

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

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 <u>www.lifetechnologies.com/instrumentsetup</u> 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 <u>drugdiscoverytech@lifetech.com</u>.

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



Version No.: 8 Oct 12

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			



Version No.: 8 Oct 12

Page 3 of 10

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

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.

Home Protocols View Ope	rations Help	
Home Protocols View Ope Home Protocols View Ope Folder Save As Protocol Locations Default Protocol Manager Protocol Library*	rations Help Export for Sharing Assay Development Associates of Cape Cod Basics Binding and Enzymology Cell Growth & Viability Cell Signaling & Transport Early ADME-Permeability & Solubility Cell Signaling & Transport Early ADME-Permeability & Solubility ELISA-Endpoint ELISA-Endpoint ELISA-Kinetic FilterMax Protocols Fluorescence Polarization IMAP MicroMax Low Volume Plate Molecular Devices Nucleic Acids Paradigm Protocols Pipettor Validation Protein Quant Reader Validation-Cuvette Abs	
	Reader Validation-Plate Abs	
	TR-FRET	HTRF Assay Optimization HTRF Reader Control
		HTRF Standard Assay Terbium HTRF Standard Assay LANCE TR-FRET LanthaScreen TR-FRET



Version No.: 8 Oct 12

Page 4 of 10

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

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.

GeneBLAzer 📖 Plate1 🔍 🙀 🙀 🙀

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:

9	Settings								
	Read Mode	ABS	M FL			TRF	F	P P	
	Read Type	© Endpoint	Kinetic	Sp	o ectrum	Well Scan			
(Category								
	Wavelengths		Wavelength Se	ttings					
	Plate Type Read Area TRE Settings				Number o	of wavelength pa	irs 2	~	
	PMT and Optics			Excitati	on	Emission Cuto	ff	Emission	
	Shake					Auto Cutof	f		
	More Settings		Lm1	332	nm	550 🗸		620	nm
			Lm2	332	nm	550 🗸		665	nm



Page 5 of 10

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

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.

Category			
Wavelengths	Plate Type Settings		
Plate Type			
Read Area	Plate Format	384 Wells 💌	
TRF Settings	Select Specific	384 Well Standard cirbtm	~
PMT and Optics		384 Well Standard opaque	
Shake	Edit Plate	384 Well Greiner blk/clr	
More Settings	Remove Plate	384 Well Greiner clear	
Wore beamgs	Incinove ridee	384 Well Costar wht/clr	
		384 Well Costar blk/clr	
		384 Well Costar black	
		384 Well Falcon blk/clr	
		384 Well Corning flatbtm	
		384 Well Corning clr/flatbtm	
		384 Well Corning low vol/rndbtm	
		384 Well MDC HE PS	
		μMax 64 Well Low Volume	~
		204 M/-II Considered and an International Data 204 LITE Landscore -1	

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

Category	
Wavelengths	Read Area Settings
Plate Type	384 Well Corning low vol/rndbtm Select All
Read Area	
PMT and Optics	A 000000000000000000000000000000000000
Shake More Settings	B OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO
	O O



Version No.: 8 Oct 12

Page 6 of 10

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

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		
Wavelengths	TRF Settings	
Plate Type	Integration Delay	50 🔽 μs
Read Area		400
TRF Settings	integration lime	400 💌 μs
PMT and Optics		
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	
	Wavelengths	PMT and Optics Settings
	Plate Type	
	Read Area	
	TRF Settings	Flashes per read 100
	PMT and Optics	
	Shake	
	More Settings	Read From Bottom

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

Category		
Wavelengths	More Settings	
Plate Type	Coliberto	
Read Area		
TRF Settings	Carriage Speed	Normal 🗸 🗸
PMT and Optics	Read Order	Column 🗸
Shake	Settling Time	
More Settings	Dura	tion 100 ms
	Dara	113



Version No.: 8 Oct 12

Page 7 of 10

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

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:

Lantha	Scre.			Pla	te0	1								Q			Σ	Σ	Ş				Q		•
											Pl	ate0	1												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
А												56.8 36.0	2e4 39	2e4 26	2e4 18	2e4	2e4	54.8 31.4]
В												56.3	2e4	2e4	2e4	2e4	2e4	59.4							
с												38.3 52.4	37 2e4	25 2e4	15 2e4	11 2e4	532 2e4	37.5 64.9							
												40.6 60.6	36 2e4	26 2e4	16 2e4	10 2e4	556 2e4	51.7 76.6							
U												40.0	36	24	16	10	552	44.1							
E												70.6 32.3	2e4 35	2e4 25	2e4 15	2e4 11	2e4 572	84.3 42.7							
F												59.5 42.0	2e4 36	2e4	2e4	2e4	2e4	67.5 38.1							
G												71.3	2e4	2e4	2e4	2e4	2e4	62.5							
												40.4 62.1	35 2e4	25 2e4	15 2e4	11 2e4	543 2e4	38.0 60.0							
н												44.Z	36	25	15	10	541	42.4							
Ι												56.2 39.7	2e4 35	2e4	2e4	2e4	2e4	58.4 40.9							
1												59.2	2e4	2e4	2e4	2e4	2e4	59.8							
1												39.6	35	25	16	10	557	46.1 67.6							
К												39.5	44.0	38.0	40.4	24.5	38.8	37.9							
L																									
М																									
N																									
о																									
Ρ																									



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

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.

🔳 Te	mol	ate	Edit	tor																						
Select	well	ls, th	en ac	dd or	sele	ct a g	grou	p (or	blan	k) ar	id ass	sign.														2
																									Groups	
	ору		Pas	te '	r	C	Cle	ear							Vie	w	0	Samp	ole N	ame	0	Des	crip	tor	Add Edit Delete	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	4	
A B C D E F G H I J K L M N O P													01 01 01 01 01 01 01 01 01	0°56 02 02 02 02 02 02 02 02 02	ampl 03 03 03 03 03 03 03 03 03	es4 04 04 04 04 04 04 04 04	05 05 05 05 05 05 05 05								Custom Buffer Samples	
Assig	nmer	nt Op	otion	s —																						
Blan	Assignment Options Blanks Plate Blank Group Blank Assign Series																									



Setup Guide on Molecular Devices SpectraMax $^{\odot}$ M5/M5e Microplate Reader

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

Lar	nthaScre	Samples			<u>fo</u>	મહ્ય્
					Samples	
	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



Version No.: 8 Oct 12

Page 10 of 10

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

C. Results:

 Table 1. LanthaScreen[®] Europium TR-FRET testing on the SpectraMax[®] M5. Data obtained from running the diffusionbased 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.

	TR-FRET		
Acceptor (nM)	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		