

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

Setup for GeneBLAzer[®] 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 GeneBLAzer[®] 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 <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>.



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A. Recommended Optics

Parameter	Specification
Detection Cartridge Name	SpectraMax [®] Paradigm [®] Fluorescence Intensity (FI) GeneBLAzer [®] Detection Cartridge
Part Number	0200-7006
Detection Technique	FRET, Fluorescence Intensity
Light Source	LED, ultra high power
Filter Set	EX: 460-15
	EM1: 465-35
	EM2: 535-25
Applications	Designed for use with GeneBLAzer® reagents



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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 "GeneBLAzer[®]" protocol and click to open.

Home Protocols View Ope	erations Help	
Folder Save As Protocol Locations Default Manager Protocol Horr Page	ne Export for Sharing	
Protocol Mana Protocol Library >	Assay Development Associates of Cape Cod Basics Binding and Enzymology Cell Growth & Viability 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 A Pipettor Validation B Protein Quant F Reader Validation-Cuvette Abs G	JphaScreen 384 HTS IRET2 P Rhodamine GeneBLAzer



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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 of the screen...

Settings	Reduction	Display	Mask Glone Plate
		Plate Tools	

... or in the plate section header.



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3. This opens the Settings window. The GeneBLAzer[®] (G-BLAZER) cartridge and its wavelengths already appear under Wavelength Settings.

Settings		X
Cartridges	ABS-MONO	
Read Modes FL	FRET	
Read Type End Point	Kinetic Well Scan	
Category	Properties	Settings Information
Wavelengths Plate Type Read Area PMT and Optics Shake More Settings	Wavelength Settings Excitation Wavelength 406 nm Excitation Wavelength 535 nm Emission 2 Wavelength 535 nm	 G-BLAZER (s/n 2003) Lm1 406, 445 Lm2 406, 535 FRET Endpoint Piate Type 384 Well Corning cir/flatbtm Landscape Read Area EntirePlate More Settings Shake Off Read/drer Column Show Optimizer On
		Read from Top Integration Time 100 ms

4. 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.

Category	Properties		
Wavelengths	Plate Type Setting	5	
Plate Type			
Read Area	Plate Format	384 Wells 🗸	
PMT and Optics	Select Specific	384 Well Standard cirbtm	~
Shake		384 Well Standard opague	-
More Settings		384 Well Greiner blk/clr	
-		384 Well Greiner clear	E
	Edit Plate	384 Well Costar wht/cir	
		384 Well Costar blk/clr	
	Import Plate	384 Well Costar black	
		384 Well Falcon blk/clr	
	Remove	384 Well Corning flatbtm	_
		384 Well Corning clr/flatbtm	~
	Orientation	Landscape 🗸	
	Is Lidded		



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

Category	Properties
Wavelengths	Read Area Settings
Plate Type	384 Well Corning clr/flatbtm
Read Area	
PMT and Optics	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Shake	
More Settings	
	K <u>000000000000000000000000000000000000</u>
	You can choose to read an entire plate or a subset of wells. Drag the cursor to select the wells to be read.



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

Category	Properties	
Wavelengths	PMT and Optics Sett	ings
Plate Type	On the Fly Detection	Off - Stop and Go
Redu Ared		
Pivit and Optics	Integration Time	140 ms
Snake		
More Settings		
	For more information abou	t the FRET Fluorescence mode, see More Information.
		More Information



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7. In the category "More Settings", choose the read order corresponding to 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	Settings Information
Wavelengths Plate Type Read Area PMT and Optics Shake More Settings	More Settings Read Order Show Pre-Read Optimization Options	G-BLAZER Lm1 406,465 Lm2 406,535 FRET Endpoint Plate Type 384 Well Corning cir/flatbtm Landscape Read Area EntirePlate More Settings Shake Off ReadOrder Row Show Optimizer On PVMT and Optics Read from Top Integration Time 140 ms
	For information on configurable settings, see More Information.	OK Cancel



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





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- 9. 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."

Pre-Read Optimization Option	ns	
		?
Optimization Options		
Run Microplate Optimization before measurement accuracy, run Micro microplate lot changes.	ore reading the plate. To improve the oplate Optimization each time the	
Run Read Height Adjustment bef measurement accuracy, run Read volume changes. Current read her	ore reading the plate. To improve the d Height Adjustment each time the eight: 1.00 mm above the Plate.	
	Run Optimization	
Microplate Options		
	Plate is Lidded	
	Microplate Orientation	
	Landscape	
	🔵 Portrait	
	Opposite Landscape	
	Opposite Portrait	
	Read Plate Cancel	



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

Microplate Optimization Wizard	d - 384 Well Corning clr/flatbtm [G-BLAZER-Landscape]	
	Insert the Microplate	
Insert the Microplate Optimize Select the Center of the Upper-Lef Select the Center of the Lower-Rig Select the Center of the Lower-Rig Verify Microplate Dimensions	Insert the prepared microplate in the reader and select its orientation. As illustrated below, the samples corner wells will be scanned to optimize the microplate. Clck Next to continue.	4
	Cancel and A	lext >

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

Well Scan in Progress		
Please wait while the wells are scanned for optimization.		
Scanning wells for optimization		
Remaining Time	00:00:09	
Click Stop Optimization to stop the scan	Stop Optimization	
Wizard.		



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12. 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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13. 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 Dim	ensions		
Verify the dimensions of the microplate. You can ec its center. Type a name for the microplate definitio microplate definition.	lit the values in the fia n in the Microplate Na	elds or return to a well me field. Click Save to	step to redefine save the
Microplate Dimensions			
Bottom-row y offset (mm)	8.99		
Column spacing (mm)	4.5		
Left-column × offset (mm)	12.12		
Right-column × 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-BLA	ZER-Landscape]
Bottom-row y offset (mm) The distance in millimeters from the lower edge of	the microplate to the	horizontal center of th	ne bottom row.
	Cancel	< Back	Save



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14. 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.

🧖 Read Height Optimization W	Vizard																				3
	Select Well																I				
Select Well Optimize Optimization Complete	Select Select	the well in ed: Well A:	the plai	te layout ig over: '	below	that c	ontains:	your	sampl	le at t	he des	ired vo	lume.	Click M	Next	to cor	ntinu	e.			
	1 A B C D E F G H I J K L M N 0											5 16									
	P								•					Ca	ancel			•	Next	•	

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

🧕 Read Height Optimization	Wizard
	Optimization Complete
Select Well Optimize	Verify the Optimized Read Height. You can adjust the height, if desired, by editing the value in the Custom Read Height field. Click Save to save the read height.
Optimization Complete	Optimized Read Height 11.91 mm Custom Read Height 11.91 👘 mm



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

Pre-Read Optimization Option	is 🛛 🔀
Optimization Options	
Run Microplate Optimization before measurement accuracy, run Micro microplate lot changes.	re reading the plate. To improve the plate Optimization each time the
Run Read Height Adjustment befor measurement accuracy, run Read volume changes. Current read hei	ore reading the plate. To improve the Height Adjustment each time the ight: 11.91 mm above the Plate.
	Run Optimization
Microplate Options	
	Plate is Lidded
	Microplate Orientation
	Landscape
	Portrait
	Opposite Landscape
	Opposite Portrait
	Read Plate Cancel



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

Navigation Tree 🛛 🐼 🗸	Document Comparison	
Expt1	Exp1 🔲 Plate1 😣 💷 🗵 🖳 📾 🔍 🏕	•
Plate1 Background Distimulat Stimulated Graph1	Lipit Plate1 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 [55] 55 <td>tings Information G-BLAZER (/n 2003) m1 406, 455 m2 406, 535 FRET Endpoint More Settings Data Office Settings More Settings Data Office Settings Nore Settings Nore Settings</td>	tings Information G-BLAZER (/n 2003) m1 406, 455 m2 406, 535 FRET Endpoint More Settings Data Office Settings More Settings Data Office Settings Nore Settings Nore Settings
	r 1e5 1e5 1e5 1e6 2e6 2e6 2e6 2e6 2e6 2e6 2e6 2e6 2e6 2	pectraMax Paradigm IOM vV1.2 b51 20.05.2011 itart Read : 1:51 PM J/11/2011 Wean Temperature : 22 5 °C

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

шт	emp	lat	e Edi	tor																						X
Sele	elect wells, then add or select a group (or blank) and assign.															0										
																									Groups	
ſ	Copy		Pa	ste 1	7	ſ	Cle	ear	٦						Vie	w	0	Samp	ole N	ame	0	Des	ript	or	Add Edit Delete	
	1	_		4	-	-	7	0	_	10	11	12	12	14	15	16	17	19	10	20	21	22	22	24		
	-			-			·	0	-	10		12	15	14	-13	10	1/	10	15	20	21	22	2.5	24		
A	Un	stin	· · · ·	+	-	-				-		<u> </u>		_						_				_	Custom	
	01	. 0 1 0	1	+	-	-	-		-	-		-		-		-									Unstimulated	
	01	1 0	1	-	-	+	-		-			-				-									Stimulated	
E	01	LO	1		1	1													_						Background	
F	01	ιO	1																							
6	01	ιO	1																							
H	01	LO	1																							
1	01	LO	1																							
J	01	LO	1		_																					
K	01	LO	1	-	-	<u> </u>	<u> </u>		<u> </u>																	
L	01		1	-	-	<u> </u>			-	<u> </u>		<u> </u>														
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	01		1	+	+	-	-		-			-				-			_	-						
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Assi	gnme	ent (Optior	IS —																						
⊂ Bla	nks –						Uns	timu	lated	d —																
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	_																									
		Grou	p Bla	nk																						
	_						ſ	Δc	cian	ור	Se	riec														
								m2:	21611		30															

Template with wells assigned to different template groups:

🕮 Te	mpl	ate	Edit	or																						X
Select	jelect wells, then add or select a group (or blank) and assign.																?									
	Groups															Groups										
	Copy Paste Clear View Sample Name Descriptor 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		
А	Unst	im	01	02	03	04	05	06	07	08	09.5	timu	late	d 12	13	14	15	16	17	18	19	20	Bac	gr	Custom	
В	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01	Unstimulated	
С	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01	Stimulated	
D	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01	Background	
E	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01	Dackground	
F	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
G	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
н	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
1	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
К	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
L	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
M	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
N	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
0	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		
Р	01	01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	01	01		



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

Navigation Tree	k 😡	Doc	cument	Con	mparison					
Expt1 Plate1			Expt1		🔛 Stimu	lated			fo fo	કે મહ્ય
Background Unstimulat									Stimulated	
Stimulated			Sam	ple (Concentration nM	CV%465	CV%535	AvgRatio	SDratio	Zprime
				01	100000.000	2.6	2.3	4.05	0.102	0.752
				02	25000.000	5.6	6.1	4.19	0.150	0.718
				03	6250.000	5.6	5.0	4.22	0.138	0.731
				04	1562.500	6.5	5.8	4.11	0.354	0.516
				05	i 390.625 6.6 6.9	4.31	0.351	0.547		
				06	97.656	7.0	6.9	4.23	0.196	0.679
				07	24.414	7.8	4.7	2.56	0.174	0.382
				08	6.104	11.0	7.3	1.33	0.112	-1.189
				09	1.526	10.0	7.0	1.04	0.092	-9.374
				10	0.381	13.0	4.6	0.97	0.153	



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C. Results



Concentration

Figure 1: GeneBLAzer® Assay. GeneBLAzer® assay performed using the Molecular Devices SpectraMax® Paradigm® Microplate Detection Platform and GeneBLAzer® MC3R CRE-bla CHO-K1 cell line stimulated with NDP-α-MSH. Z' = 0.75.