

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.

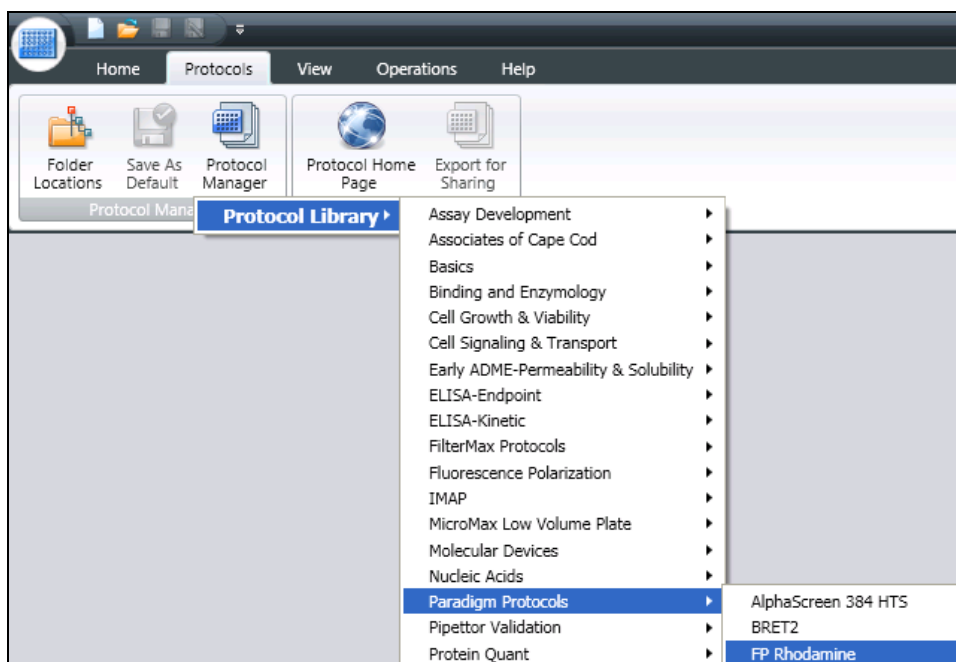
Setup Guide on the Molecular Devices SpectraMax® Paradigm® Microplate Detection Platform

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

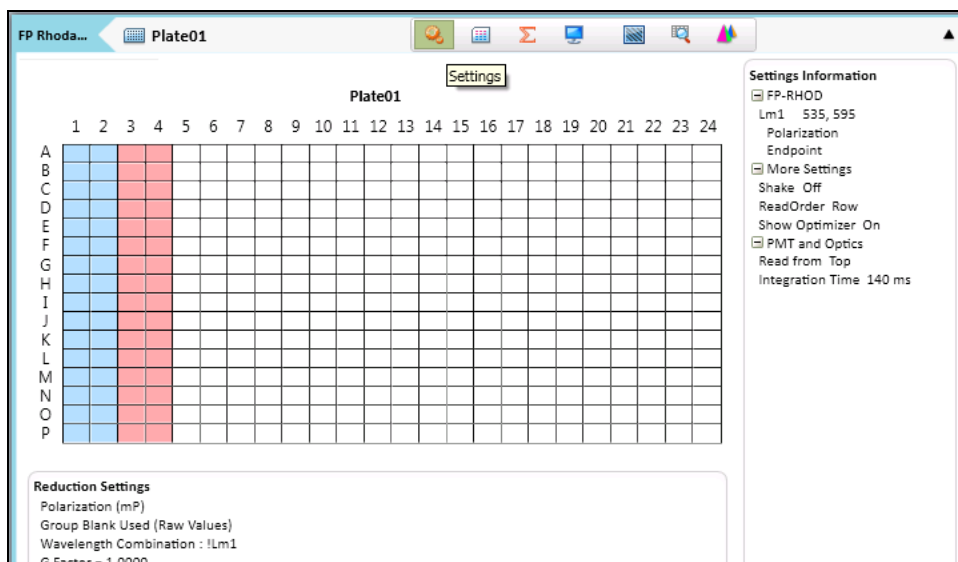
B. Instrument Setup:

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.



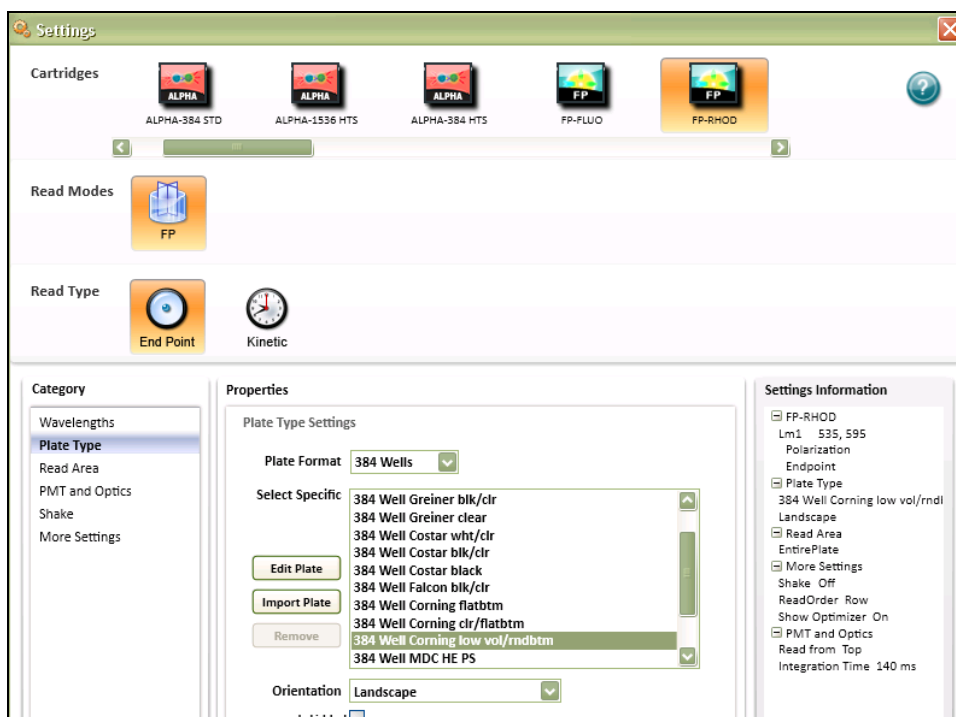
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- Click on "Plate01" in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen or in the plate section header.



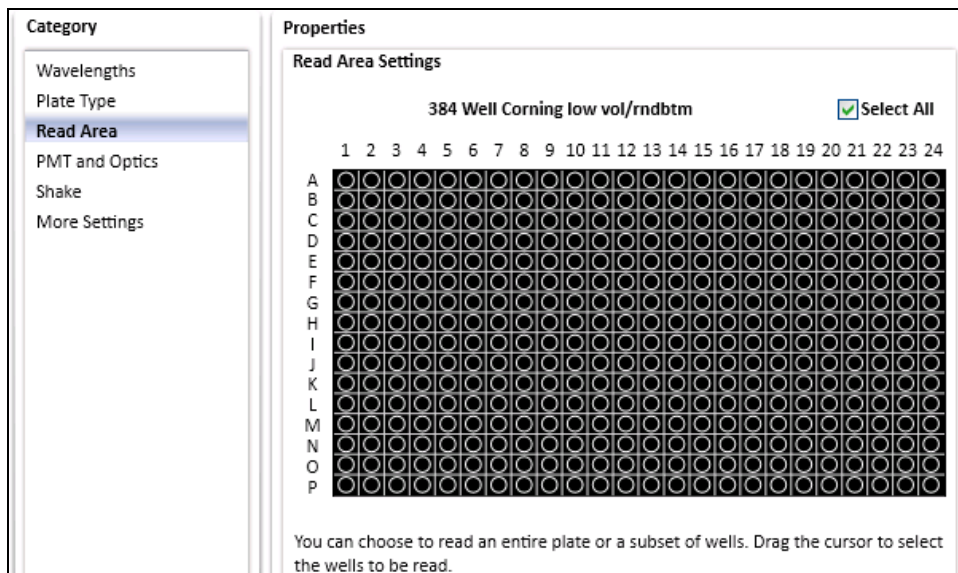
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3. This opens the Settings window. The FP-Rhodamine cartridge and its wavelengths already appear under Wavelength Settings. Choose the desired plate type, using the upper dropdown menu to choose plate format (96, 384, or 1536 wells) and the "Select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



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- Now select the area of the plate to read.



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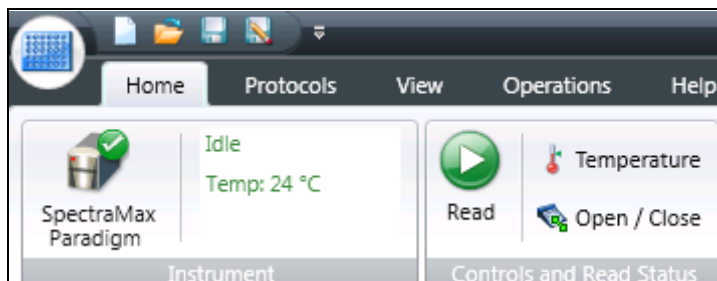
5. PMT and Optics Settings include the option to read using On the Fly detection and adjust the integration time if desired. "Off – Stop and Go" is the default setting. To select On the Fly for faster read times, use the dropdown menu to choose Performance or Speed (faster) On the Fly options. The default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

Category	Properties
- Wavelengths - Plate Type - Read Area PMT and Optics - Shake - More Settings	PMT and Optics Settings On the Fly Detection: Off - Stop and Go ▼ Integration Time: Off - Stop and Go Performance - On the fly Speed - On the fly Read From Bottom: ☐ Specify the measurement time per measurement point in milliseconds by typing a value in the Integration Time field. More Information

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.

Category	Properties
- Wavelengths - Plate Type - Read Area - PMT and Optics - Shake More Settings	More Settings Read Order: Row ▼ Show Pre-Read Optimization Options: ☒

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

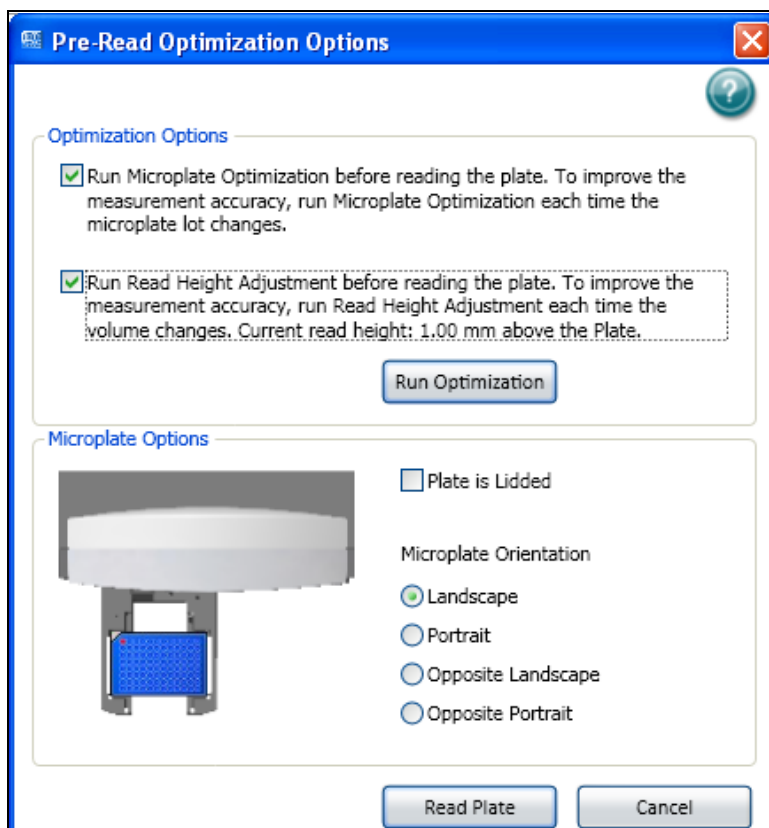


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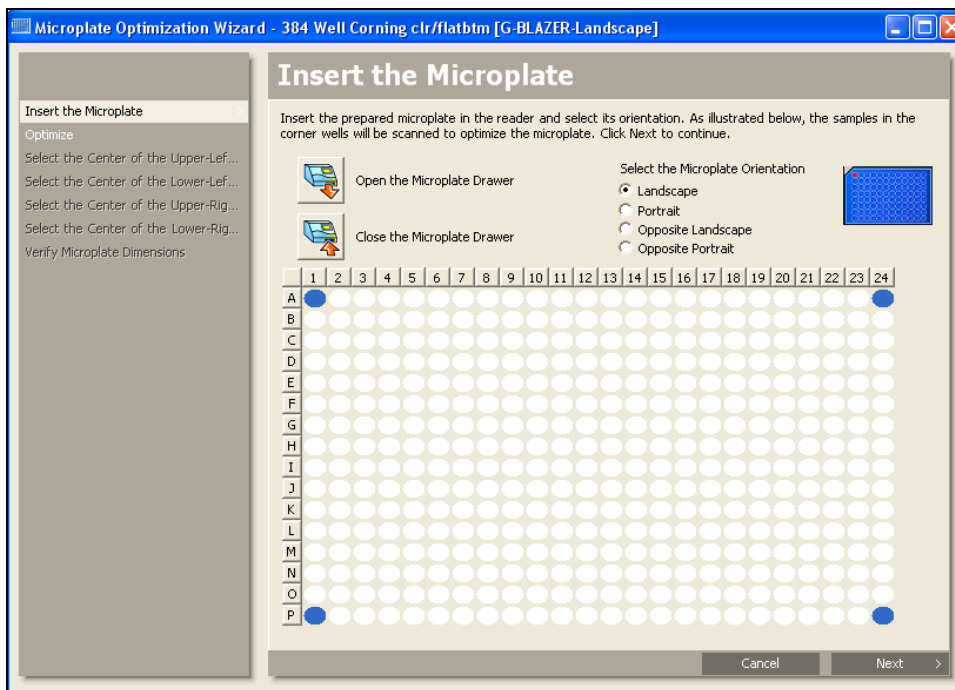
8. If selected, pre-read optimization options will appear:

- 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.

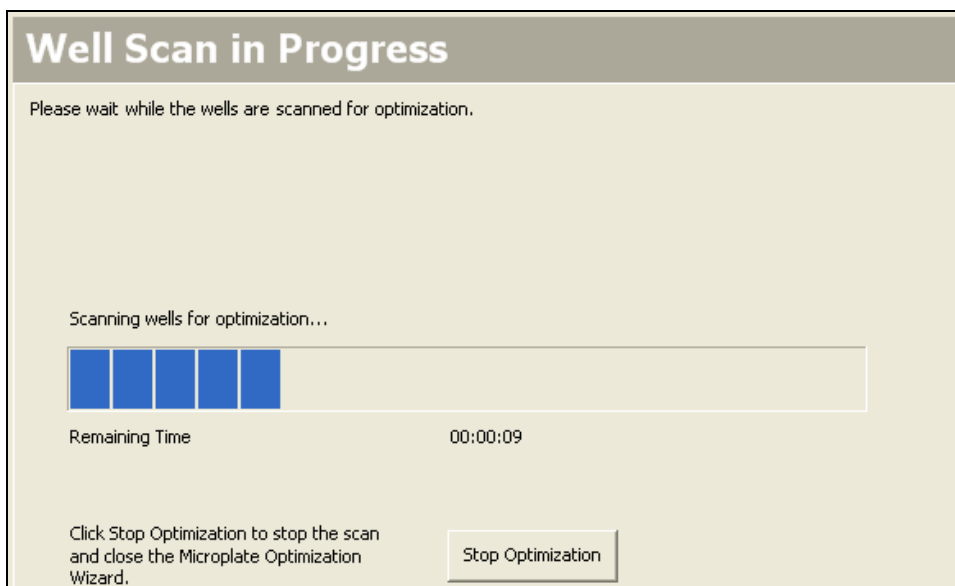
Click "Run Optimization" to proceed. Alternatively, if no optimization is desired, leave the boxes unchecked and click "Read Plate."



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

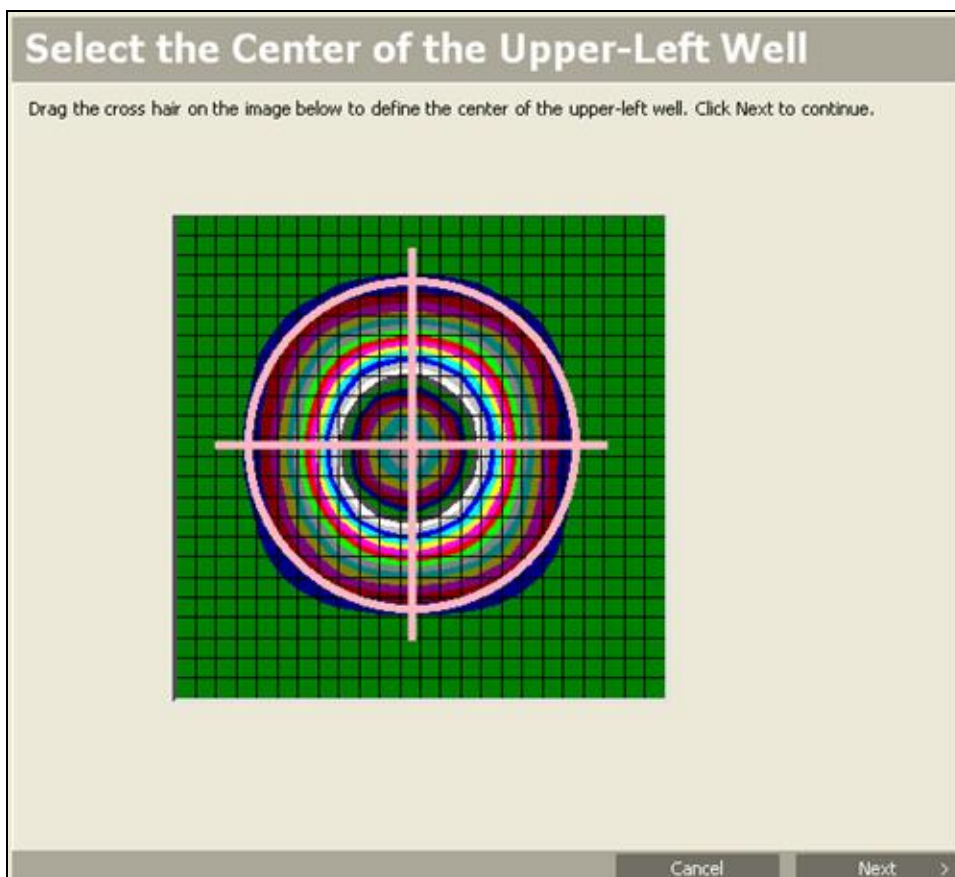


10. When you select "Next," a progress screen will appear as wells are scanned.



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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.



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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.

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.

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
Microplate Name	
Microplate Name	384 Well Corning clr/flatbtm [G-BLAZER-Landscape]

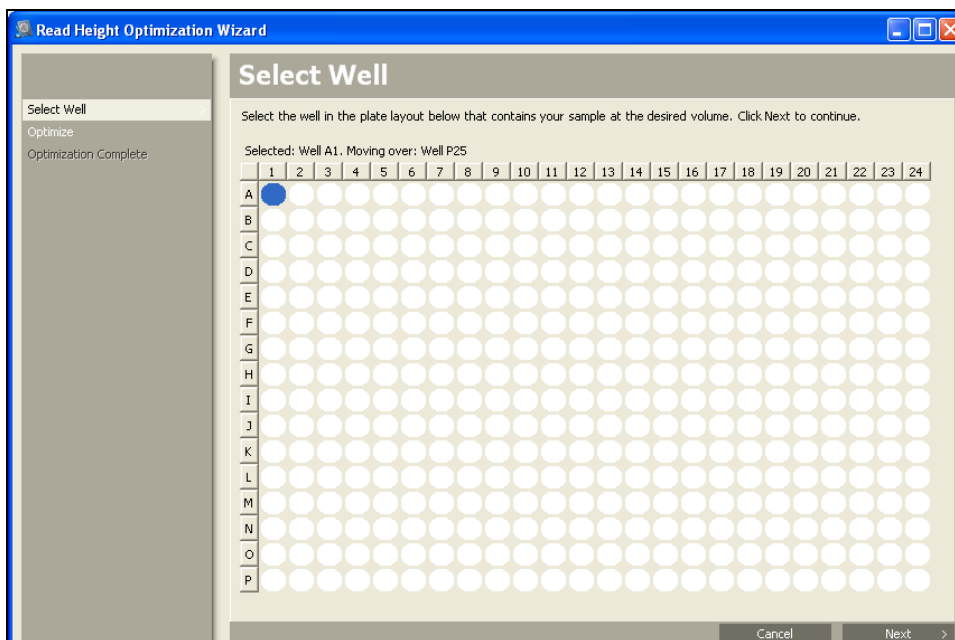
Bottom-row y offset (mm)

The distance in millimeters from the lower edge of the microplate to the horizontal center of the bottom row.

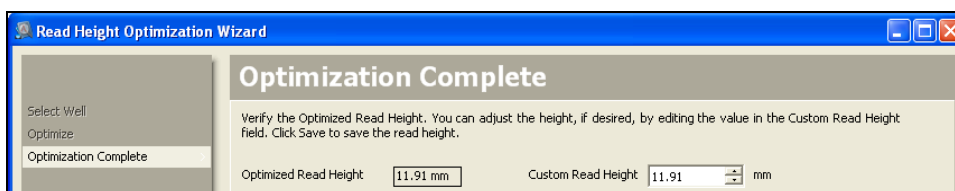
Cancel
< Back
Save

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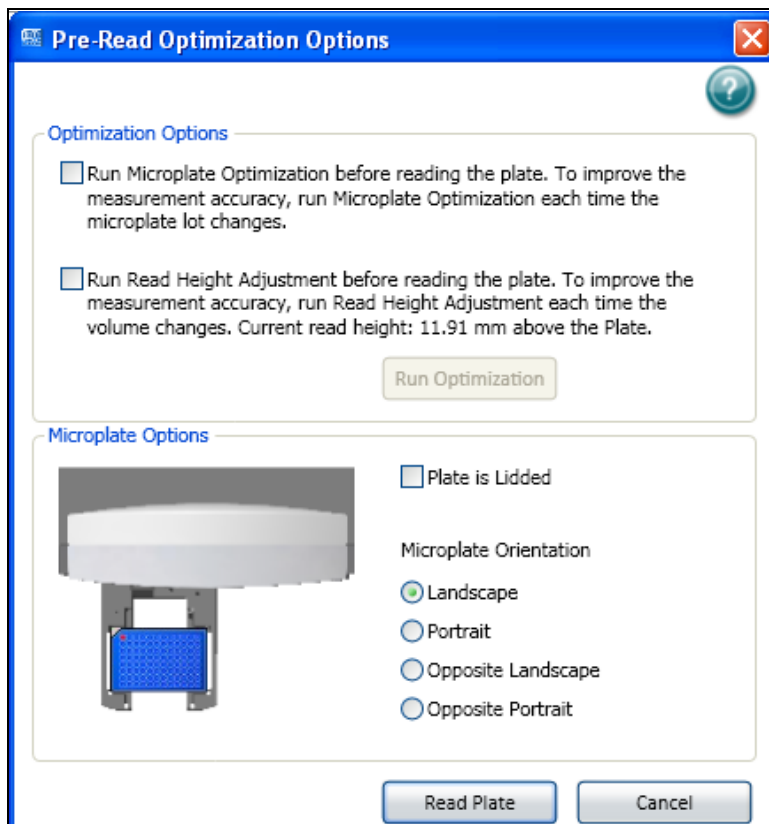
13. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control. Click "Next" to read.



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

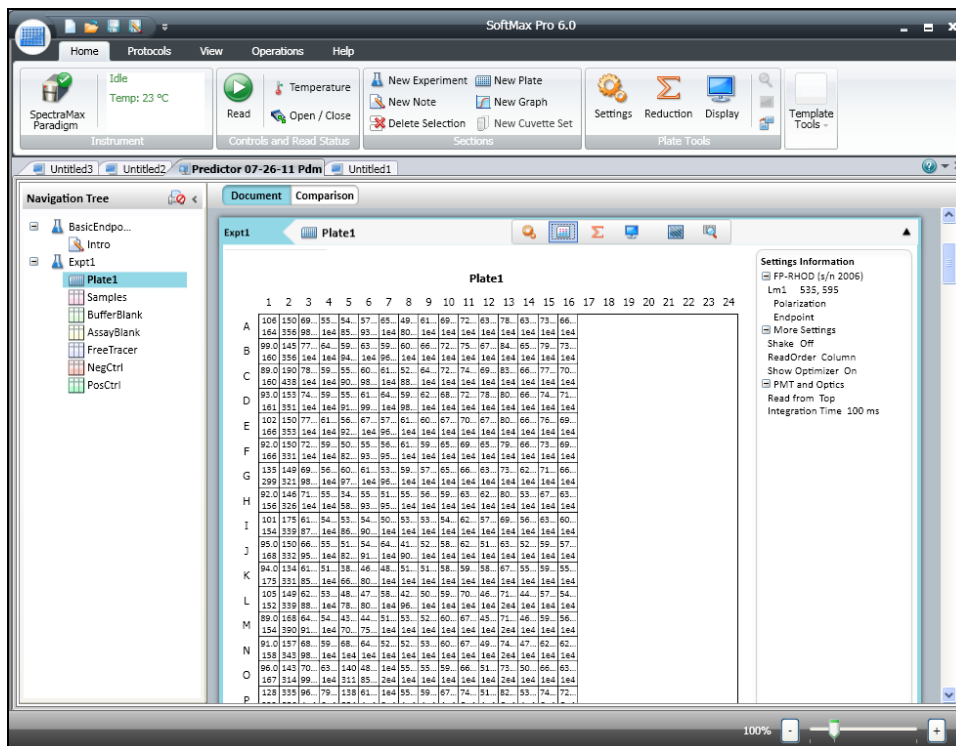


15. After optimization is complete, click on "Read Plate" to proceed.

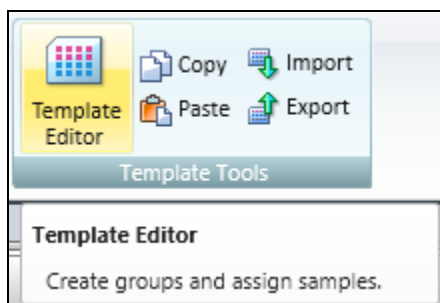


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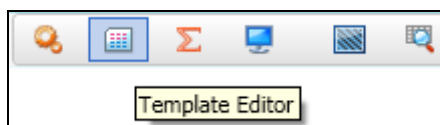
16. After the plate is read, data will appear in the plate section:



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



...or on the plate section header.



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

The screenshot shows the 'Template Editor' window. At the top, it says 'Select wells, then add or select a group (or blank) and assign.' Below this is a 96-well plate grid with columns 1-24 and rows A-P. To the right of the grid is a 'Groups' panel with buttons 'Add', 'Edit', and 'Delete'. Under 'Groups', there are two sections: 'Standards' with 'Samples' and 'Custom' (containing BufferBlank, AssayBlank, FreeTracer, NegCtrl, PosCtrl). Below the grid is the 'Assignment Options' section. It has a 'Blanks' section with 'Plate Blank' and 'Group Blank' buttons. To the right, there's a 'BufferBlank' section with a 'Sample' dropdown set to '01' and 'Assign' and 'Series' buttons.

Template with wells assigned to different template groups:

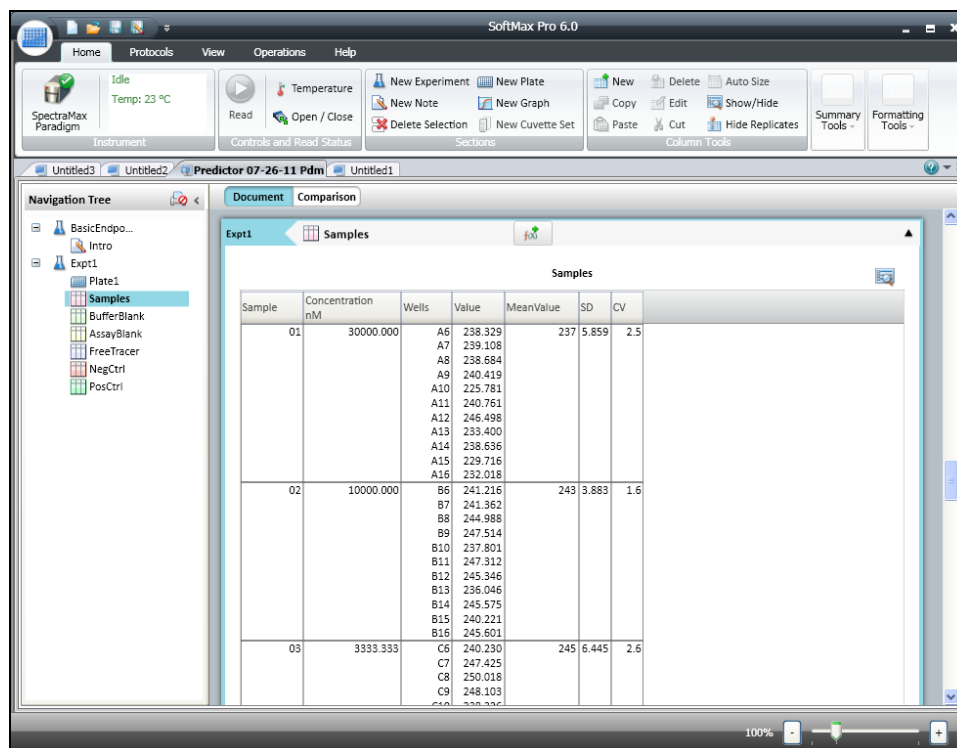
The screenshot shows the 'Template Editor' window with the 96-well plate grid populated with various template group assignments. The grid shows columns 1-24 and rows A-P. The assignments are as follows:

	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	Blank	AssayBlank	FreeTracer	NegCtrl	PosCtrl	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
B	01	01	01	01	01	02	02	02	02	02	02	02	02	02	02	02	02	02	02	02	02	02	02	02
C	01	01	01	01	01	03	03	03	03	03	03	03	03	03	03	03	03	03	03	03	03	03	03	03
D	01	01	01	01	01	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04
E	01	01	01	01	01	05	05	05	05	05	05	05	05	05	05	05	05	05	05	05	05	05	05	05
F	01	01	01	01	01	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06
G	01	01	01	01	01	07	07	07	07	07	07	07	07	07	07	07	07	07	07	07	07	07	07	07
H	01	01	01	01	01	08	08	08	08	08	08	08	08	08	08	08	08	08	08	08	08	08	08	08
I	01	01	01	01	01	09	09	09	09	09	09	09	09	09	09	09	09	09	09	09	09	09	09	09
J	01	01	01	01	01	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
K	01	01	01	01	01	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
L	01	01	01	01	01	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
M	01	01	01	01	01	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
N	01	01	01	01	01	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
O	01	01	01	01	01	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
P	01	01	01	01	01	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16

 The 'Assignment Options' section at the bottom shows 'Blanks' with 'Plate Blank' and 'Group Blank' buttons. To the right, there's a 'Samples' section with a 'Sample' dropdown set to '16' and a 'Concentration' field set to '0.000696917 nM'. 'Assign' and 'Series' buttons are at the bottom.

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



The screenshot shows the SoftMax Pro 6.0 software interface. The main window displays a table of data for Sample 01. The table has columns for Sample, Concentration nM, Wells, Value, MeanValue, SD, and CV. The data is organized into three groups: Sample 01 (Concentration 30000.000), Sample 02 (Concentration 10000.000), and Sample 03 (Concentration 3333.333). Each group contains a list of wells and their corresponding values.

Sample	Concentration nM	Wells	Value	MeanValue	SD	CV					
01	30000.000	A6	238.329	237	5.859	2.5					
		A7	239.108								
		A8	238.684								
		A9	240.419								
		A10	225.781								
		A11	240.761								
		A12	246.498								
		A13	233.400								
		A14	238.636								
		A15	229.716								
		A16	232.018								
		02	10000.000				B6	241.216	243	3.883	1.6
							B7	241.362			
							B8	244.988			
							B9	247.514			
							B10	237.801			
B11	247.312										
B12	245.346										
B13	236.046										
03	3333.333	C6	240.230	245	6.445	2.6					
		C7	247.425								
		C8	250.018								
		C9	248.103								

C. Results

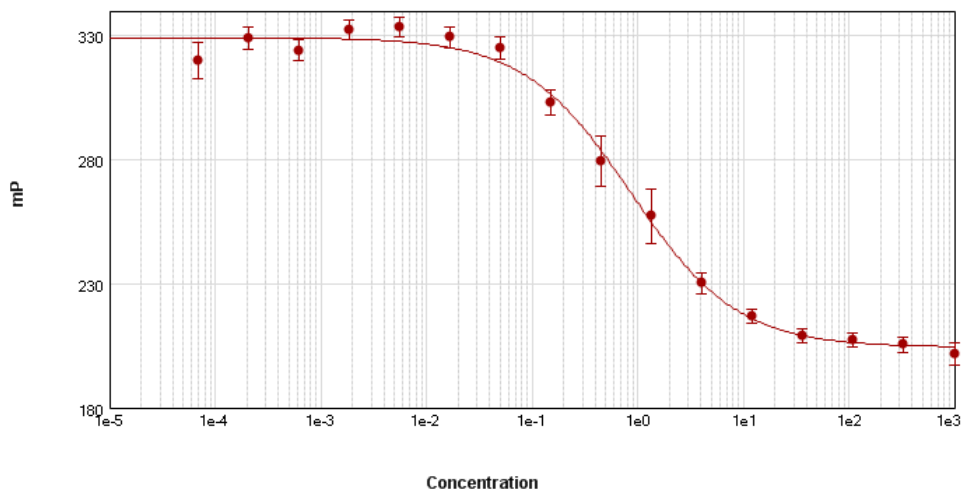


Figure 1: Predictor™ hERG FP Assay. Dose-Response Curves read on the Molecular Devices SpectraMax® Paradigm® Multilabel Plate Reader using the Predictor™ assay and 1:3 dilution series prepared for Astemizole from a starting concentration of 10 μM. Curve calculations were baseline-corrected against duplicate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