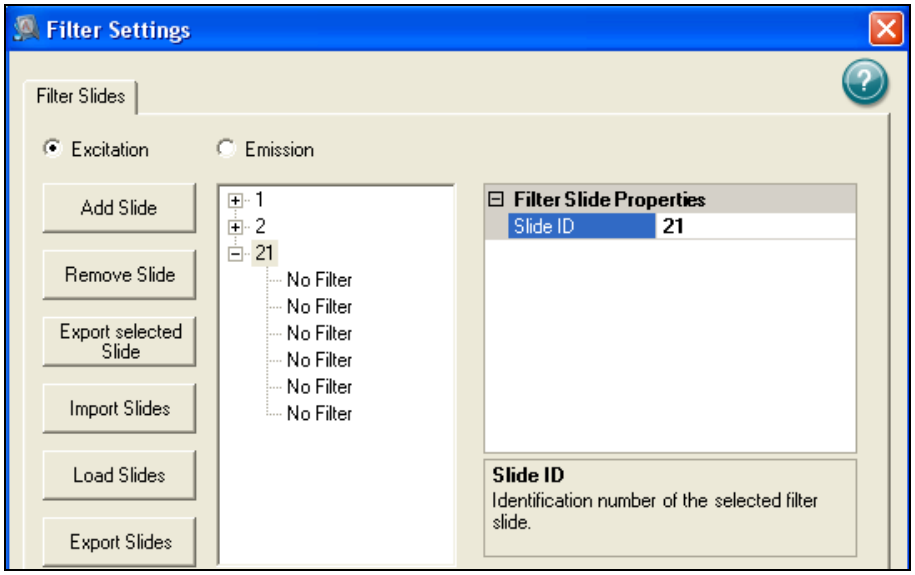
2. Click "Edit Slide Configuration". This opens the Filter Settings window:



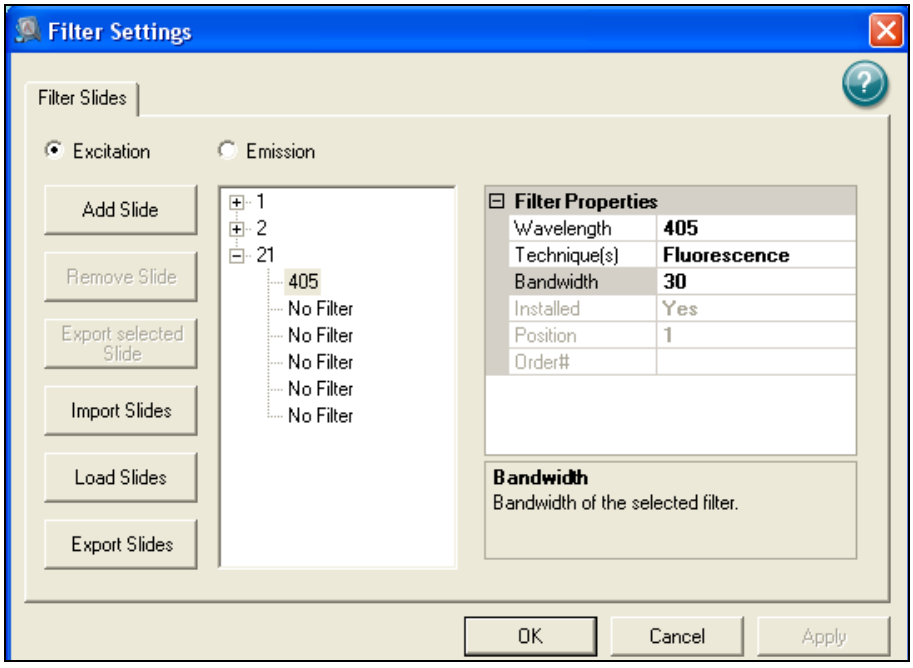
3. Click the radio button next to "Excitation" and click "Add Slide".



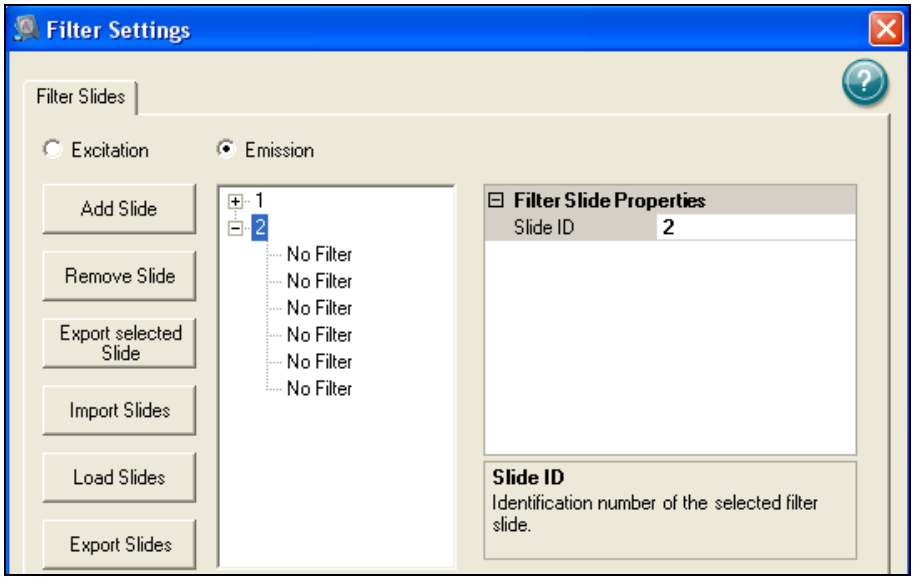
4. Under Filter Slide Properties, type "21" next to Slide ID.



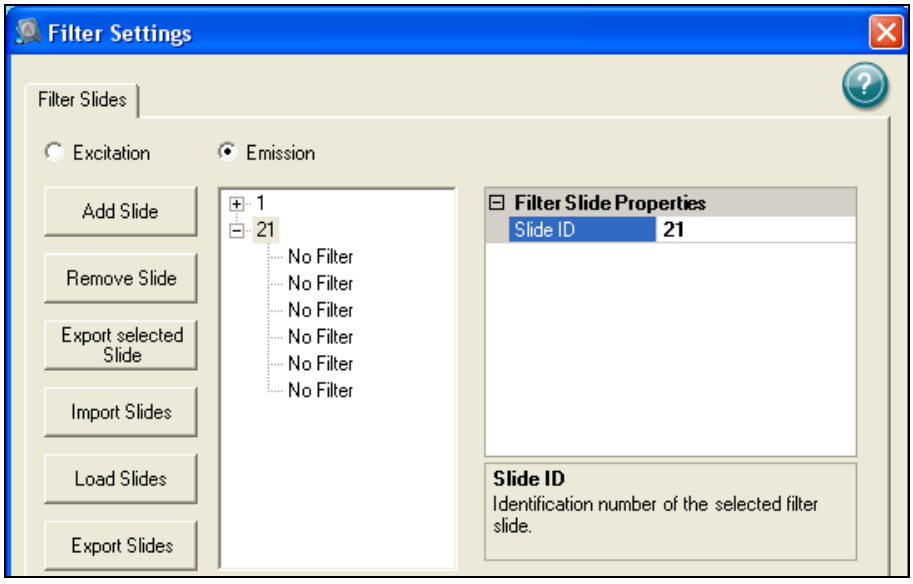
5. In the list below the slide number 21, select the position where the excitation filter is installed and enter the appropriate values under "Filter Properties". Click Apply to save.



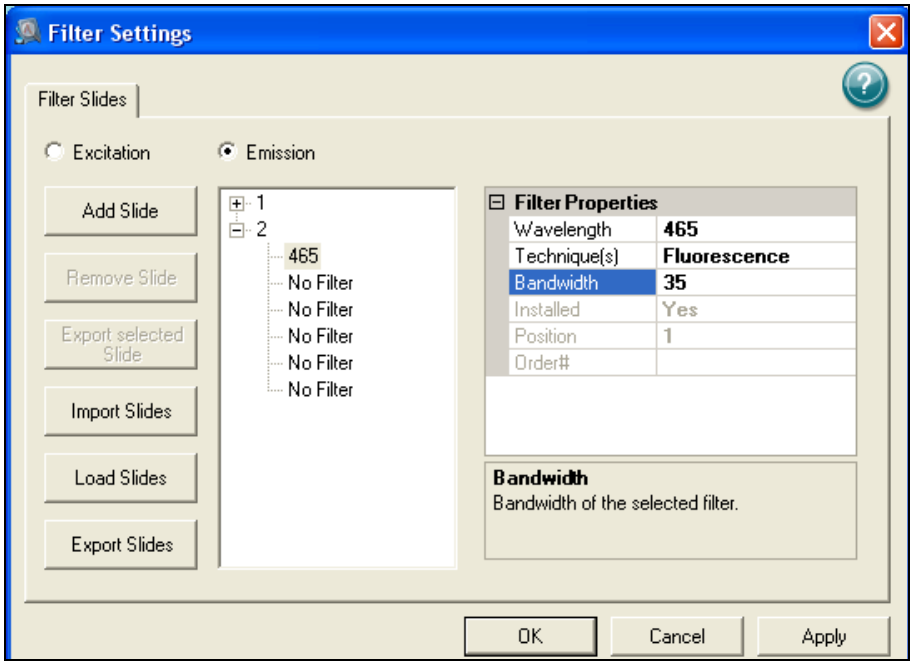
6. Click the radio button next to "Emission" and click "Add Slide"



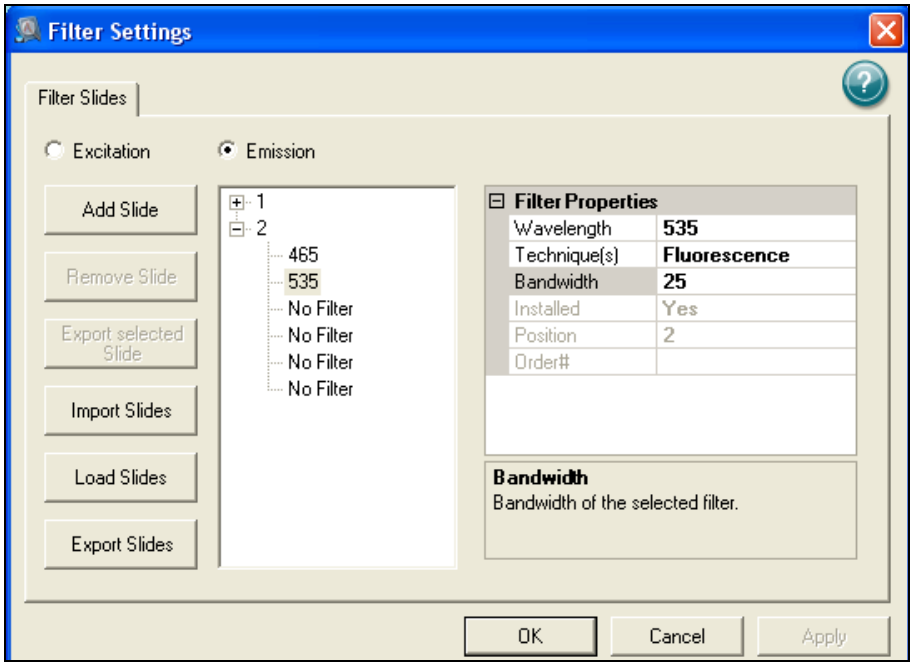
7. Under Filter Slide Properties, type "21" next to Slide ID.



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.



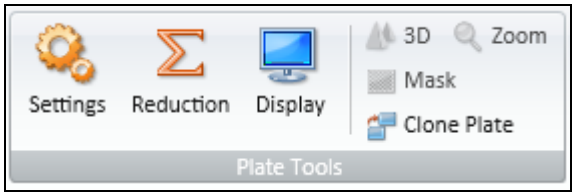
9. Repeat for second emission filter.



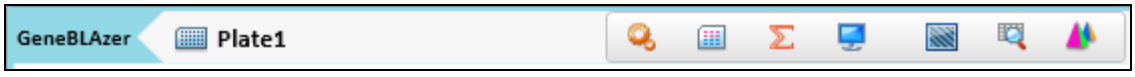
10. When finished, click Okay to exit the configuration, and then click Close to exit Instrument Information.

Instrument settings

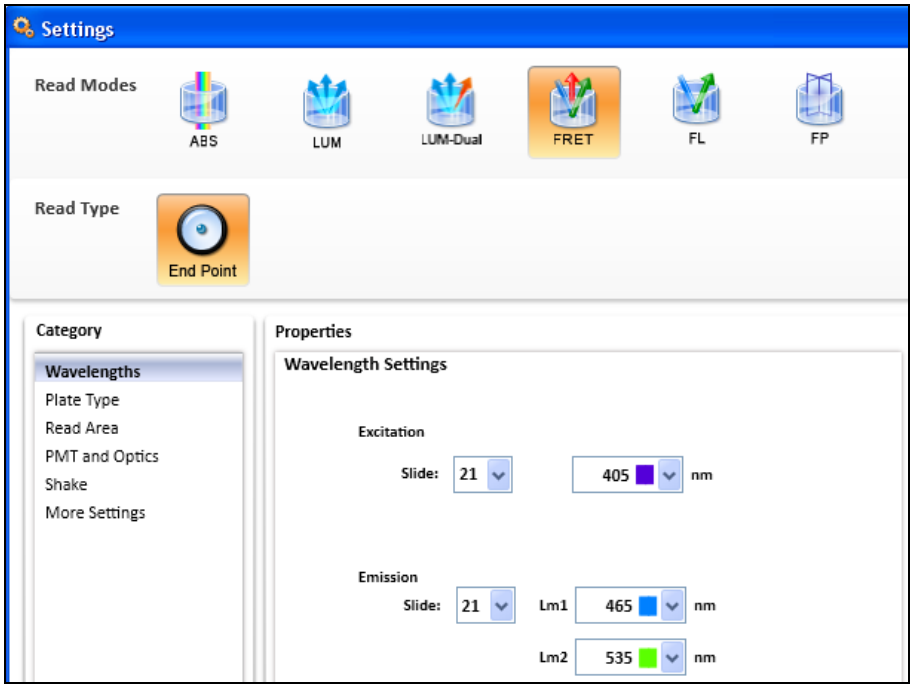
11. Click on the microplate icon in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen or in the plate section header.



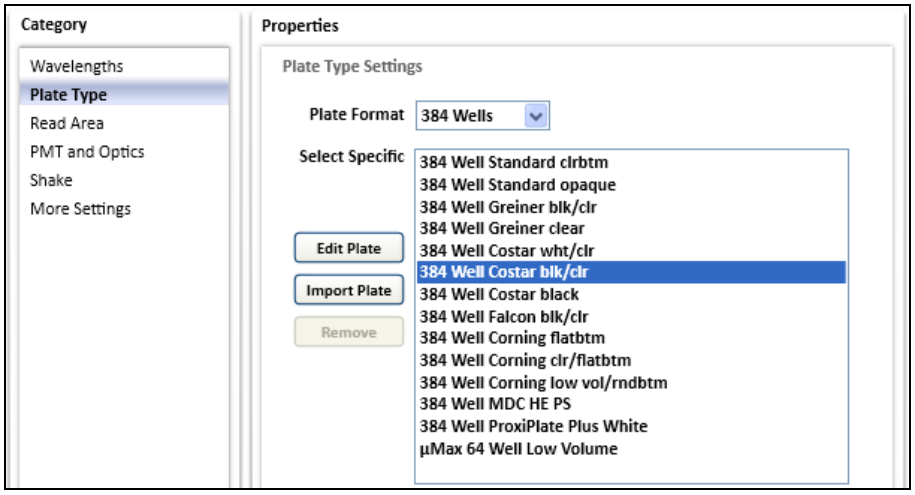
Or



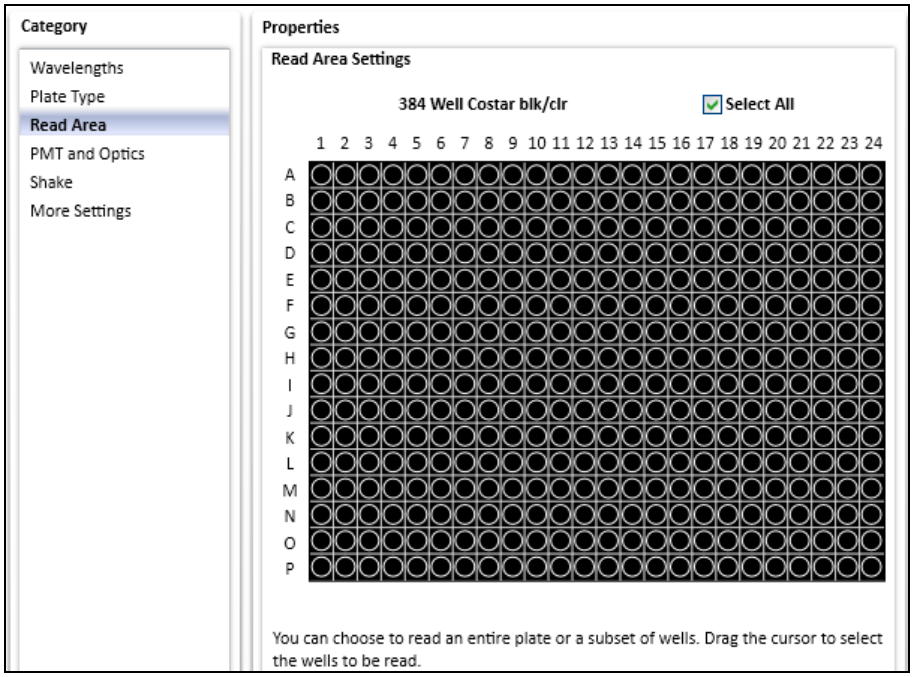
12. Select the FRET read mode and End Point read type. Select the appropriate excitation and emission slides, and select the wavelengths as shown below.



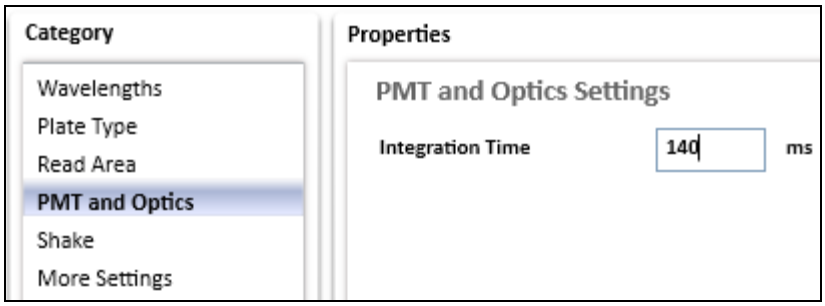
13. Choose the desired plate type, using the upper dropdown menu to choose plate format (96 or 384 wells) and the "Select Specific" menu to choose the specific plate type.



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

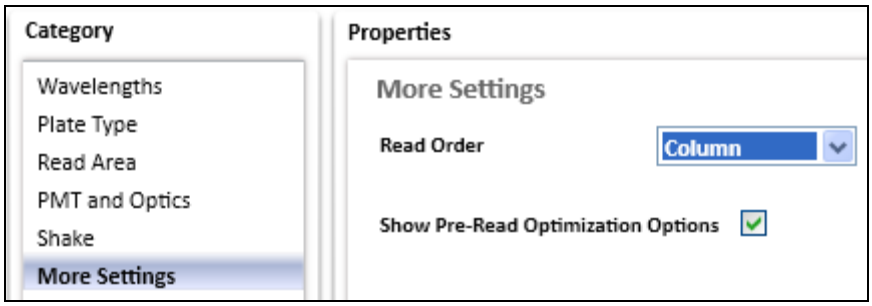


15. In the PMT and Optics Settings enter the desired integration time. Shorter integration times enable faster reading, while longer integration times enable better performance.



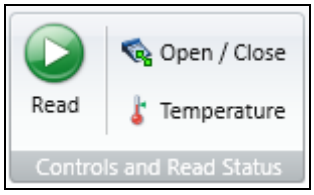
16. In "More Settings", choose the read order corresponding to how the assay plate is set up. If the entire plate is to be read, choose "Row". If entire rows of a partial plate are to be read, choose "Row"; if entire columns of a partial plate are to be read, choose "Column".

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.

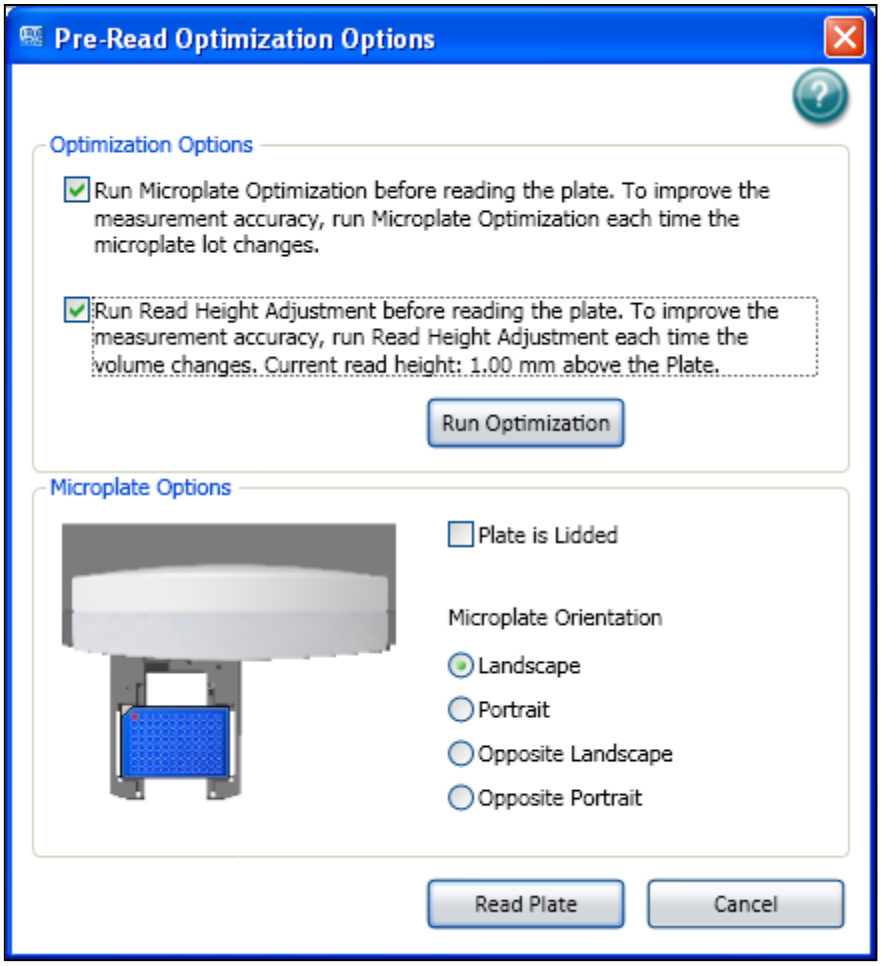
17. To read the plate, click the green "Read" button at the top of the screen.



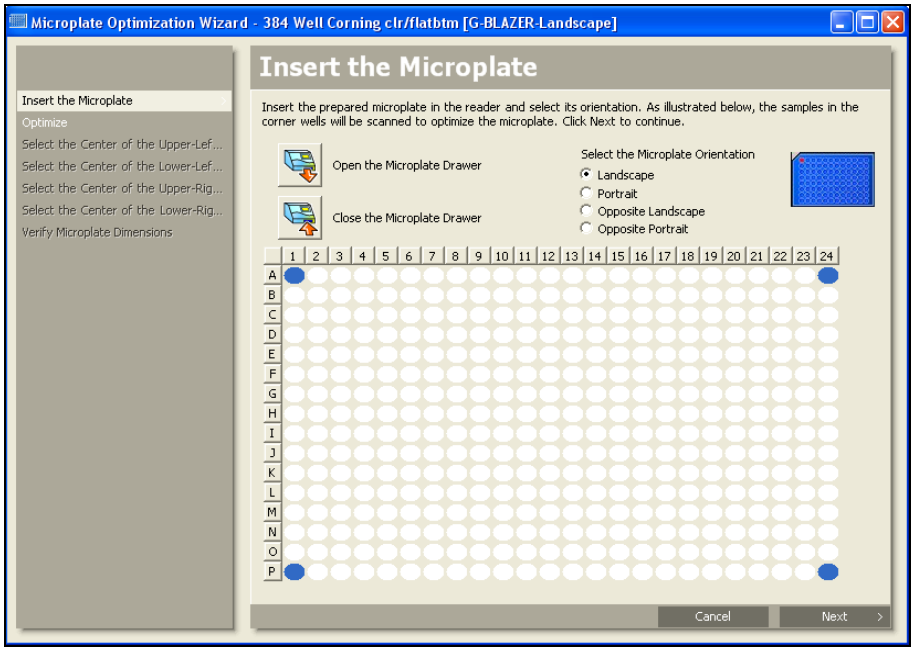
18. If selected, pre-read optimization options will appear.

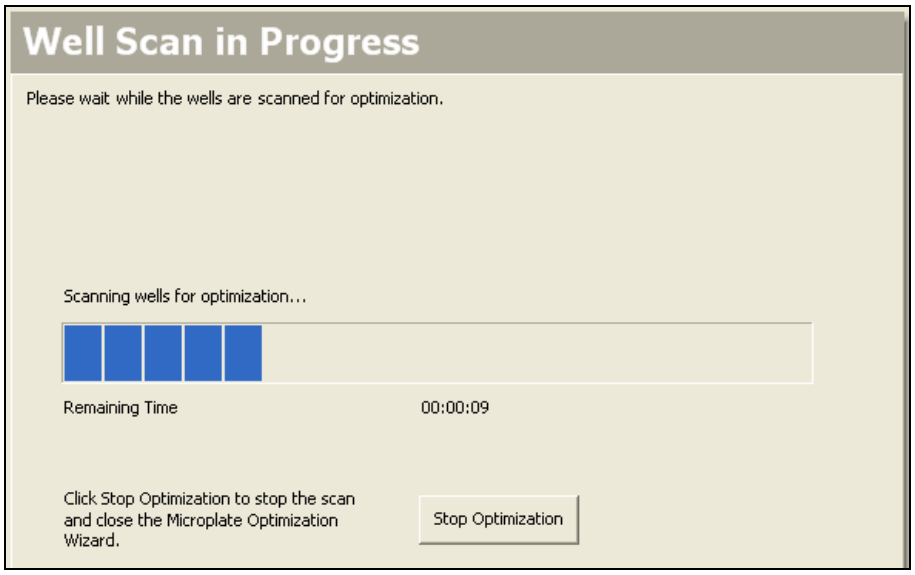
- Microplate Optimization scans the four corner wells of the plate and adjusts the microplate dimensions if necessary to improve accuracy. It requires that all four corners of the microplate contain detectable fluorescent material (i.e. positive control samples).
- Read Height Adjustment determines the 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".

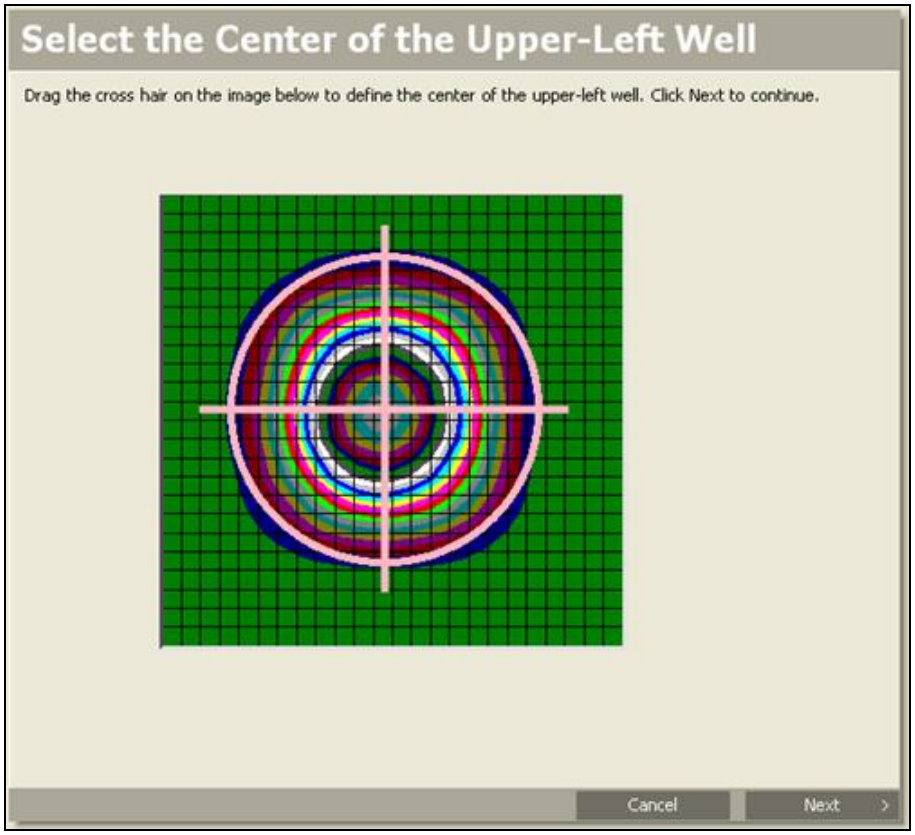


19. If optimization was selected, a wizard will pop up. Follow the steps outlined in the wizard.

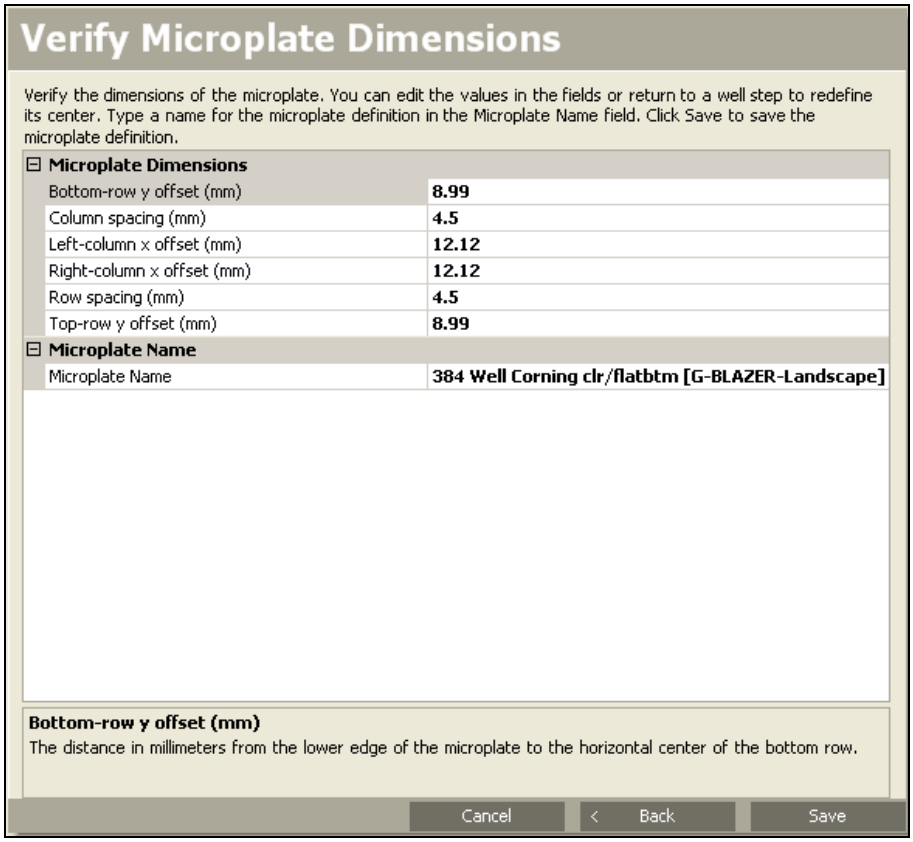




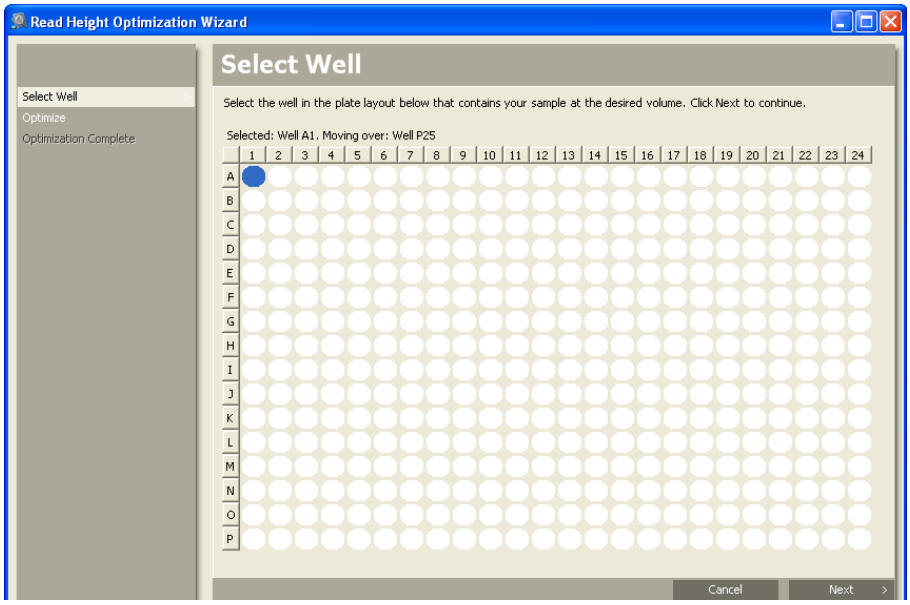
20. Center the pink target over the image of the scanned well. Click "Next" and repeat for the remaining three wells. This adjusts the microplate definition to match the actual plate.



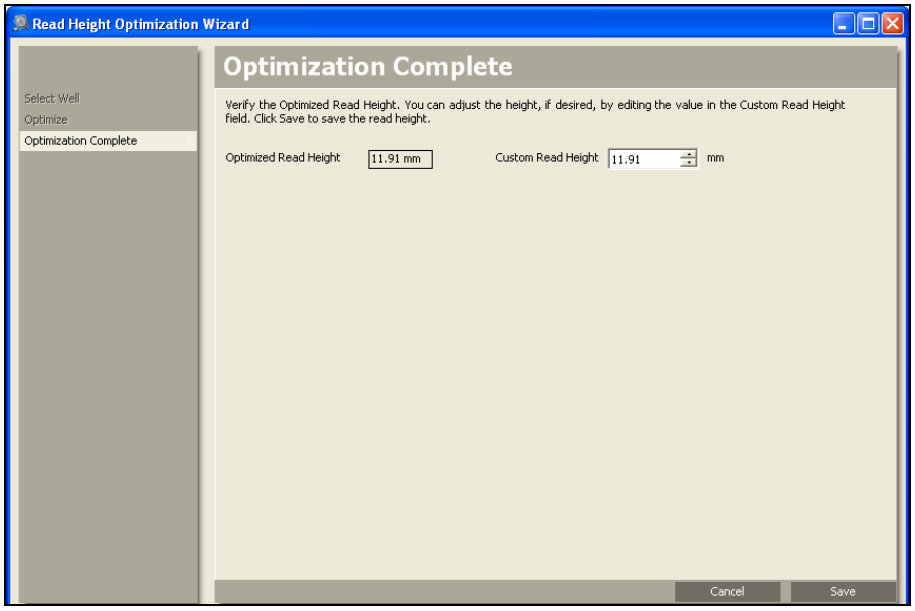
21. Click "Save" to save the modified plate dimensions with the Microplate Name as shown. This optimized microplate type will be available in the Settings for future use.



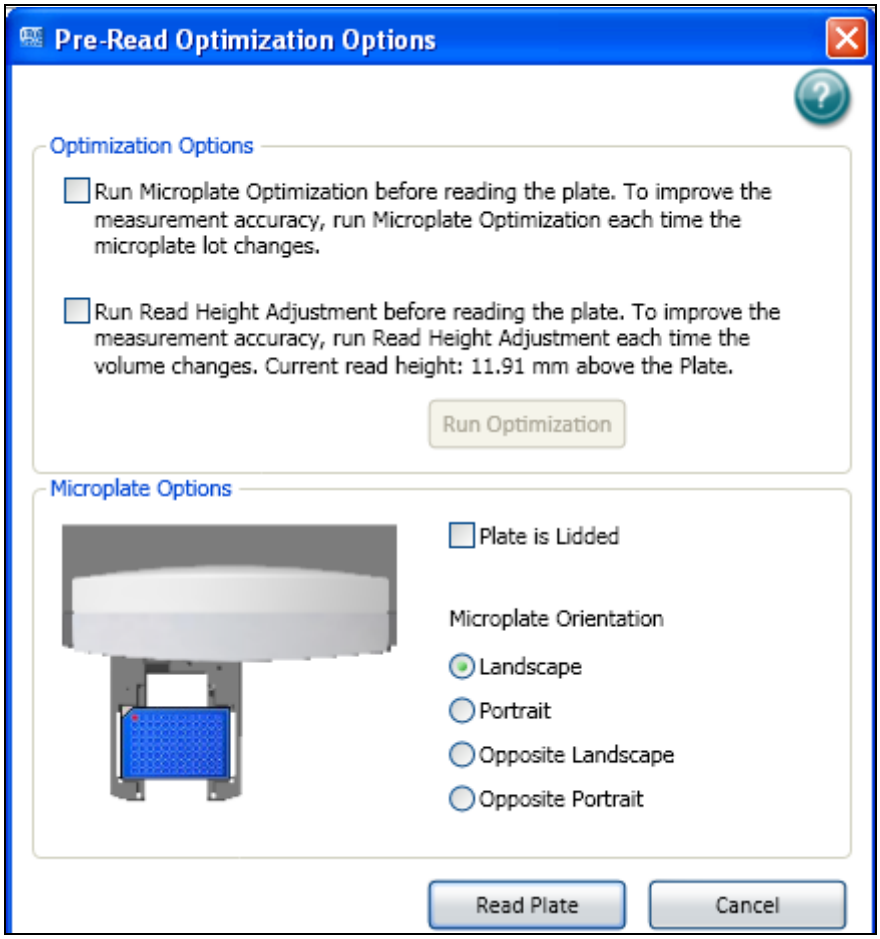
22. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control.



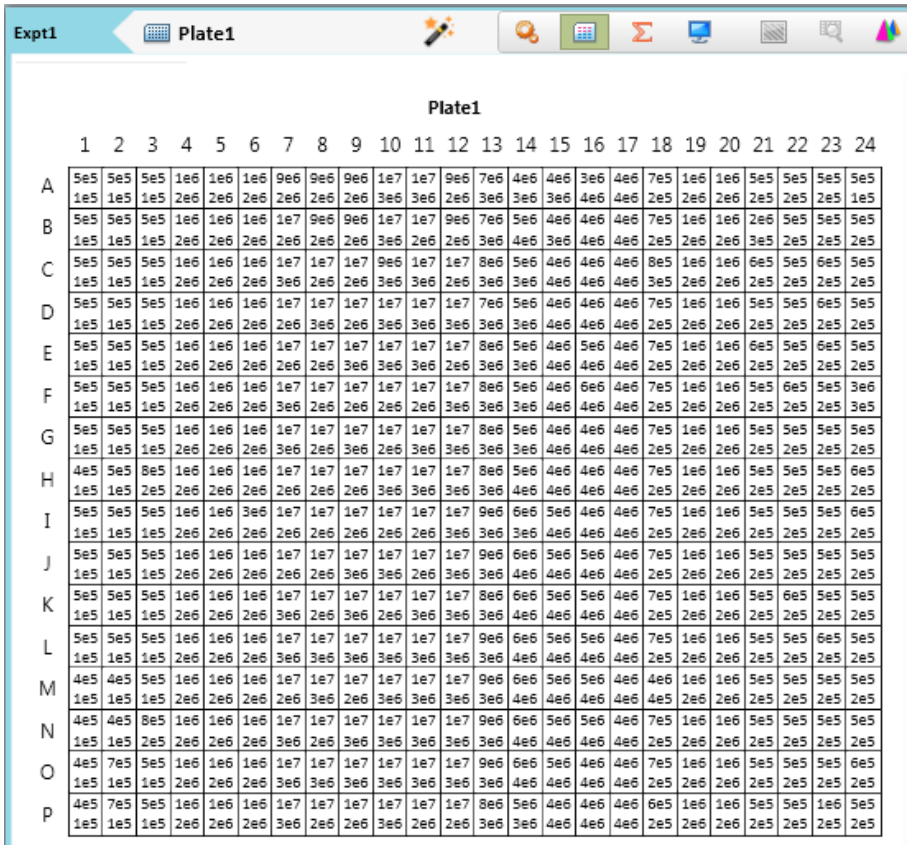
Click "Next"



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

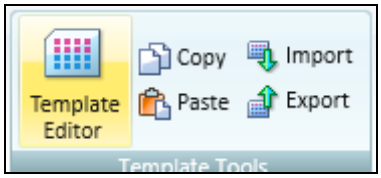


24. After the plate is read, data will appear in the plate section:

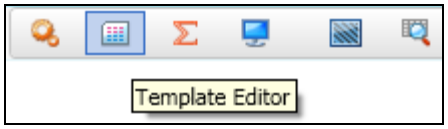


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	5e5 1e5	5e5 1e5	5e5 1e5	1e6 2e6	1e6 2e6	1e6 2e6	9e6 2e6	9e6 2e6	9e6 2e6	1e7 3e6	1e7 3e6	9e6 2e6	7e6 3e6	4e6 3e6	4e6 3e6	3e6 4e6	4e6 4e6	7e5 2e5	1e6 2e6	1e6 2e6	5e5 2e5	5e5 2e5	5e5 2e5	5e5 1e5
B	5e5 1e5	5e5 1e5	5e5 1e5	1e6 2e6	1e6 2e6	1e6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

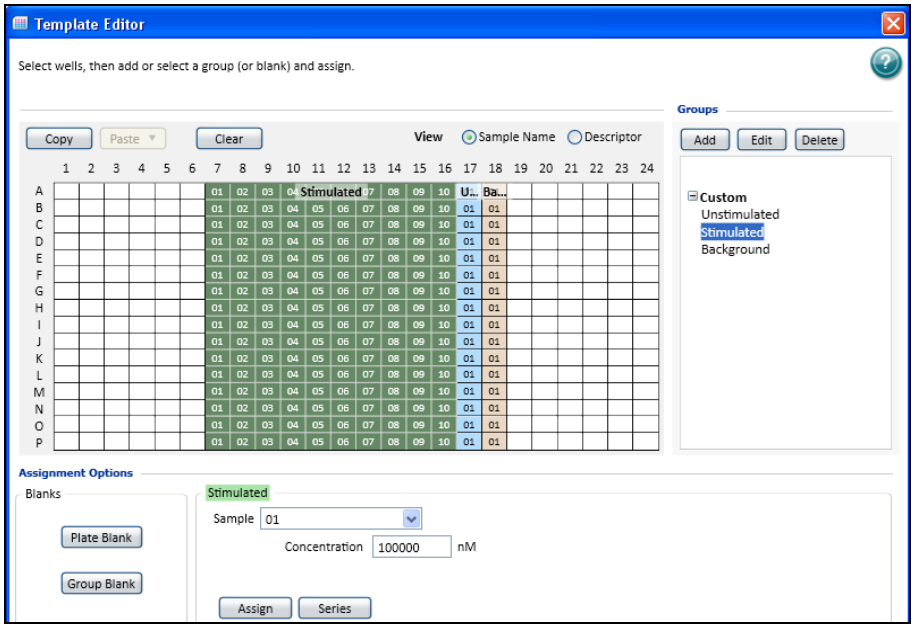
25. To set up template for data analysis, click on Template Editor icon in the Template Tools section of the top toolbar, or on the plate section header:



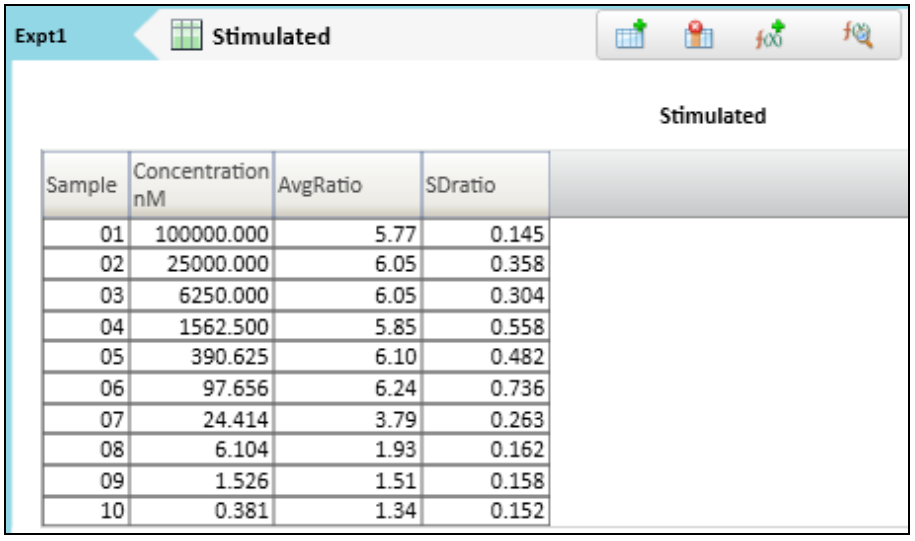
Or



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:



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

C. Results

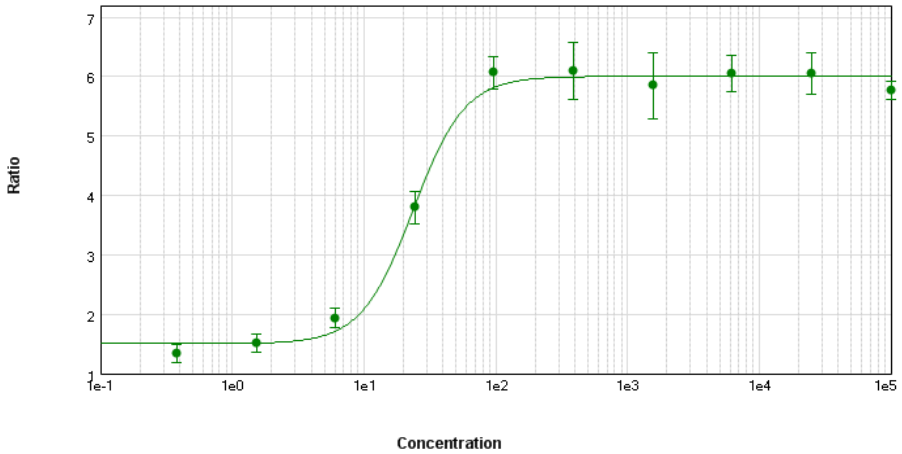


Figure 1: GeneBLAzer® Assay. GeneBLAzer® assay performed using the Molecular Devices FilterMax™ F5 microplate reader and GeneBLAzer® MC3R CRE-bla CHO-K1 cell line stimulated with NDP-α-MSH. Z' = 0.80.