

Setup for LanthaScreen® Europium Assays on SpectraMax® M5/M5e Microplate Reader with SoftMax® Pro 6 Software

IMPORTANT INFORMATION

Test your plate reader set-up before using LanthaScreen® Terbium and Europium assays

We have developed two technical notes which provide a method for verifying that a fluorescent plate reader is able to detect a change in time-resolved fluorescence energy transfer (TR-FRET) signal, confirming proper instrument set-up and a suitable response. The method is independent of any biological reaction or equilibrium and uses reagents that are on-hand for the LanthaScreen® assay.

For complete instructions, visit www.lifetechnologies.com/instrumentsetup and click on “[Download Terbium assay application note](#)” or “[Download Europium assay application note](#).”

Molecular Devices SpectraMax M5/M5e Microplate Reader was tested for compatibility with Life Technologies LanthaScreen® Kinase Binding and Adapta™ Europium-based TR-FRET assays. The following document is intended to demonstrate setup of this instrument and provide representative data. **These settings are also valid for the SpectraMax M3/M4 and FlexStation® 3 Multi-Mode Microplate Readers.**

For more detailed information and technical support of Life Technologies assays including specific conditions for assay windows between 2-3 fold, 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.

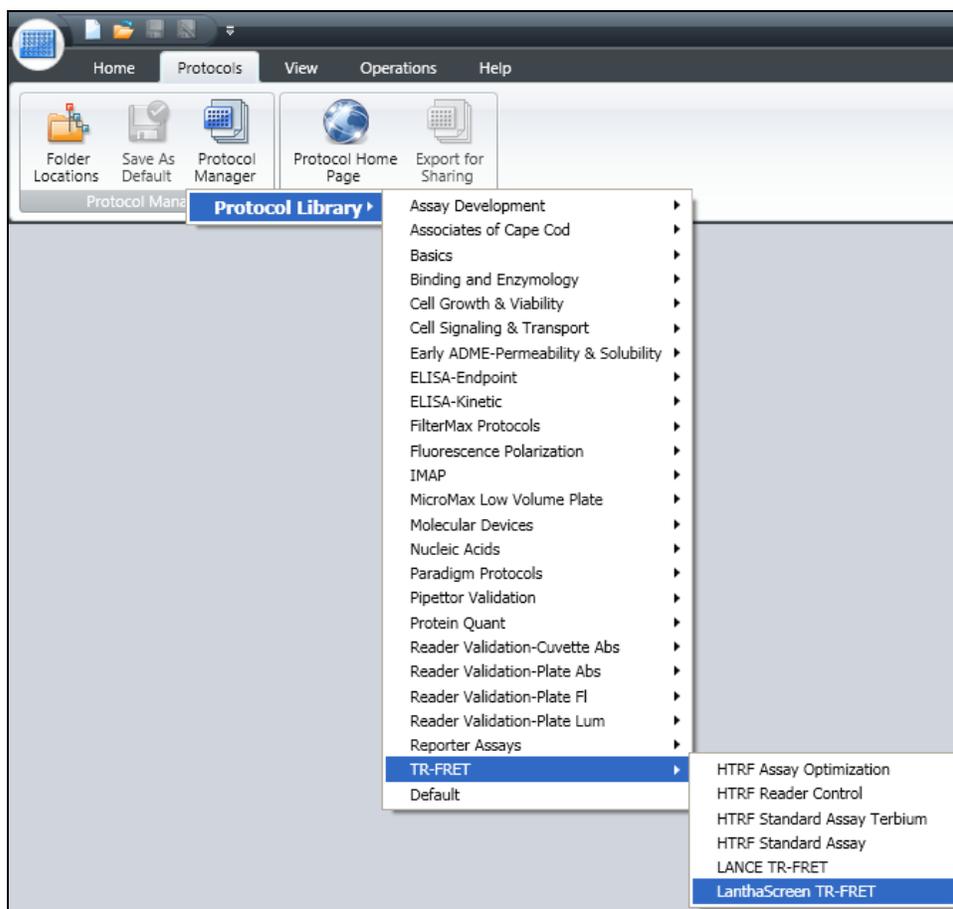
Setup Guide on Molecular Devices SpectraMax® M5/M5e Microplate Reader

A. Recommended Optics

	Wavelength (nm)	Wavelength selection
Excitation	332/9	Monochromator
Emission 1	620/15	Monochromator
Emission 2	665/15	Monochromator
Emission 1 Cutoff	550	Filter
Emission 2 Cutoff	550	Filter

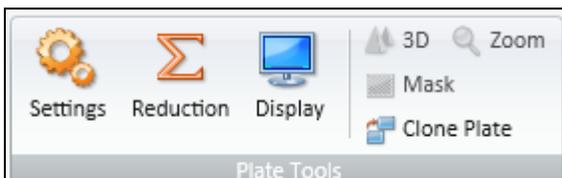
B. Instrument Setup

1. Open SoftMax® Pro 6 software. Click on "Protocol Manager" to open the Protocol Library. Within the "TR-FRET" folder, locate the "LanthaScreen TR-FRET" protocol and click to open.



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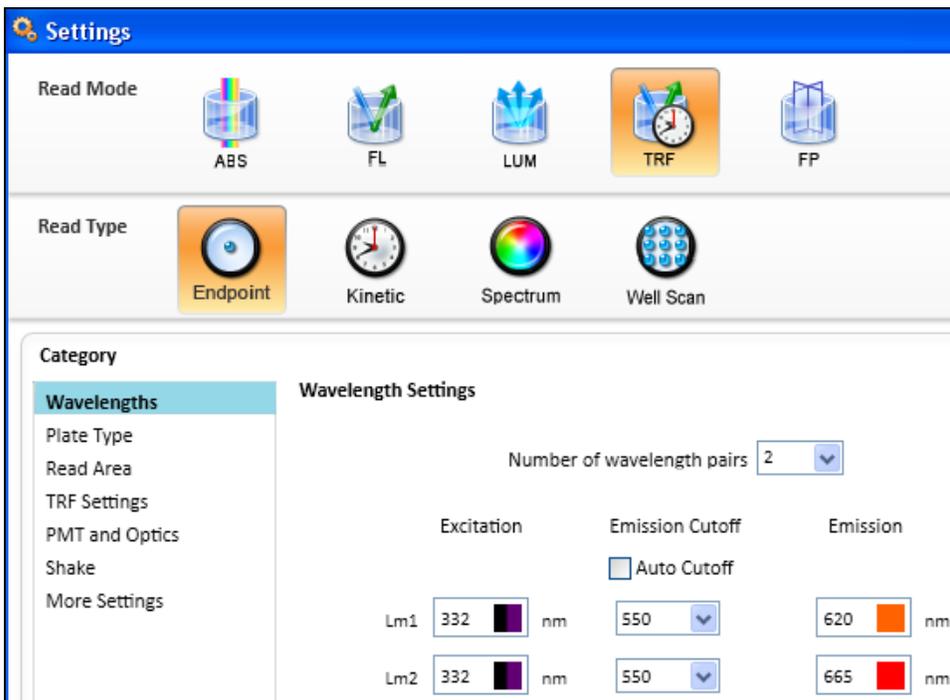
2. Click on the microplate icon in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen...



...or in the plate section header.

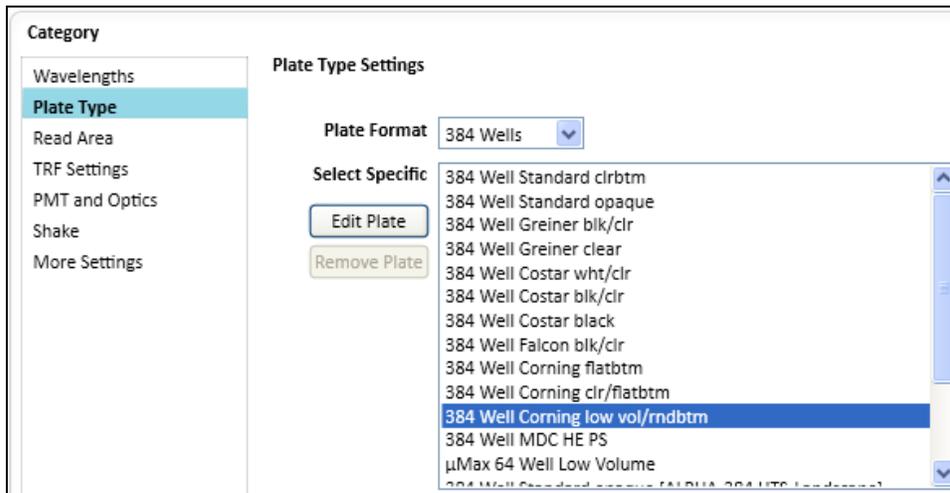


3. This opens the Settings window. TRF read mode and Endpoint read type are already selected in the pre-configured protocol. The default LanthaScreen protocol contains optimal settings for assays using a Terbium donor and fluorescein or similar acceptor. To use the protocol for assays with a Europium donor and Alexa Fluor 647 or similar acceptor, the wavelengths must be modified to match those in the screenshot below:

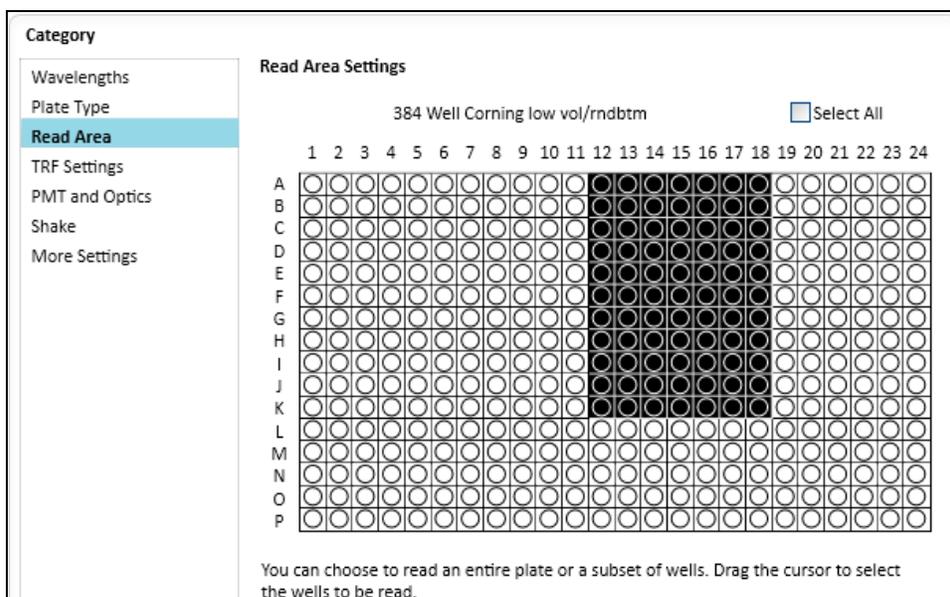


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- Choose the desired plate type, using the upper dropdown menu to choose plate format (96 or 384 wells) and the "Select Specific" menu to choose the specific plate type.

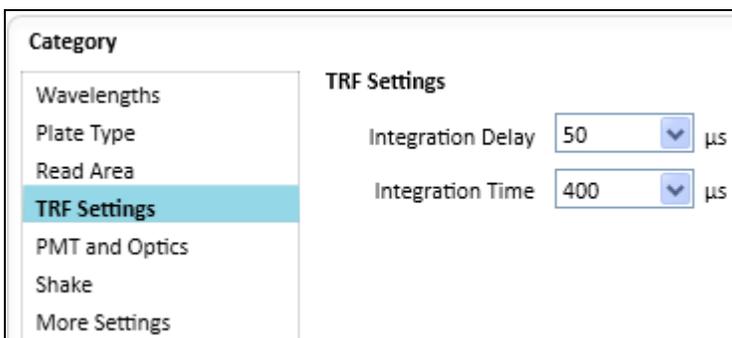


- Now select the area of the plate to read.



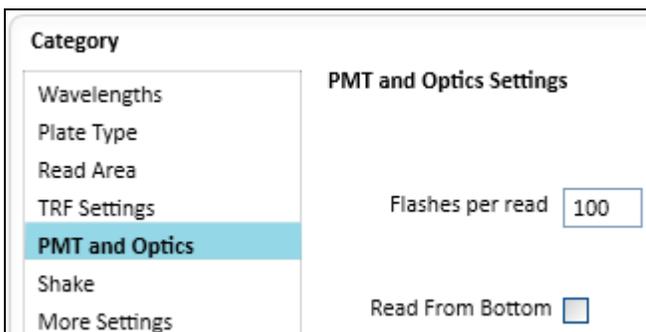
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6. In TRF Settings, set the Integration Delay to 50 μ s and the Integration Time to 400 μ s. Note: typical settings for LanthaScreen assays are 100 μ s delay and 200 μ s integration; optimizing the delay and integration may improve assay window, but in general the SpectraMax® performs better with the delay and integration times listed here.



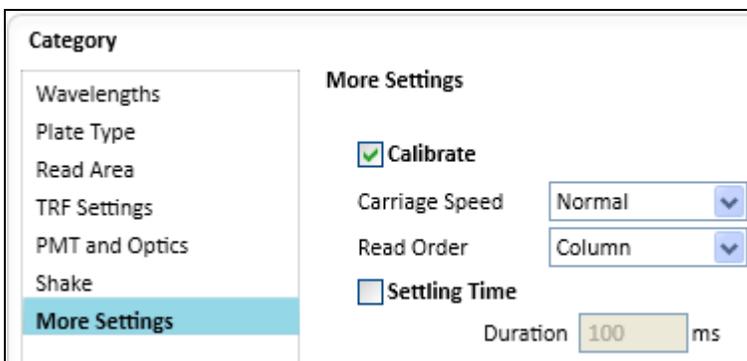
Category	TRF Settings
Wavelengths	
Plate Type	
Read Area	
TRF Settings	Integration Delay: 50 μ s
PMT and Optics	Integration Time: 400 μ s
Shake	
More Settings	

7. PMT and Optics, Flashes per read should be set to 100 for optimal performance. The number of flashes per read may be decreased for faster read times.



Category	PMT and Optics Settings
Wavelengths	
Plate Type	
Read Area	
TRF Settings	
PMT and Optics	Flashes per read: 100
Shake	Read From Bottom: <input type="checkbox"/>
More Settings	

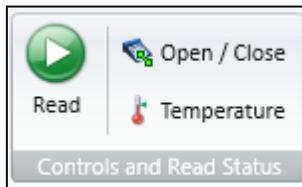
8. In the category "More Settings", the settings shown below should be used.



Category	More Settings
Wavelengths	
Plate Type	
Read Area	
TRF Settings	
PMT and Optics	
Shake	
More Settings	<input checked="" type="checkbox"/> Calibrate Carriage Speed: Normal Read Order: Column <input type="checkbox"/> Settling Time Duration: 100 ms

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9. Click OK to close the Settings window. To read the plate, click the green "Read" button at the top of the screen.



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

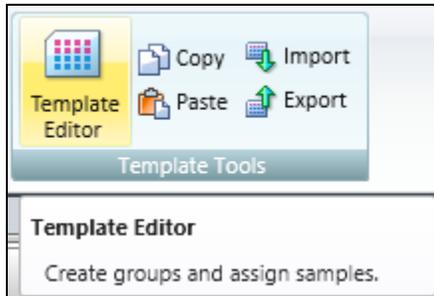
LanthaScre... Plate01

Plate01

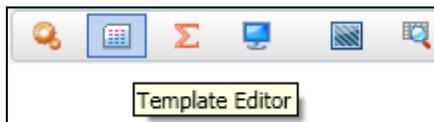
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A											56.8	2e4	2e4	2e4	2e4	2e4	2e4	54.8							
B											36.0	39...	26...	18...	11...	621	31.4								
C											56.3	2e4	2e4	2e4	2e4	2e4	2e4	59.4							
D											38.3	37...	25...	15...	11...	532	37.5								
E											52.4	2e4	2e4	2e4	2e4	2e4	64.9								
F											40.6	36...	26...	16...	10...	556	51.7								
G											60.6	2e4	2e4	2e4	2e4	2e4	76.6								
H											40.0	36...	24...	16...	10...	552	44.1								
I											70.6	2e4	2e4	2e4	2e4	2e4	84.3								
J											32.3	35...	25...	15...	11...	572	42.7								
K											59.5	2e4	2e4	2e4	2e4	2e4	67.5								
L											42.0	36...	26...	16...	11...	523	38.1								
M											71.3	2e4	2e4	2e4	2e4	2e4	62.5								
N											40.4	35...	25...	15...	11...	543	38.0								
O											62.1	2e4	2e4	2e4	2e4	2e4	60.0								
P											44.2	36...	25...	15...	10...	541	42.4								
											56.2	2e4	2e4	2e4	2e4	2e4	58.4								
											39.7	35...	25...	15...	10...	551	40.9								
											59.2	2e4	2e4	2e4	2e4	2e4	59.8								
											39.6	35...	25...	16...	10...	557	46.1								
											58.0	74.7	72.4	60.4	82.6	65.0	62.6								
											39.5	44.0	38.0	40.4	24.5	38.8	37.9								

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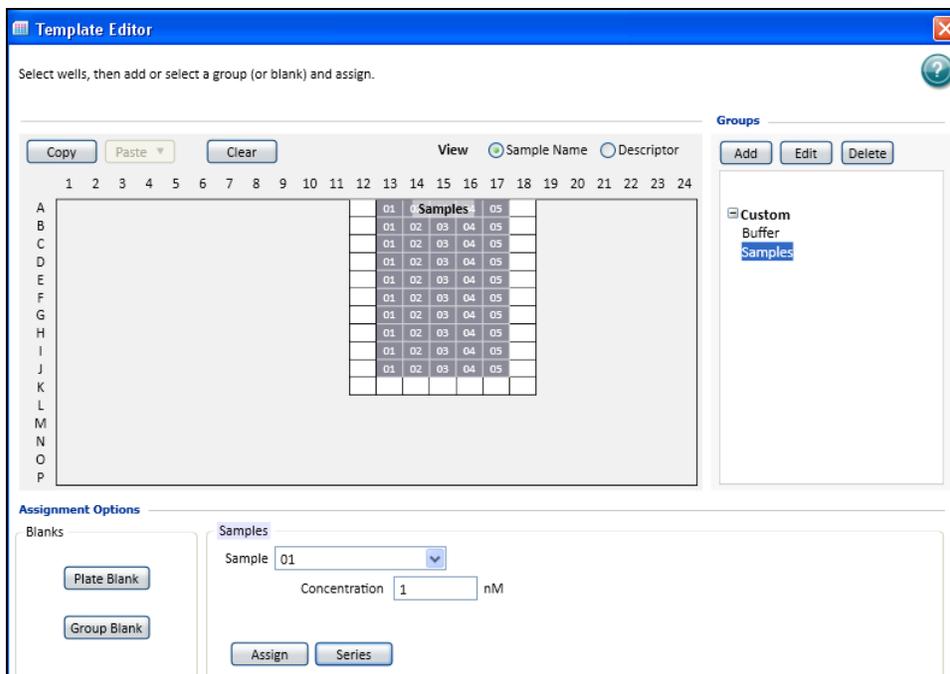
11. To set up the template for data analysis, click on Template Editor icon in the top toolbar...



...or on the plate section header.



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



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



Sample	Sample#	Concentration nM	AvgRatio	SDratio	CVratio
01	1	800.000	0.227	0.008	3.5
02	2	400.000	0.150	0.004	2.9
03	3	200.000	0.082	0.003	3.7
04	4	100.000	0.055	0.001	2.6
05	5	25.000	0.026	0.001	4.4

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C. Results:

Table 1. LanthaScreen® Europium TR-FRET testing on the SpectraMax® M5. Data obtained from running the diffusion-based TR-FRET instrument test available at Life Technologies Instrument Portal (www.lifetechnologies.com/instrumentsetup) under "[Download Europium assay Application Note.](#)" Ratios obtained, response ratio (RR = ratio at a given high concentration of acceptor divided by the TR-FRET ratio obtained at 25nM acceptor), and Z' values at each concentration are shown.

Acceptor (nM)	TR-FRET Ratio	RR	Z'
800	0.227	8.73	0.86
400	0.150	5.77	0.87
200	0.082	3.15	0.78
100	0.055	2.12	0.73
25	0.026		