Setup for Z'-LYTE® assay on SpectraMax® Paradigm® Microplate Detection Platform with SoftMax® Pro 6 software

The Molecular Devices SpectraMax® Paradigm® Microplate Detection Platform was tested for compatibility with Life Technologies' Z'-LYTE® FRET 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.

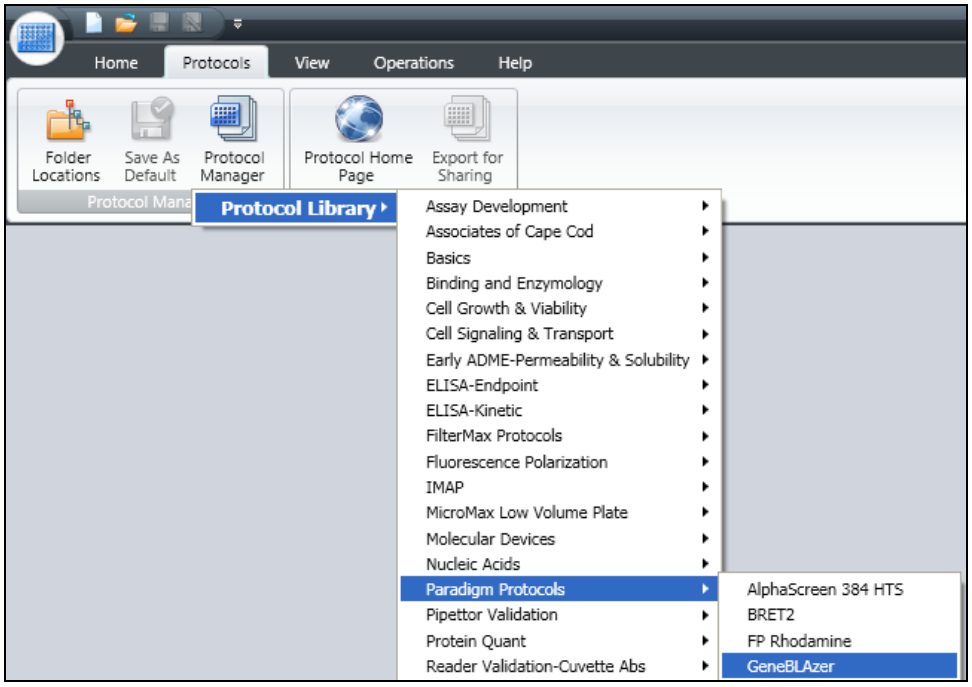
For more detailed information and technical support of Molecular Devices instruments or software, please contact Molecular Devices at 1-800-635-5577 or www.moleculardevices.com.

A. Recommended Optics

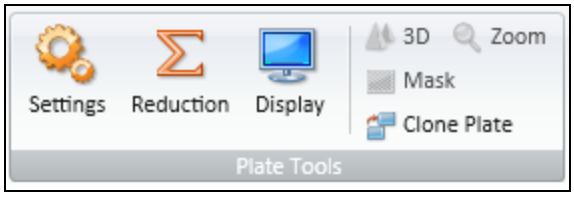
Parameter	Specification
Detection Cartridge Name	SpectraMax® Paradigm® Fluorescence Intensity (FI) GeneBLAzer® Detection Cartridge
Part Number	0200-7006
Detection Technique	FRET, Fluorescence Intensity
Light Source	LED, ultra high power
Filter Set	EX: 406/15 EM1: 465/35 EM2: 535/25
Applications	Designed for use with GeneBLAzer® reagents; also suitable for Z'-LYTE® assays

B. Instrument Setup:

1. Open SoftMax® Pro 6 software. Click on "Protocol Manager" to open the Protocol Library. Within the "Paradigm® Protocols" folder, locate the "GeneBLAzer®" protocol and click to open.

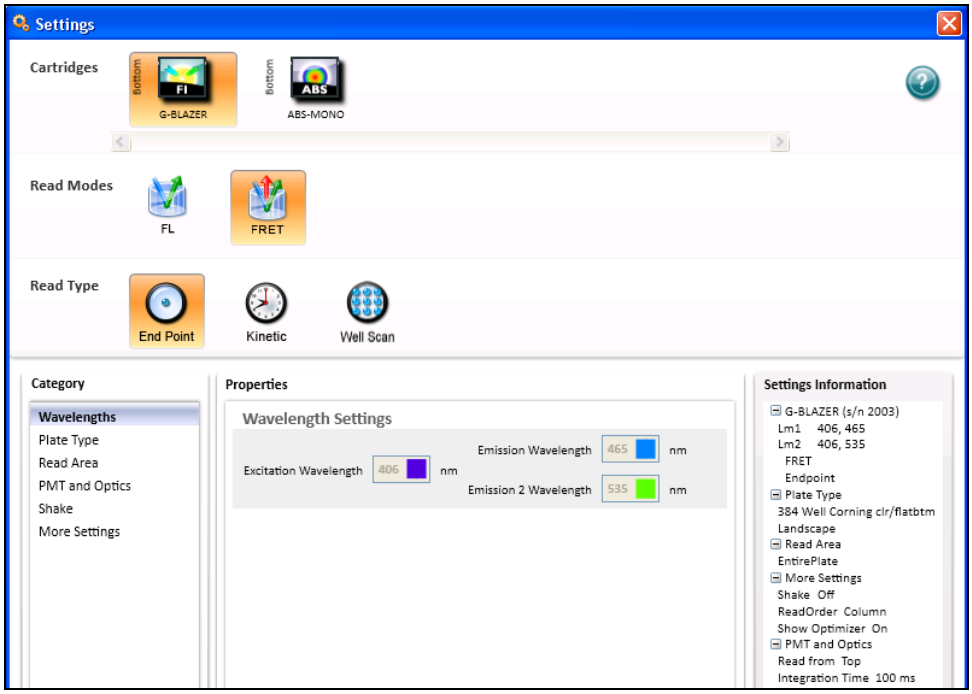


2. Click on "Plate01" in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen…

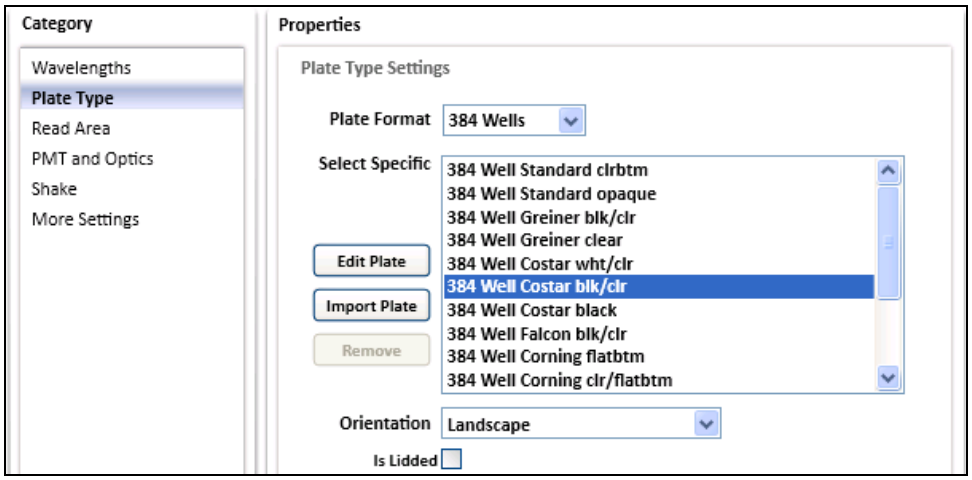


…or in the plate section header.

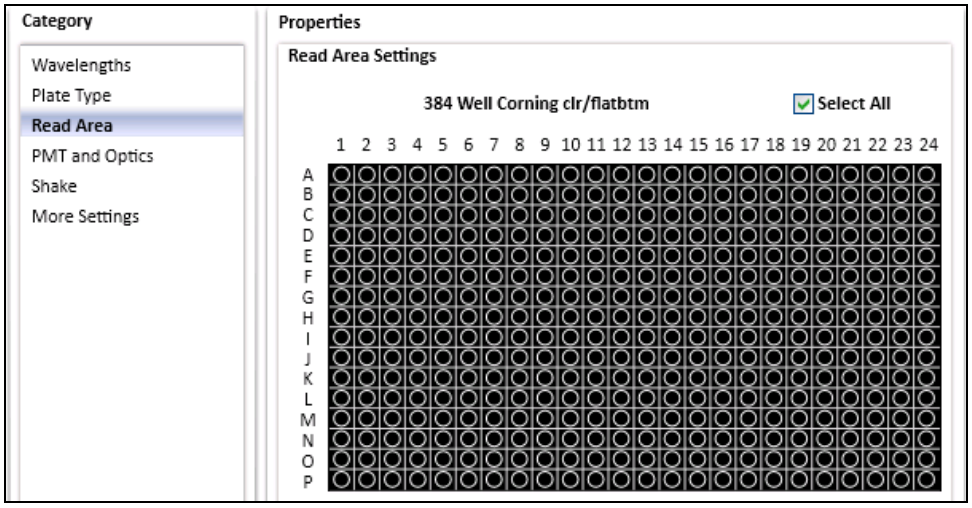
3. This opens the Settings window. The GeneBLAzer® (G-BLAZER) cartridge and its wavelengths already appear under Wavelength Settings.



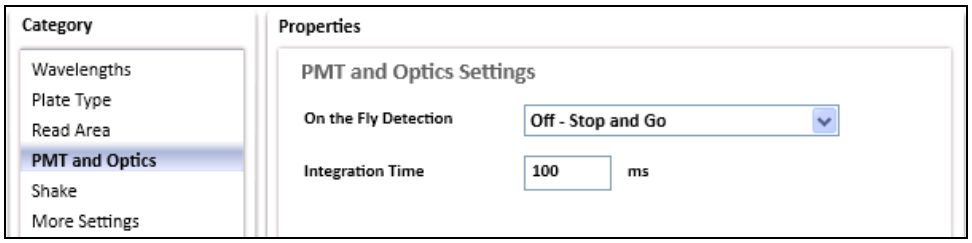
4. Choose the desired plate type, using the upper dropdown menu to choose plate format (96, 384, or 1536 wells) and the "Select Specific" menu to choose the specific plate type.



5. Now select the area of the plate to read.

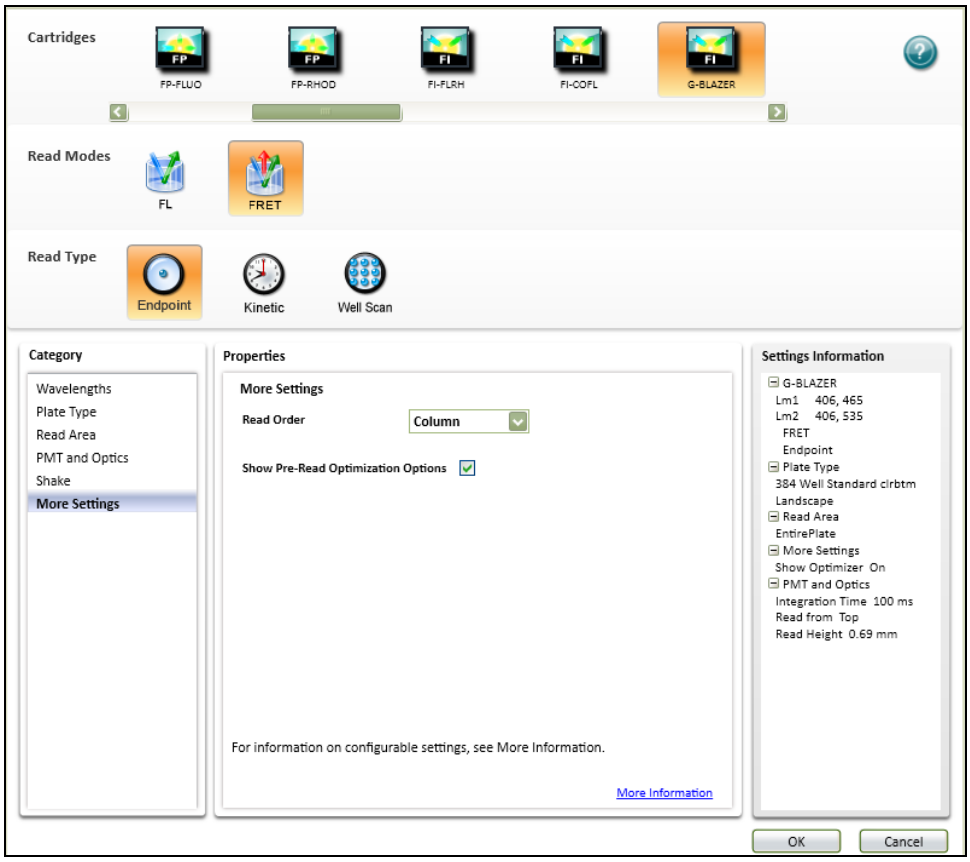


6. PMT and Optics Settings include the option to read using On the Fly detection and adjust the integration time if desired. "Off – Stop and Go" is the default setting. To select On the Fly for faster read times, use the dropdown menu to choose Performance or Speed (faster) On the Fly options. The default integration time is 140 msec. Shorter integration times enable faster reading, while longer integration times enable better performance.

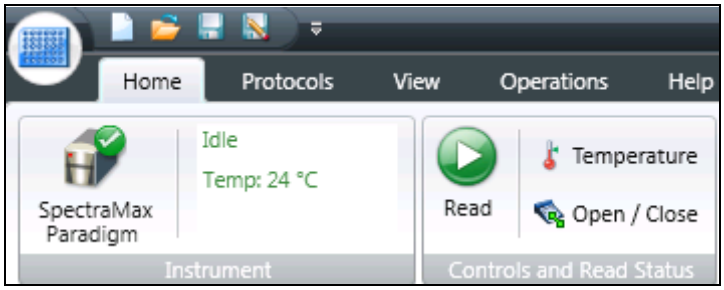


7. In the category "More Settings", choose the read order corresponding to how the assay plate is set up. If the entire plate is to be read, choose "Row". If entire rows of a partial plate are to be read, choose "Row"; if entire columns of a partial plate are to be read, choose "Column". Check the box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



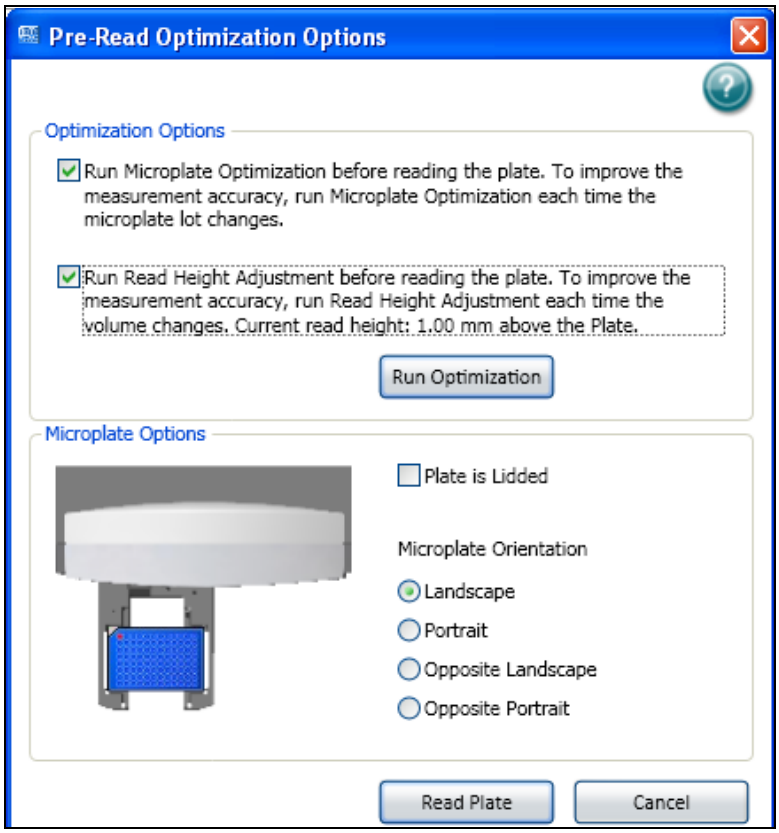
8. To read the plate, click the green "Read" button at the top of the screen.



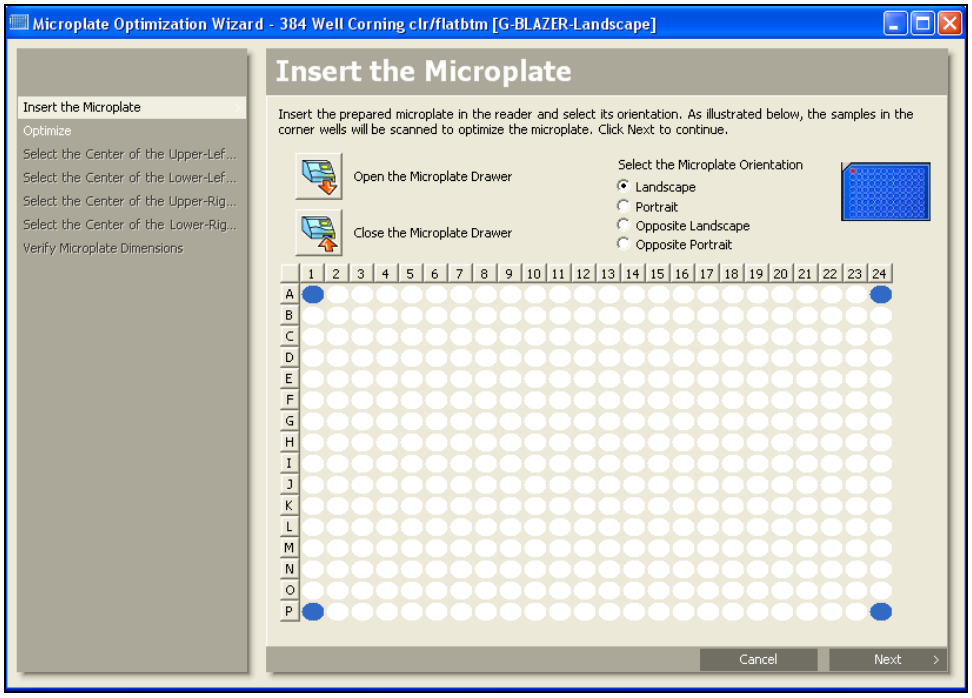
9. If selected, pre-read optimization options will appear:

- 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.

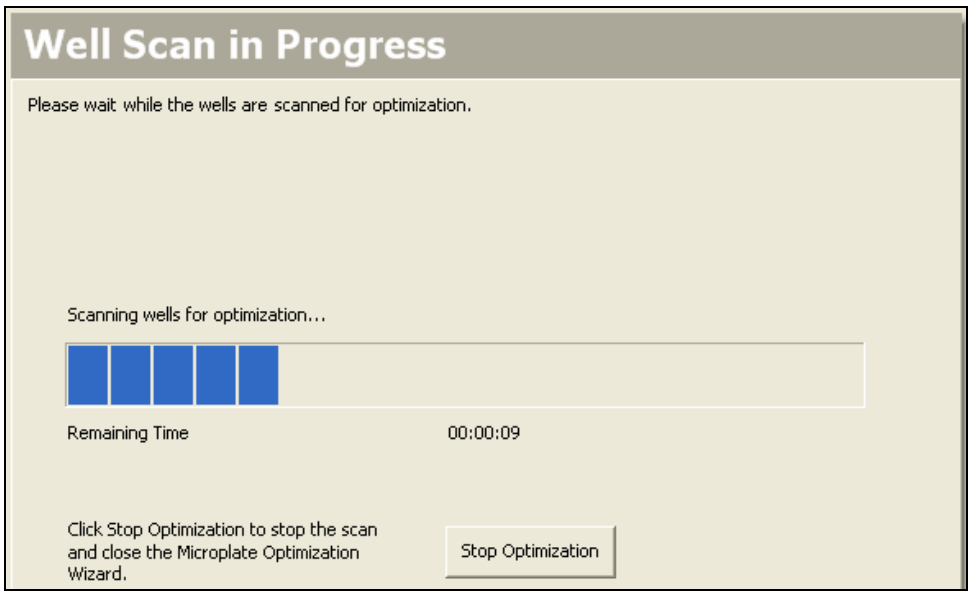
Click "Run Optimization" to proceed. Alternatively, if no optimization is desired, leave the boxes unchecked and click "Read Plate."



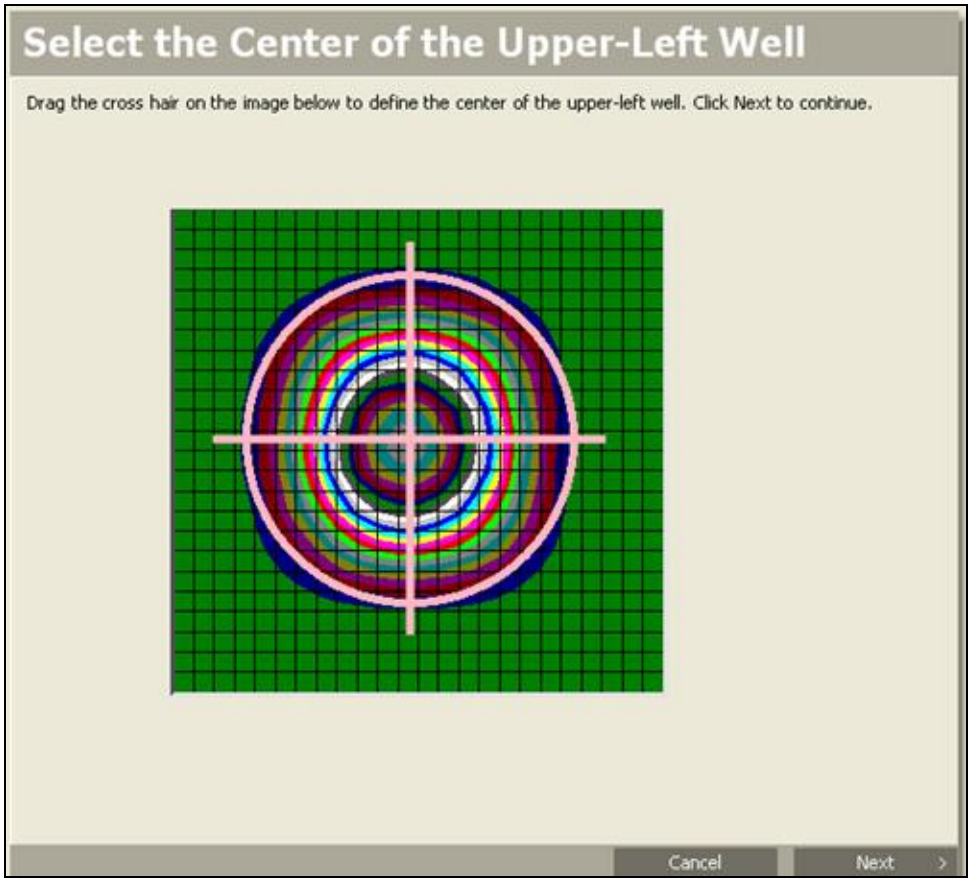
10. If optimization was selected, a wizard will pop up. Follow the steps outlined in the wizard.



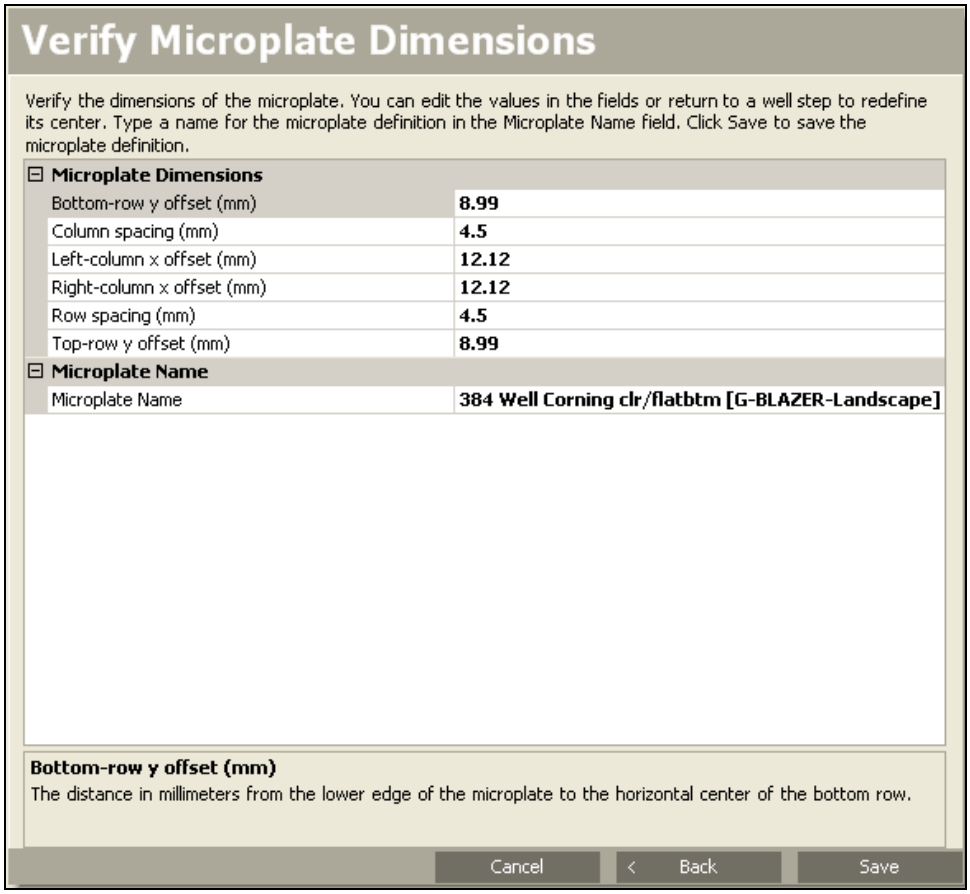
11. When you select read plate, a progress screen will appear as the plate is read.



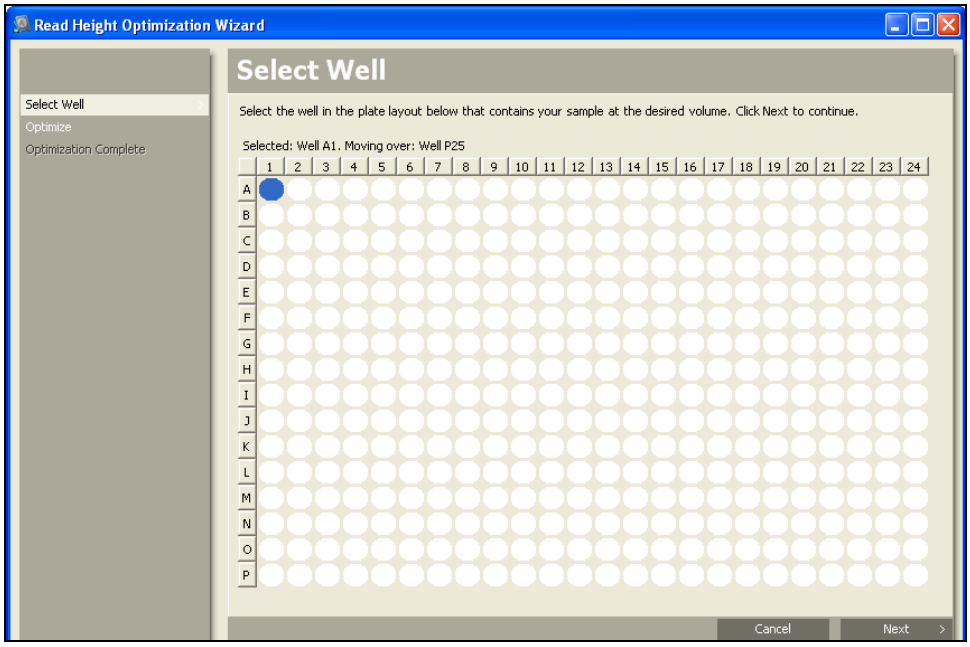
12. Center the pink target over the image of the scanned well. Click "Next" and repeat for the remaining three wells. This adjusts the microplate definition to match the actual plate.



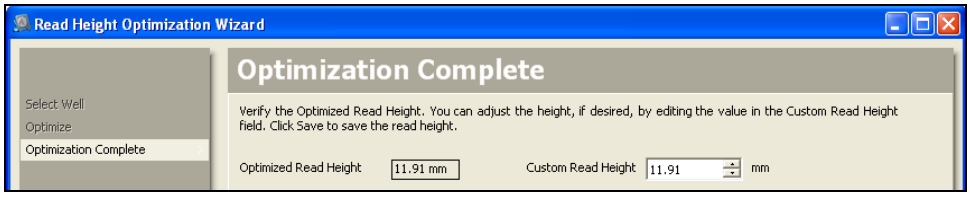
13. Click "Save" to save the modified plate dimensions with the Microplate Name as shown. This optimized microplate type will be available in the Settings for future use.



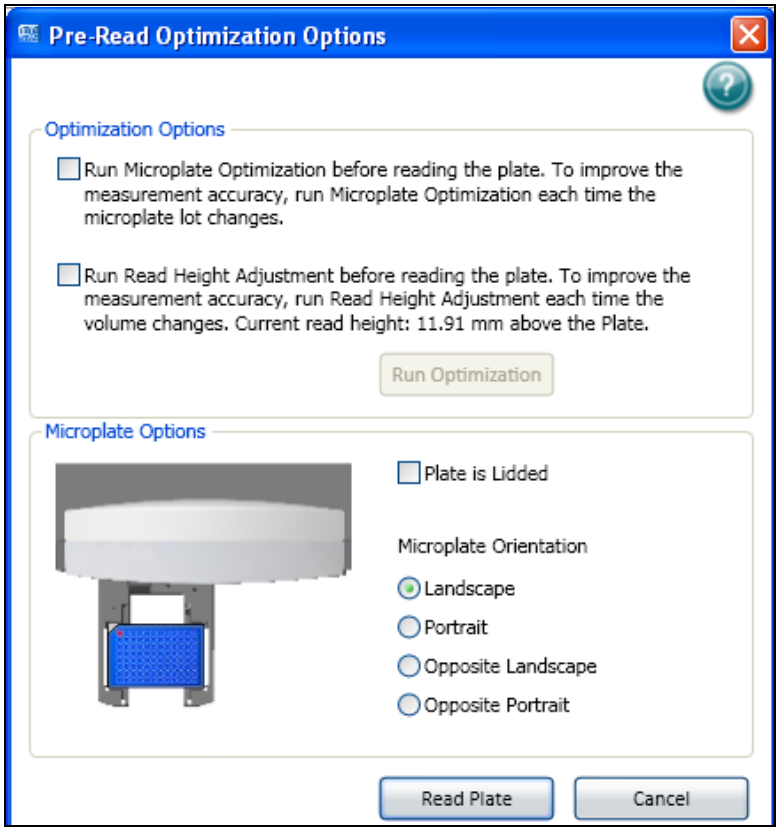
14. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control. Click "Next" to read.



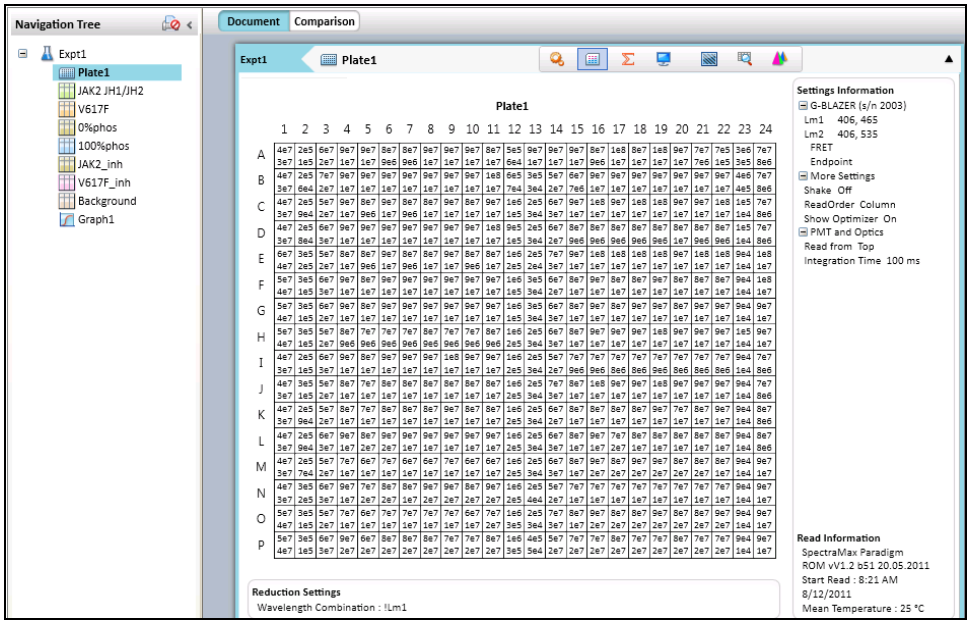
15. The instrument will calculate and report optimized read height.



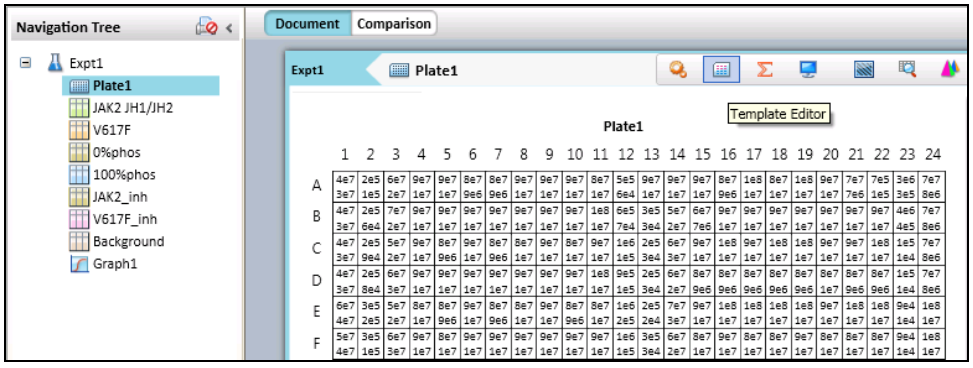
16. After optimization is complete, click on "Read Plate" to proceed.



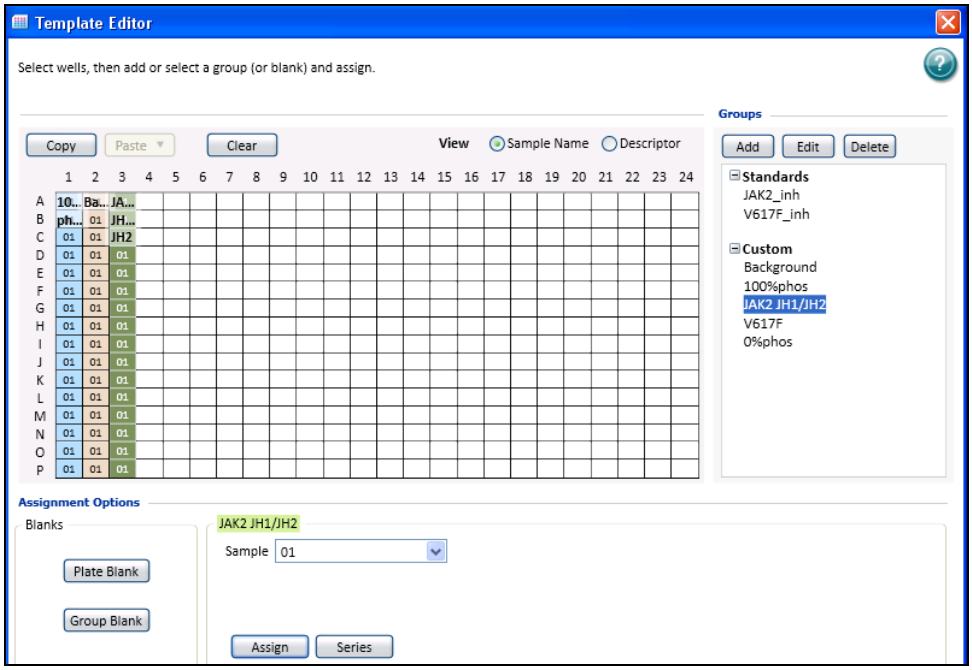
17. After the plate is read, data will appear in the plate section:



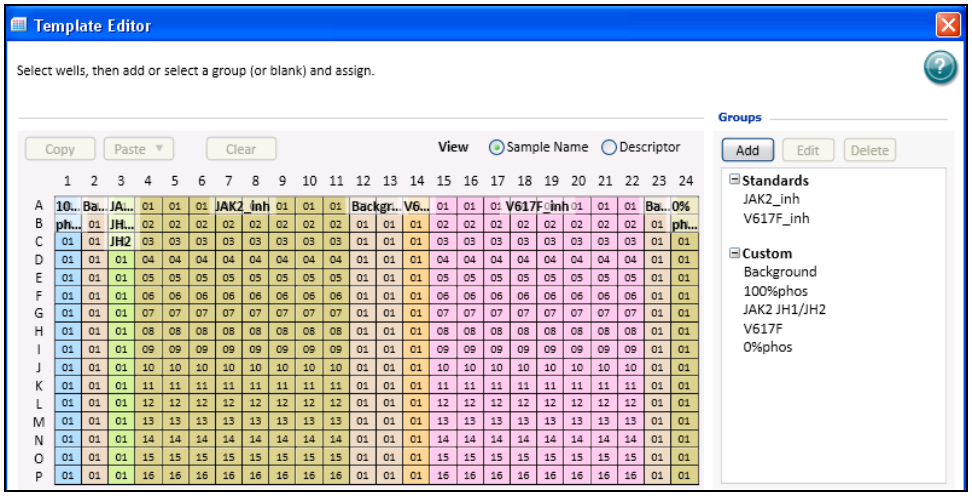
18. To set up the template for data analysis, click on Template Editor icon:



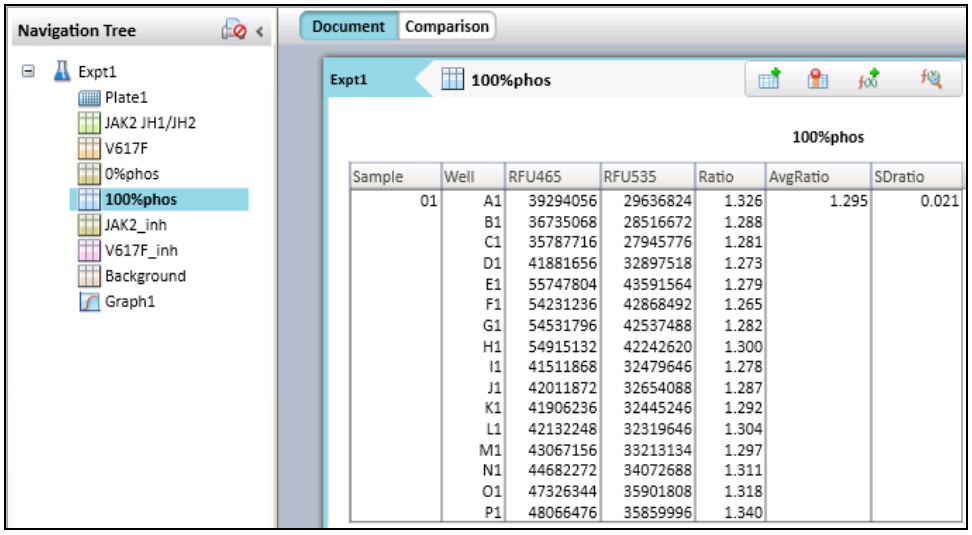
19. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.



Template with wells assigned to different template groups:



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



Sample	Well	RFU465	RFU535	Ratio	AvgRatio	SDratio
01	A1	39294056	29636824	1.326	1.295	0.021
	B1	36735068	28516672	1.288		
	C1	35787716	27945776	1.281		
	D1	41881656	32897518	1.273		
	E1	55747804	43591564	1.279		
	F1	54231236	42868492	1.265		
	G1	54531796	42537488	1.282		
	H1	54915132	42242620	1.300		
	I1	41511868	32479646	1.278		
	J1	42011872	32654088	1.287		
	K1	41906236	32445246	1.292		
	L1	42132248	32319646	1.304		
	M1	43067156	33213134	1.297		
	N1	44682272	34072688	1.311		
	O1	47326344	35901808	1.318		
	P1	48066476	35859996	1.340		

C. Results

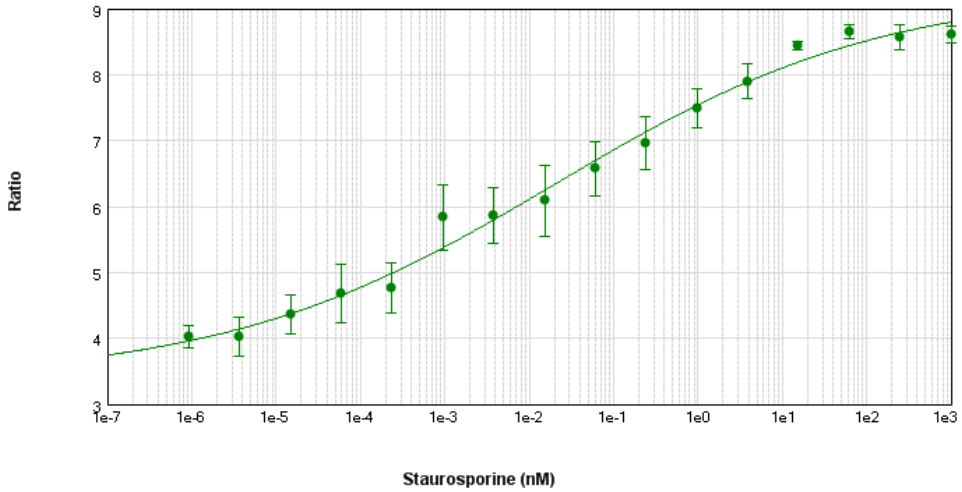


Figure 1: Z'-LYTE® Assay. JAK2 JH1/JH2 Dose-Response Curve read on the Molecular Devices SpectraMax® Paradigm® Microplate Detection Platform. Z' = 0.89.