Setup for Predictor™ hERG 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 Predictor™ FP 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.

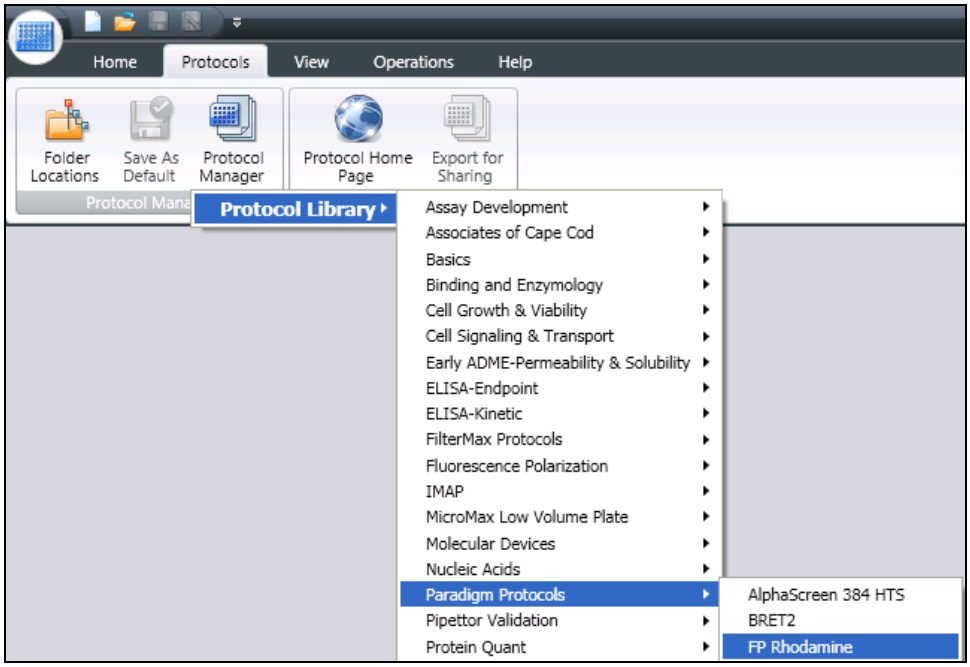
For more detailed information and technical support of Molecular Devices instruments or software, please contact Molecular Devices at 1-800-635-5577 or www.moleculardevices.com.

A. Recommended Optics

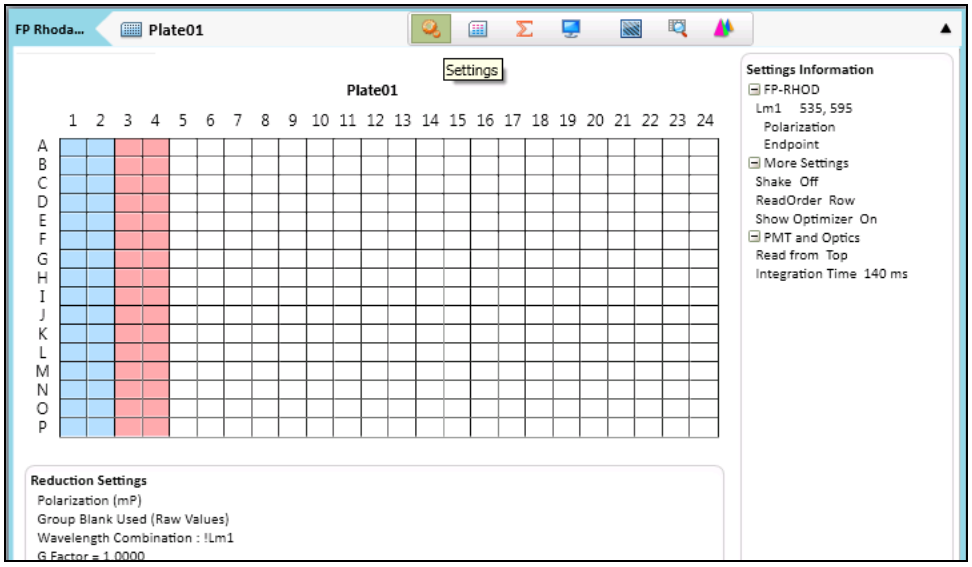
Parameter	Specification
Detection Cartridge Name	SpectraMax® Paradigm® FP Rhodamine Detection Cartridge
Part Number	0200-7010
Detection Technique	Fluorescence Polarization
Light Source	High-Power LED
Filter Set	EX: 535/25 EM Parallel: 595/35 EM Perpendicular: 595/35

B. Instrument Setup:

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

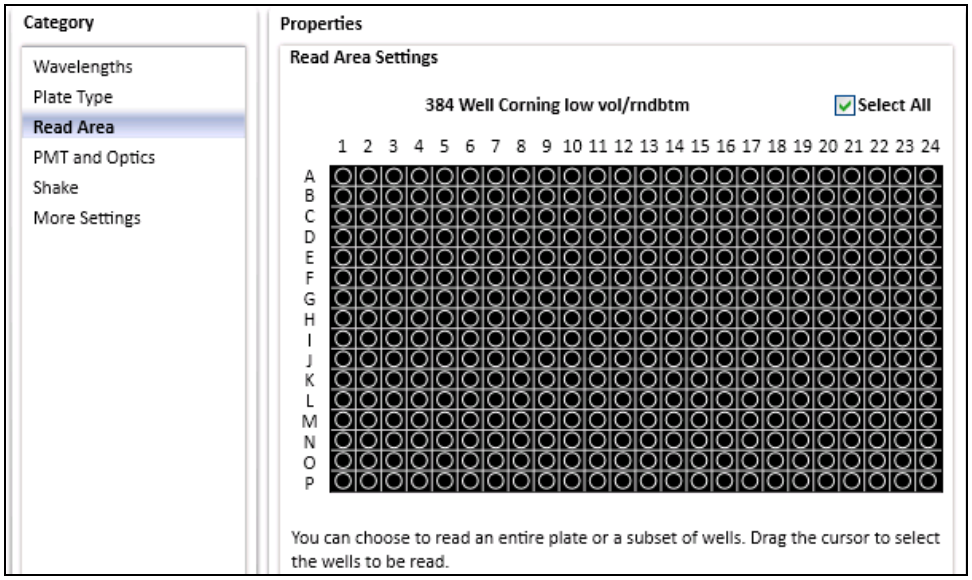


2. Click on "Plate01" in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top ofthe screen or in the plate section header.

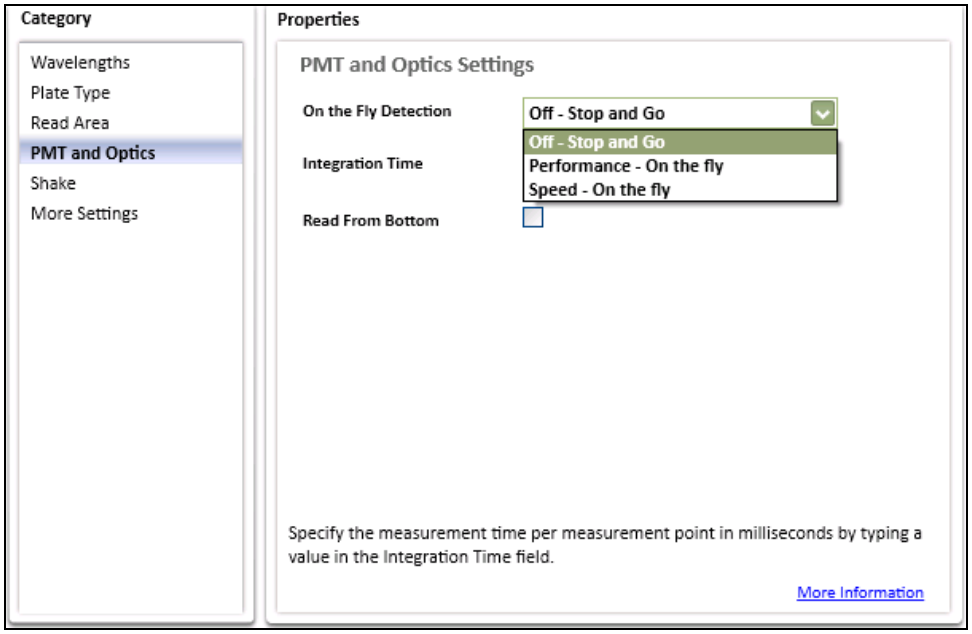


3. This opens the Settings window. The FP-Rhodamine cartridge and its wavelengths already appear under Wavelength Settings. Choose the desired plate type, using the upper dropdown menu to choose plate format (96, 384, or 1536 wells) and the "Select Specific" menu to choose the specific plate type.

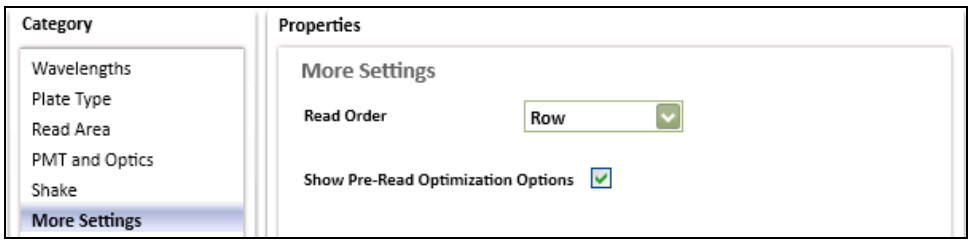
4. Now select the area of the plate to read.



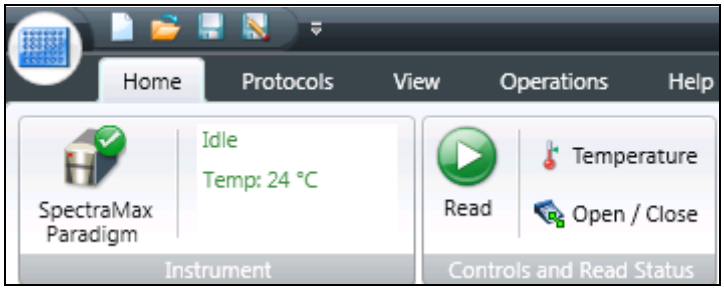
5. PMT and Optics Settings include the option to read using On the Fly detection and adjust the integration time if desired. "Off – Stop and Go" is the default setting. To select On the Fly for faster read times, use the dropdown menu to choose Performance or Speed (faster) On the Fly options. The default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



6. In the category "More Settings", choose the read order, depending on 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.



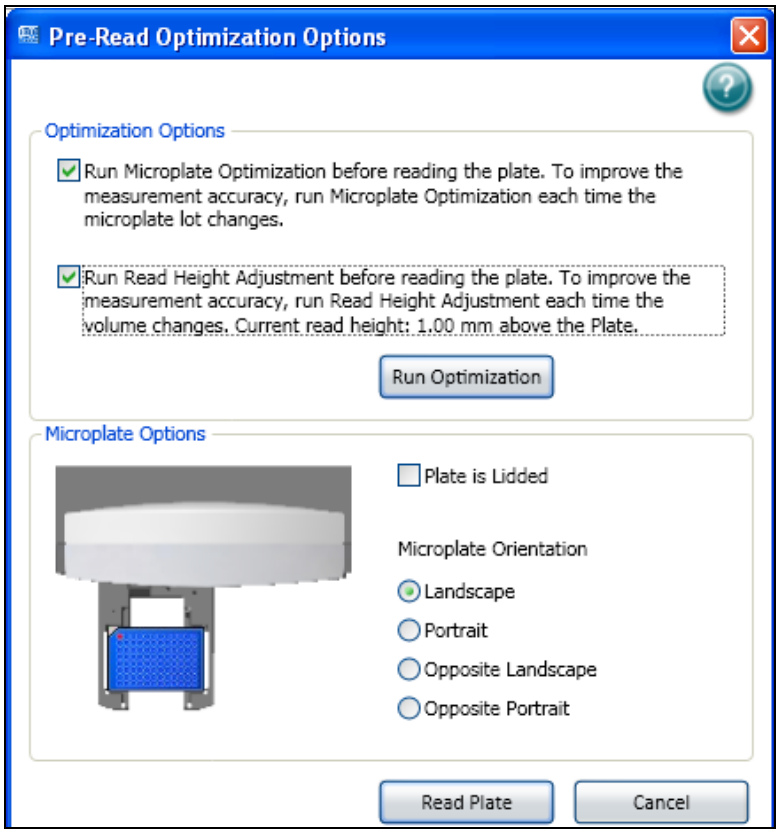
7. To read the plate, click the green "Read" button at the top of the screen.



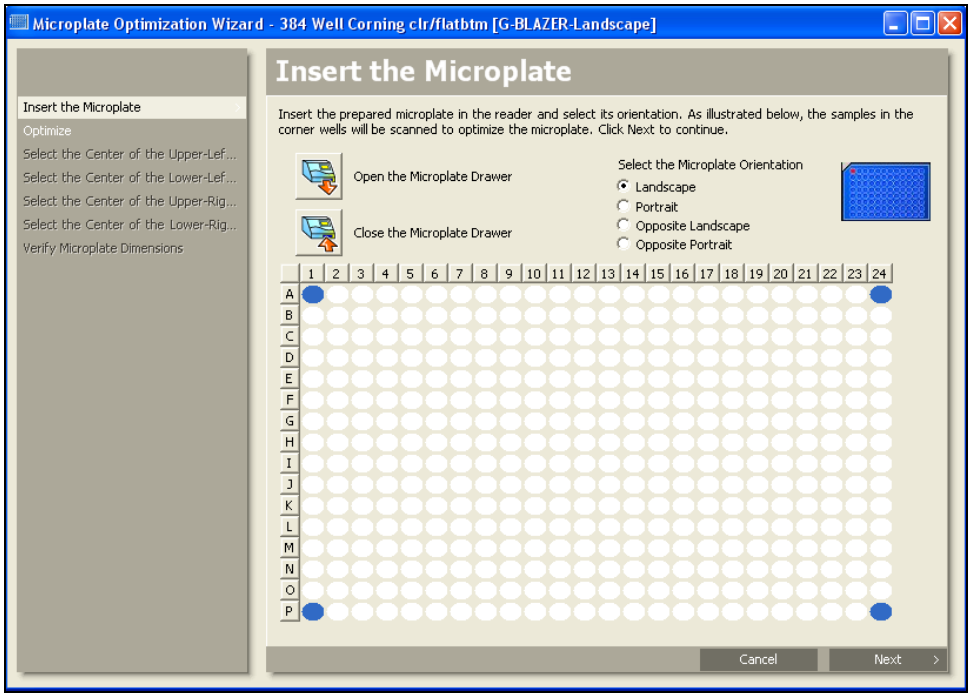
8. If selected, pre-read optimization options will appear:

   - 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.

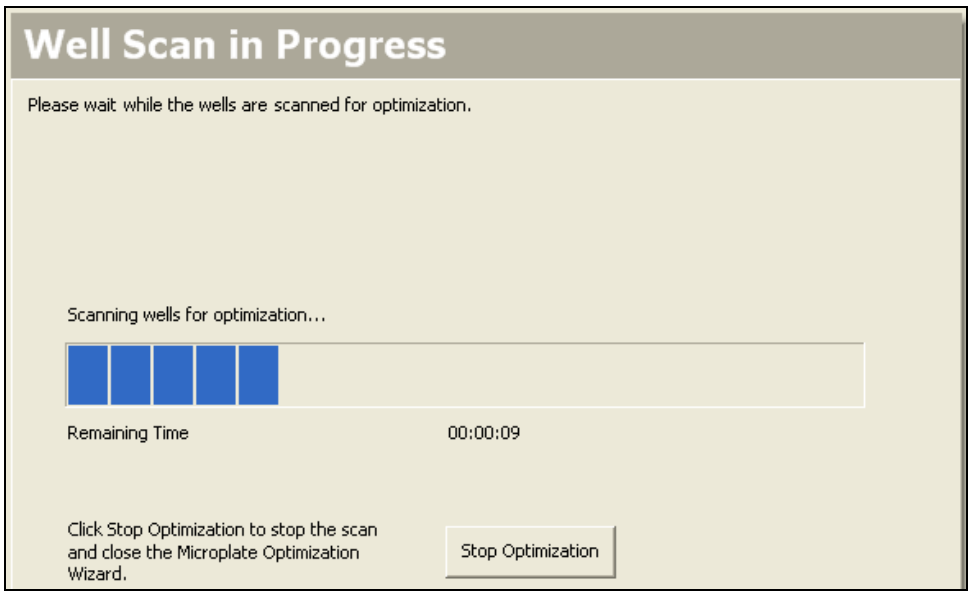
Click "Run Optimization" to proceed. Alternatively, if no optimization is desired, leave the boxes unchecked and click "Read Plate."



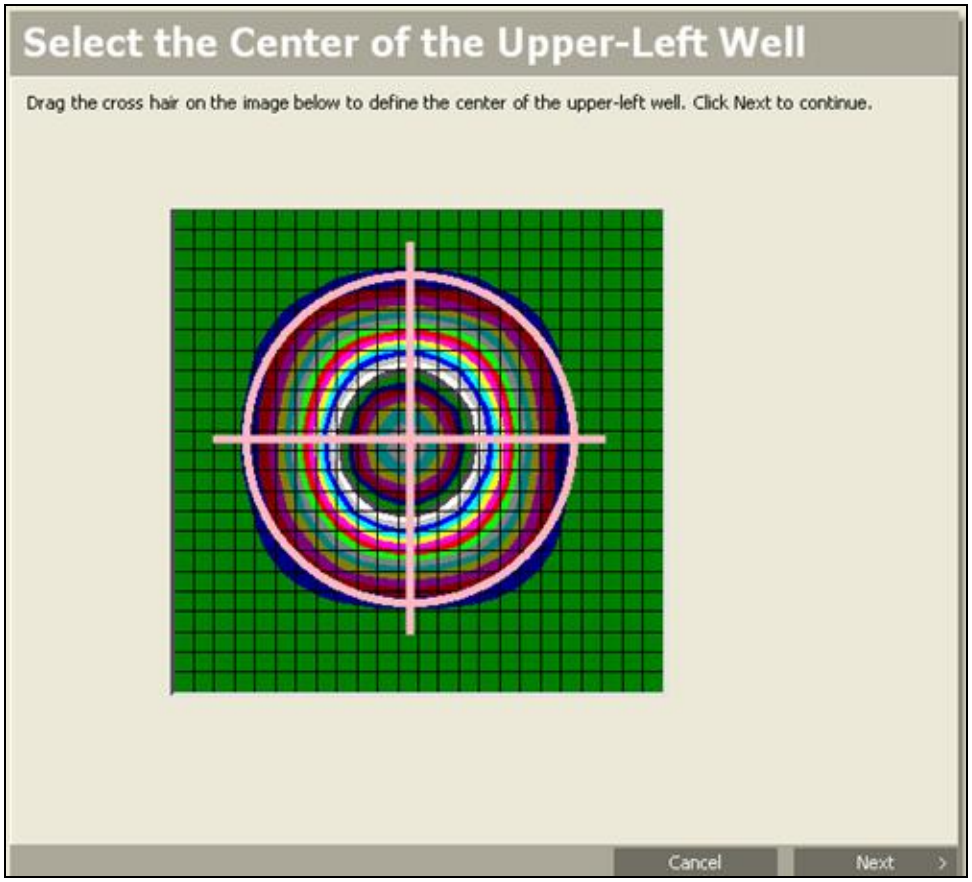
9. If optimization was selected, a wizard will pop up. Follow the steps outlined in the wizard.



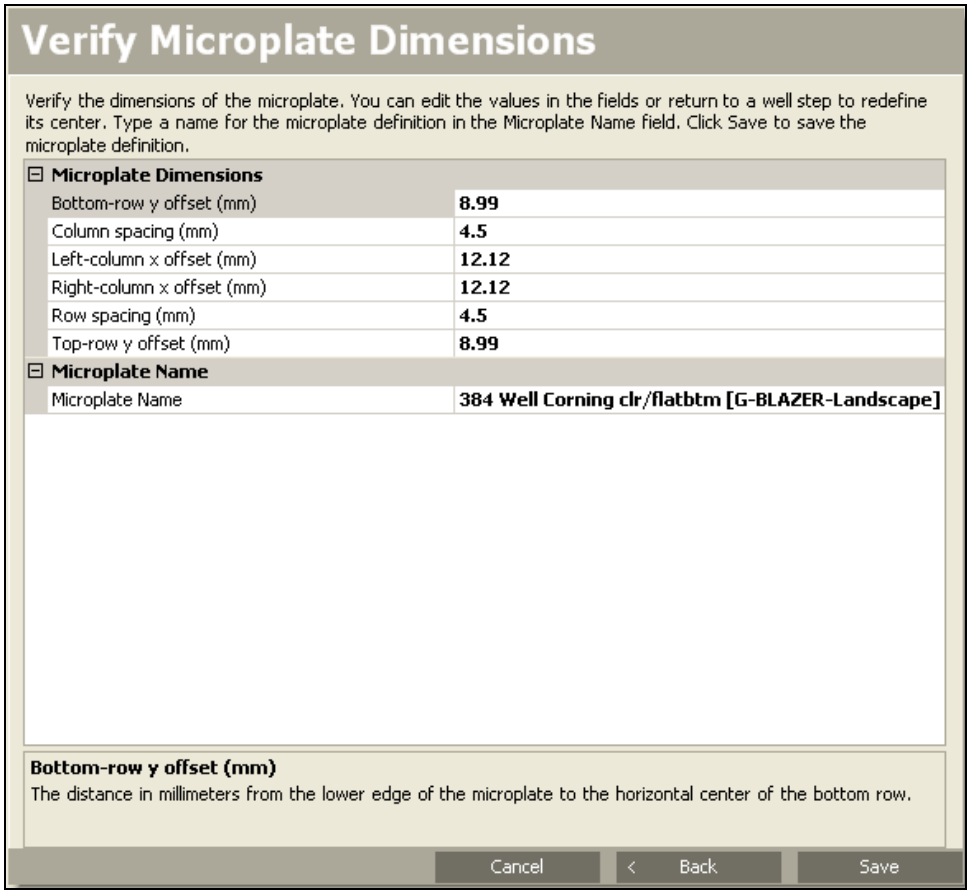
10. When you select "Next," a progress screen will appear as wells are scanned.



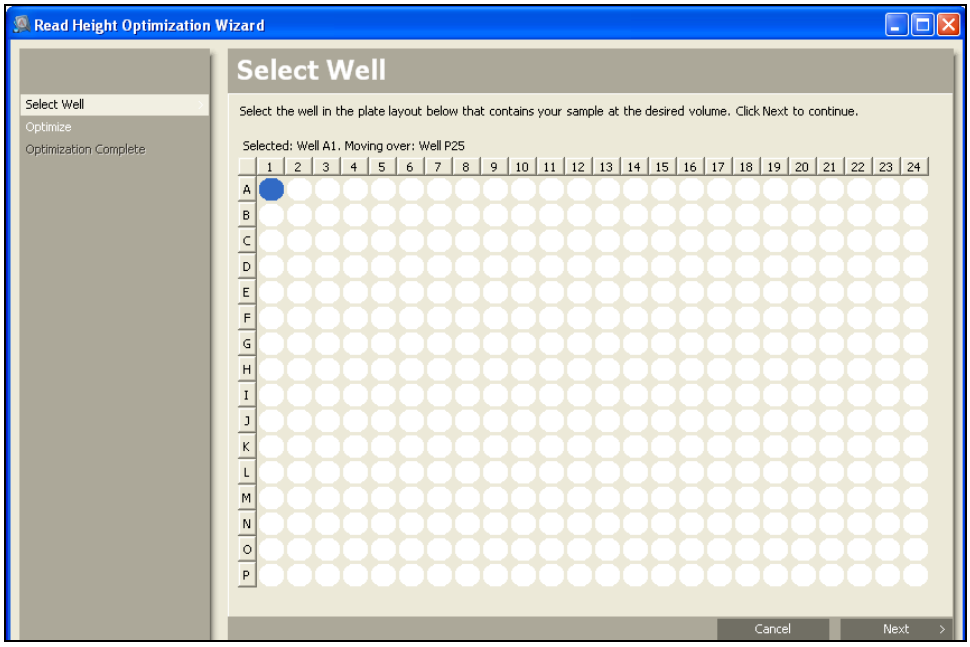
11. Center the pink target over the image of the scanned well. Click "Next" and repeat for the remaining three wells. This adjusts the microplate definition to match the actual plate.



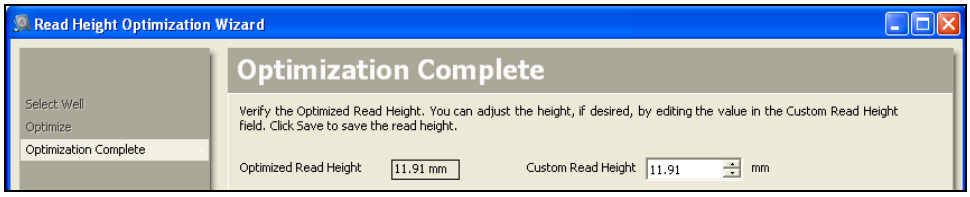
12. Click "Save" to save the modified plate dimensions with the Microplate Name as shown. This optimized microplate type will be available in the Settings for future use.



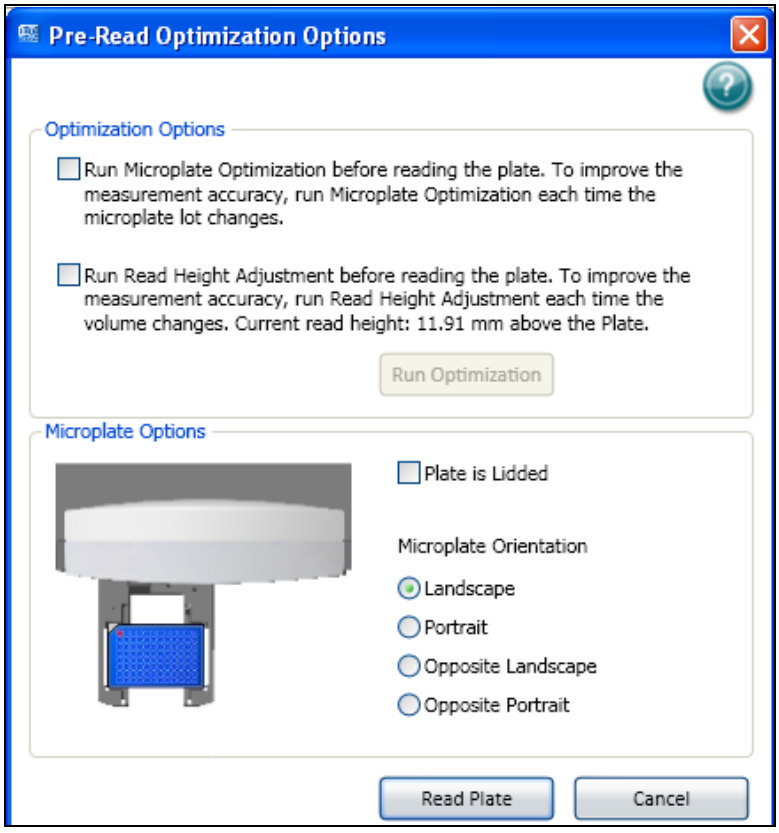
13. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control. Click "Next" to read.



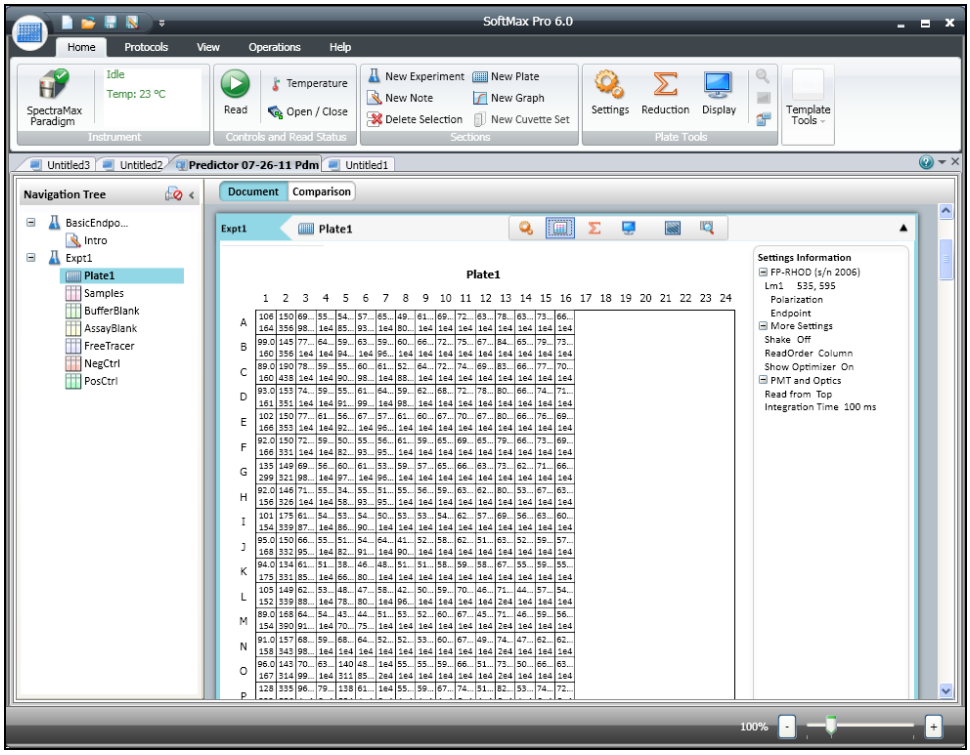
14. The instrument will calculate and report optimized read height. Click “Save.”



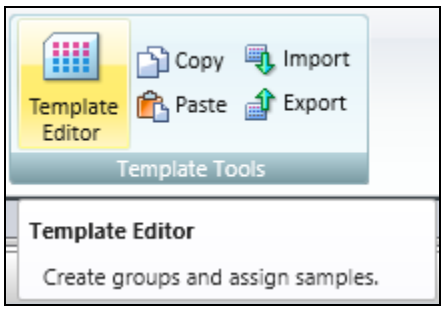
15. After optimization is complete, click on "Read Plate" to proceed.



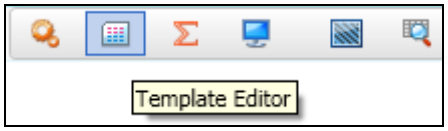
16. After the plate is read, data will appear in the plate section:



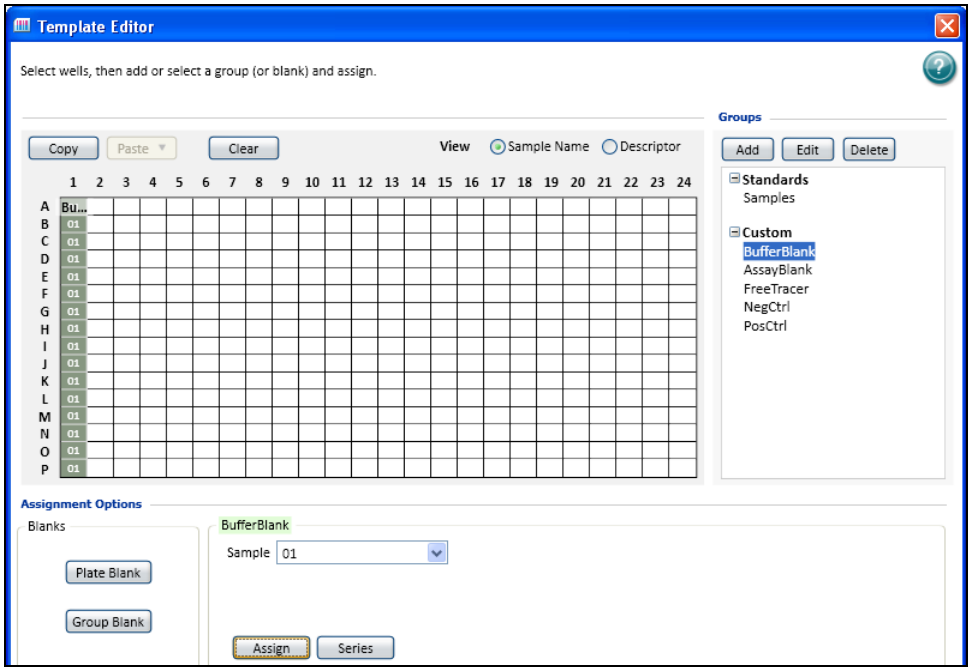
17. To set up the template for data analysis, click on Template Editor icon in the top toolbar…



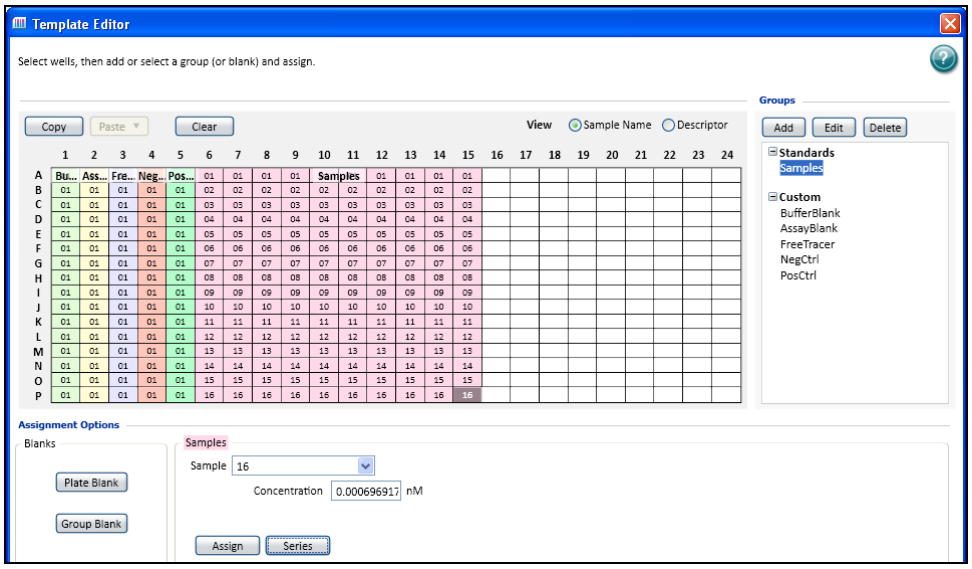
…or on the plate section header.



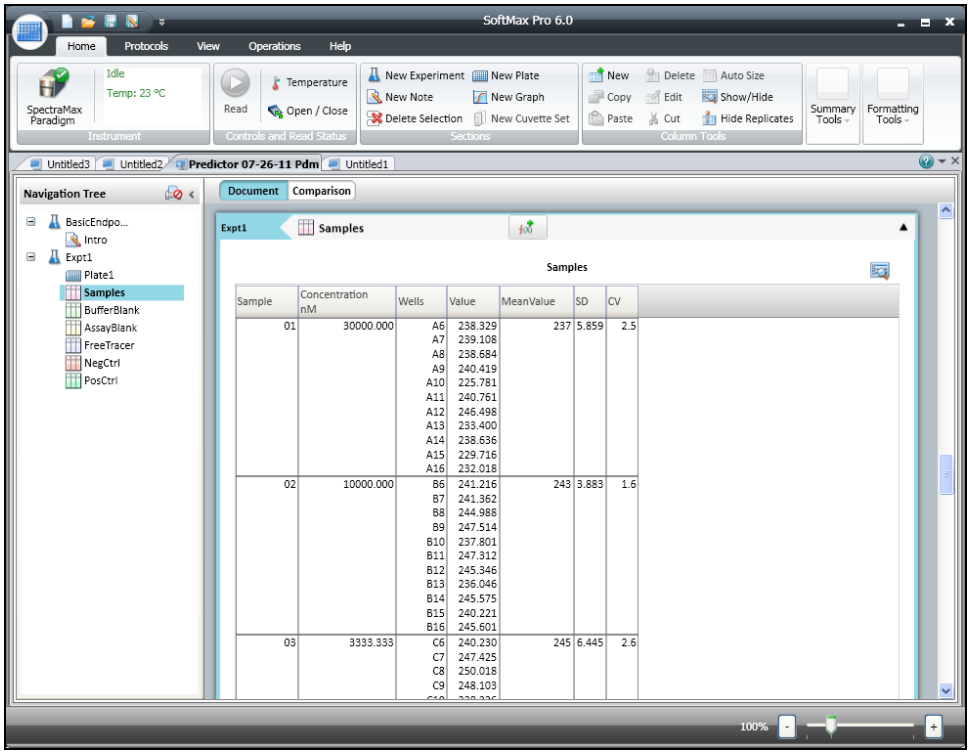
18. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.



Template with wells assigned to different template groups:



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



C. Results

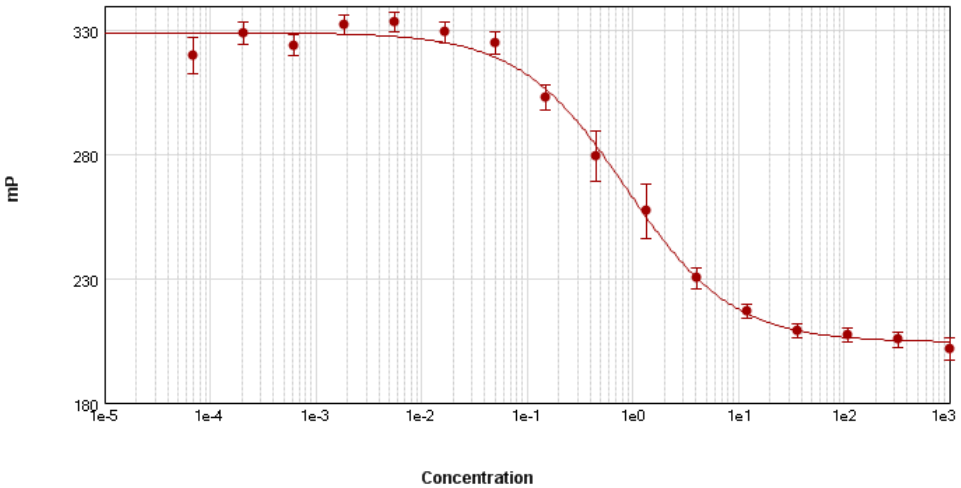


Figure 1: Predictor™ hERG FP Assay. Dose-Response Curves read on the Molecular Devices SpectraMax® Paradigm® Multilabel Plate Reader using the Predictor™ assay and 1:3 dilution series prepared for Astemizole from a starting concentration of 10 µM. Curve calculations were baseline-corrected against duplicate dilution series prepared in saturating E-4031.

Table 1. Predictor™ Assay Results on the Molecular Devices SpectraMax® Paradigm® microplate reader.

	Avg.	Std. Dev.
free tracer	143.64	5.09
no inhibitor	328.50	6.24
30 µM E-4031	194.02	3.90
ΔmP	134.48	
Z'-factor	0.77	
Astemizole IC_{50}	1.87 nM	