Setup for LanthaScreen® Terbium 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® Terbium-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.

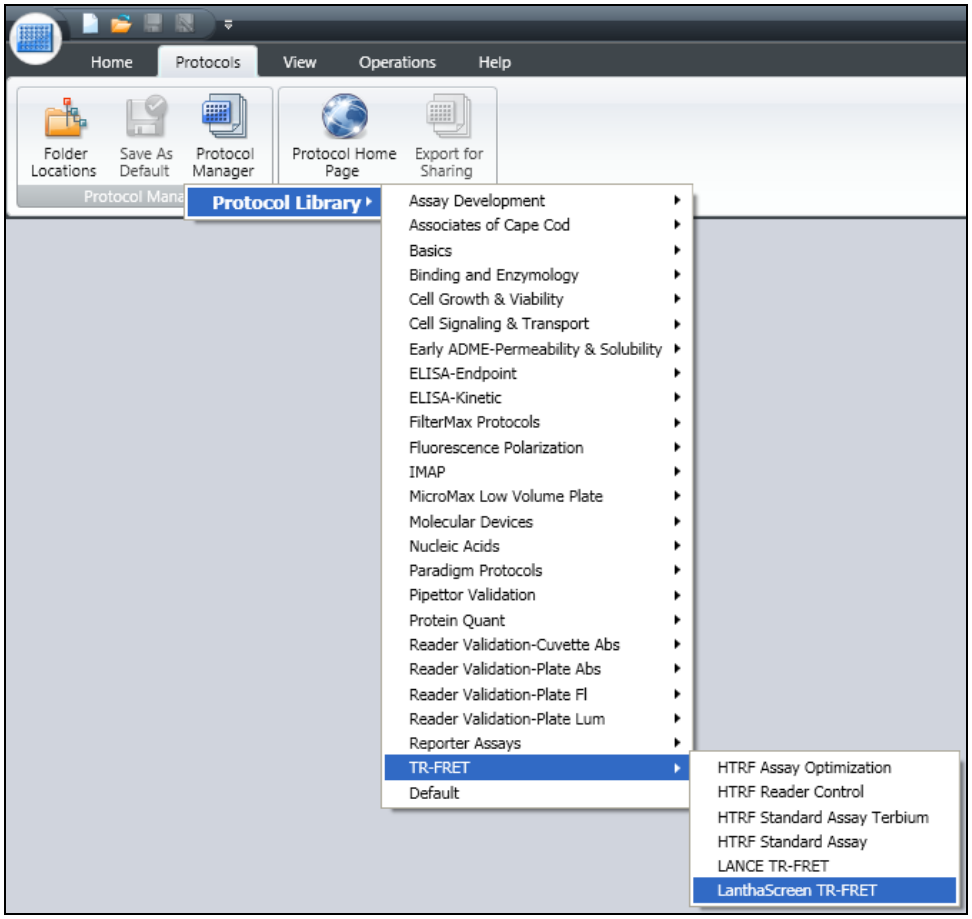
For more detailed information and technical support of Molecular Devices instruments or software, please contact Molecular Devices at 1-800-635-5577 or www.moleculardevices.com.

A. Recommended Optics

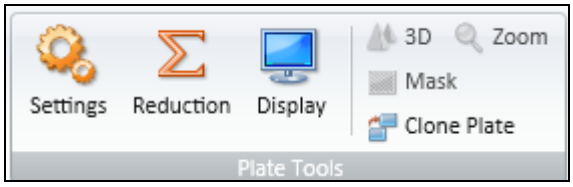
	Wavelength (nm)	Wavelength selection
Excitation	332/9	Monochromator
Emission 1	488/15	Monochromator
Emission 2	518/15	Monochromator
Emission 1 Cutoff	420	Filter
Emission 2 Cutoff	420	Filter

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.

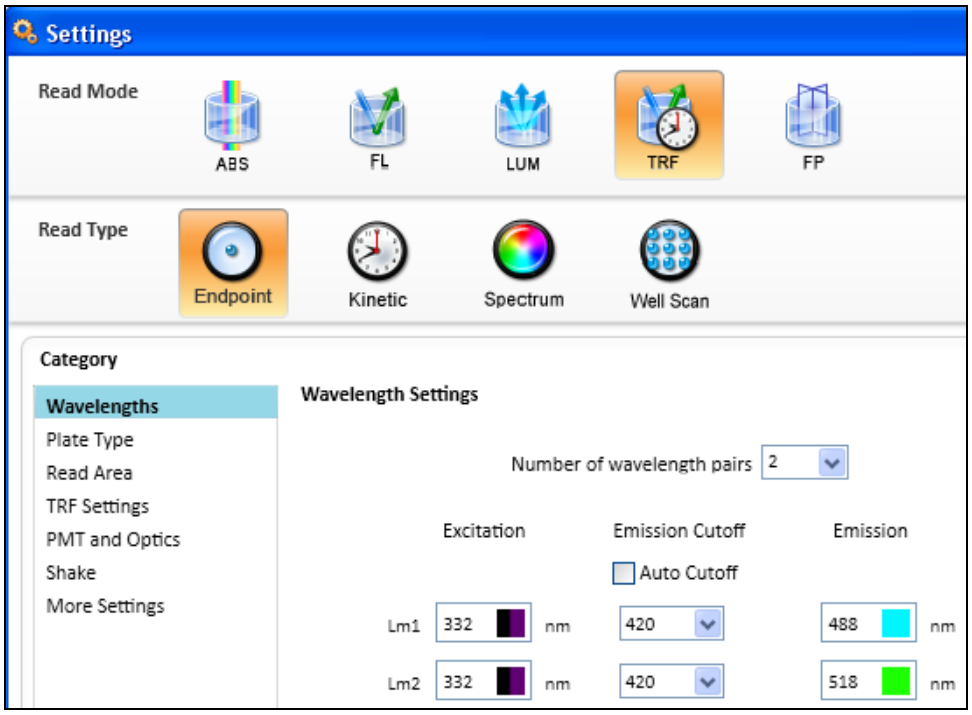


2. Click on the microplate icon in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen…

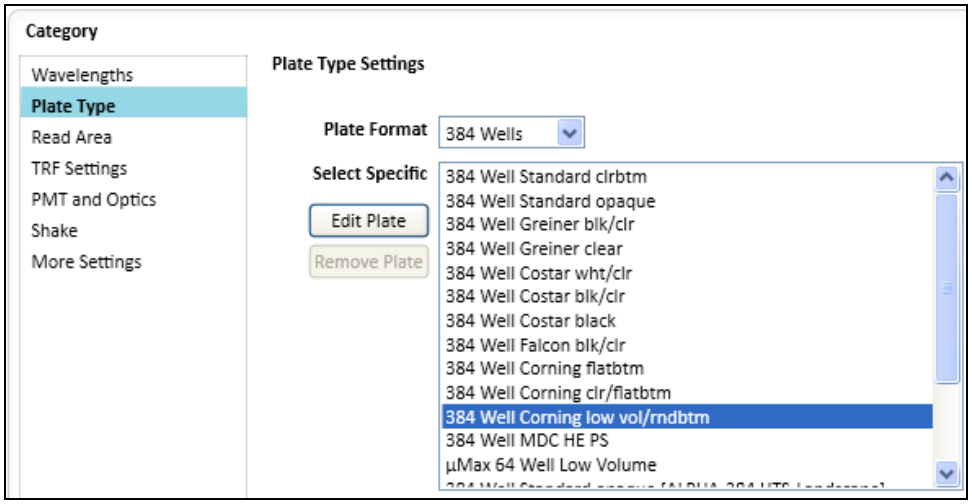


…or in the plate section header.

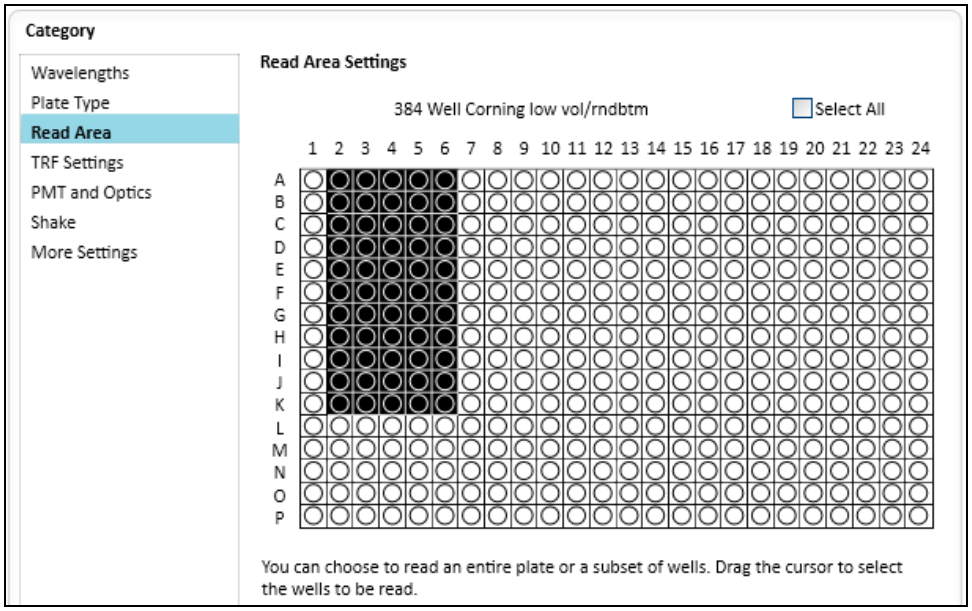
3. Click the settings icon in the toolbar to open the instrument settings window. Select the "TR-FRET BG" cartridge and TR-FRET read mode with End Point read type. When finished, select the "Plate Type" icon under Category.



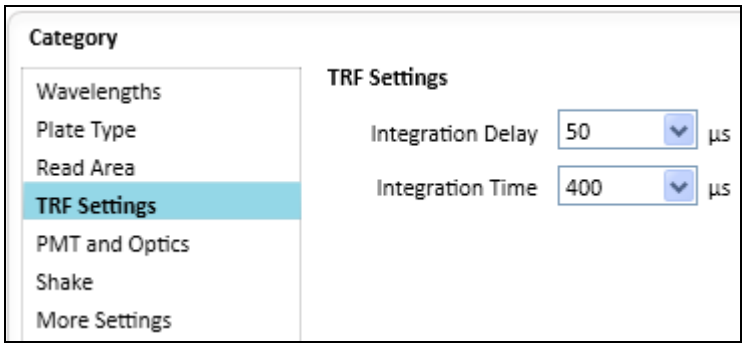
4. Choose the desired plate type, using the upper dropdown menu to choose plate format (96 or 384 wells) and the "Select Specific" menu to choose the specific plate type.



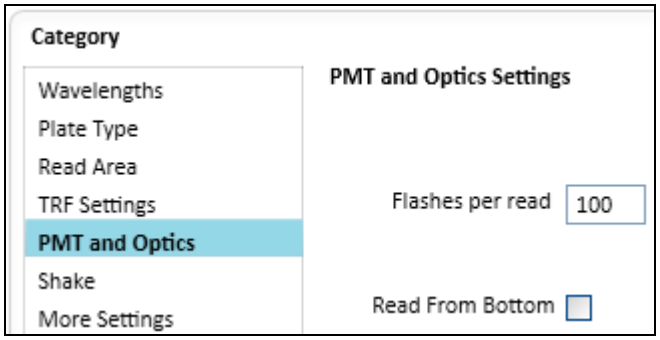
5. Now select the area of the plate to read.



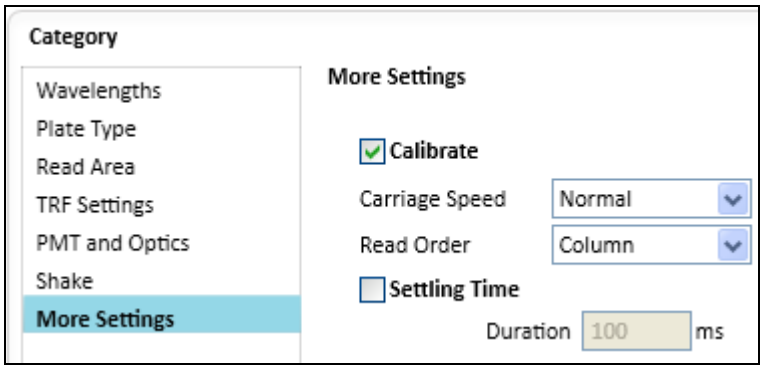
6. In TRF Settings, adjust the Integration Delay to 50 µs and the Integration Time to 400 µs. Note: typical settings for LanthaScreen assays are 100 µs delay and 200 µs integration; optimizing the delay and integration may improve assay window but in general the SpectraMax performs better with the delay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



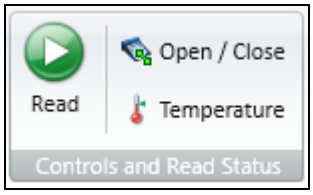
7. PMT and Optics, Flashes per read should be set to 100 for optimal performance. The number of flashes per read may be decreased for faster read times.



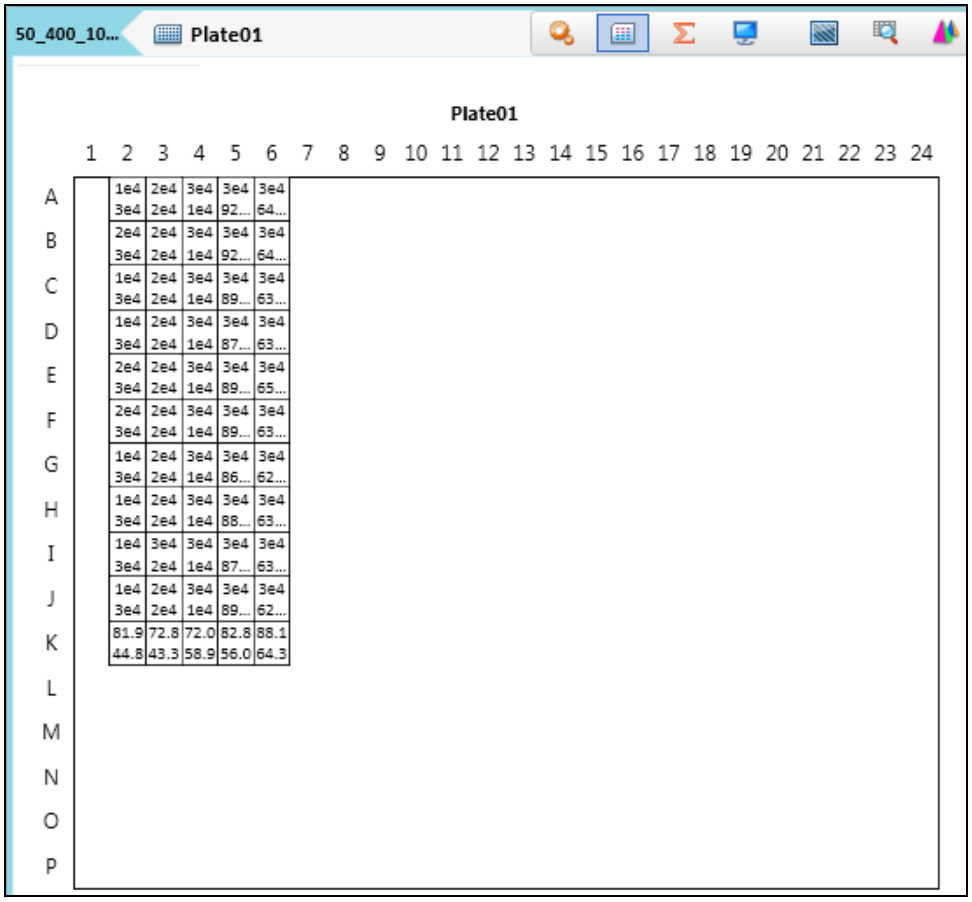
8. In the category "More Settings", the settings shown below should be used.



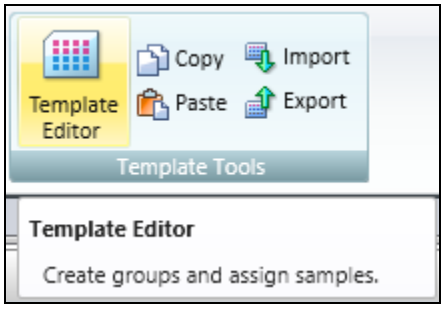
9. Click OK to close the Settings window. To read the plate, click the green "Read" button at the top of the screen.



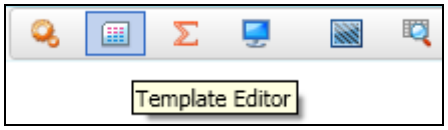
10. After the plate is read, data will appear in the plate section:



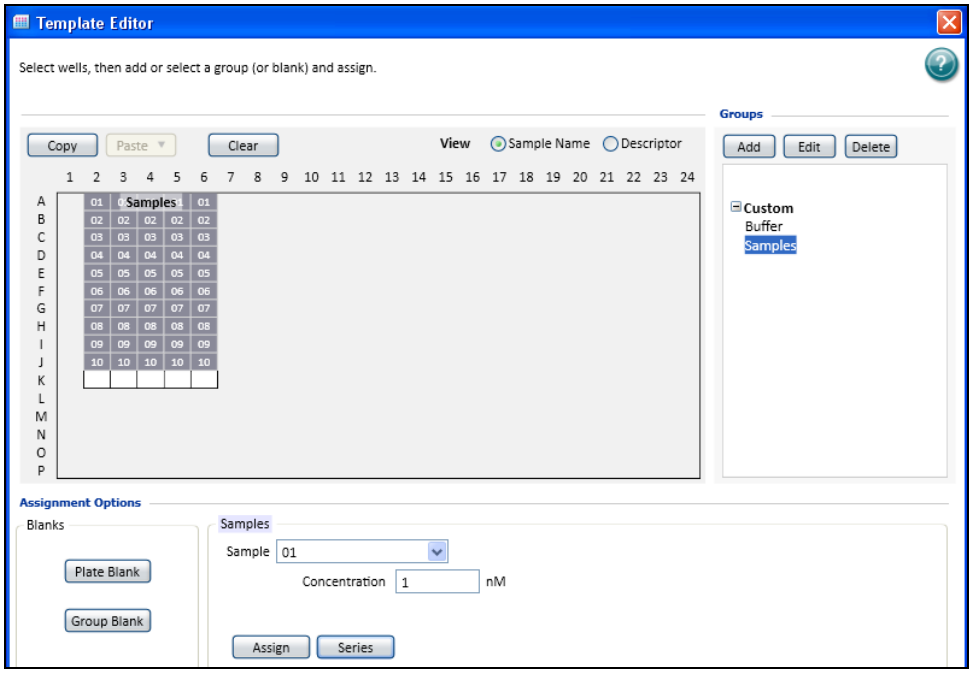
11. To set up the template for data analysis, click on Template Editor icon in the top toolbar…



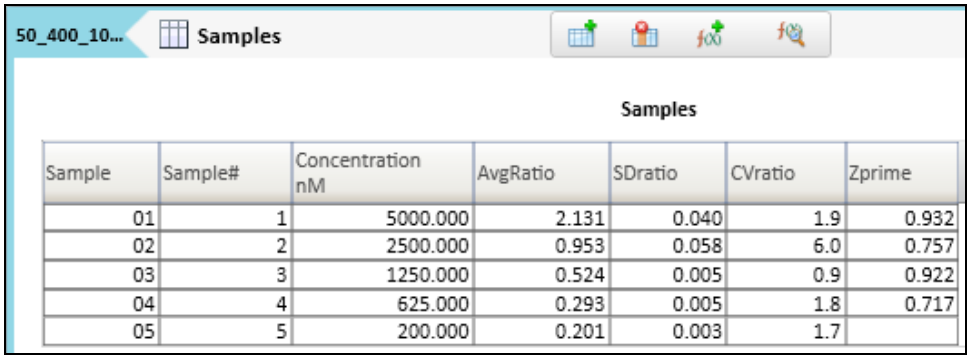
…or on the plate section header.



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



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



Samples

Sample	Sample#	Concentration nM	AvgRatio	SDratio	CVratio	Zprime
01	1	5000.000	2.131	0.040	1.9	0.932
02	2	2500.000	0.953	0.058	6.0	0.757
03	3	1250.000	0.524	0.005	0.9	0.922
04	4	625.000	0.293	0.005	1.8	0.717
05	5	200.000	0.201	0.003	1.7	

C. Results:

Table 1. LanthaScreen® Terbium 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 Terbium assay Application Note." Ratios obtained, response ratio (RR = ratio at a given high concentration of acceptor divided by the TR-FRET ratio obtained at 200nM acceptor), and Z' values at each concentration are shown.

Acceptor (nM)	TR-FRET Ratio	RR	Z'
5,000	2.131	10.60	0.93
2,500	0.953	4.74	0.76
1,250	0.524	2.50	0.92
625	0.293	1.46	0.72
200	0.201		