

Setup for GeneBLAzer® Assay on SpectraMax® M5/M5e Microplate Reader with SoftMax® Pro 6 Software

The Molecular Devices SpectraMax® M5/M5e Microplate Reader was tested for compatibility with Life Technologies GeneBLAzer® assays. The following document is intended to demonstrate setup of this instrument. **These settings are also valid for the SpectraMax M3/M4, FlexStation® 3 Multi-Mode Microplate Reader and Gemini Fluorescence Microplate Reader with bottom-read fluorescence detection.**

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® M5/M5e Microplate Reader

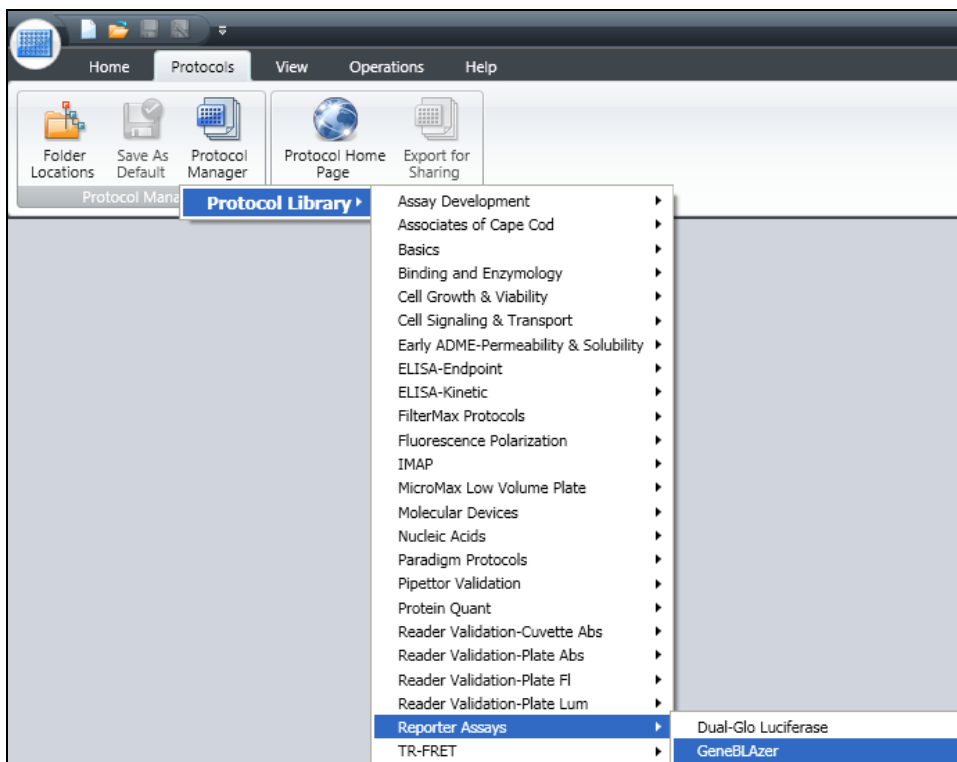
A. Recommended Optics

	Wavelength (nm)	Wavelength selection
Excitation	410/9	Monochromator
Emission 1	460/15	Monochromator
Emission 2	530/15	Monochromator
Emission 1 Cutoff	435	Filter
Emission 2 Cutoff	515	Filter

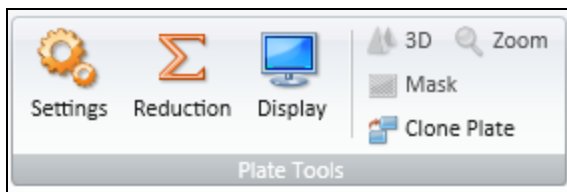
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B. Instrument Setup:

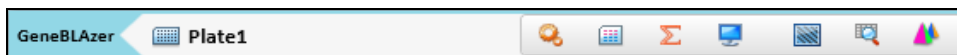
1. Open SoftMax® Pro 6 software. Click on "Protocol Manager" to open the Protocol Library. Within the "Reporter Assays" folder, locate the "GeneBLAzer" protocol and click to open.



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...

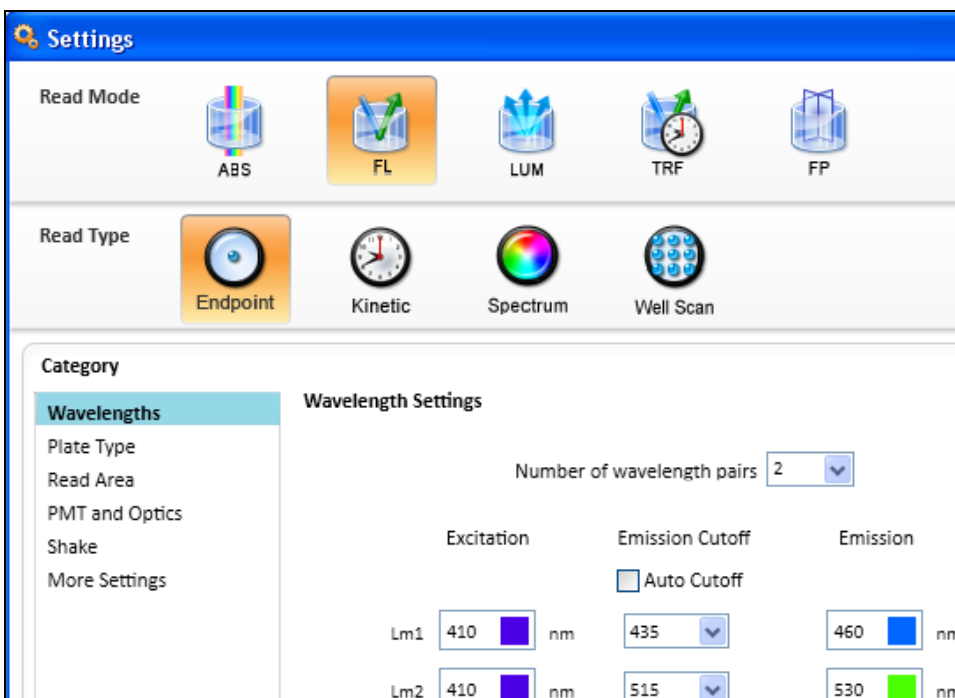


...or in the plate section header.

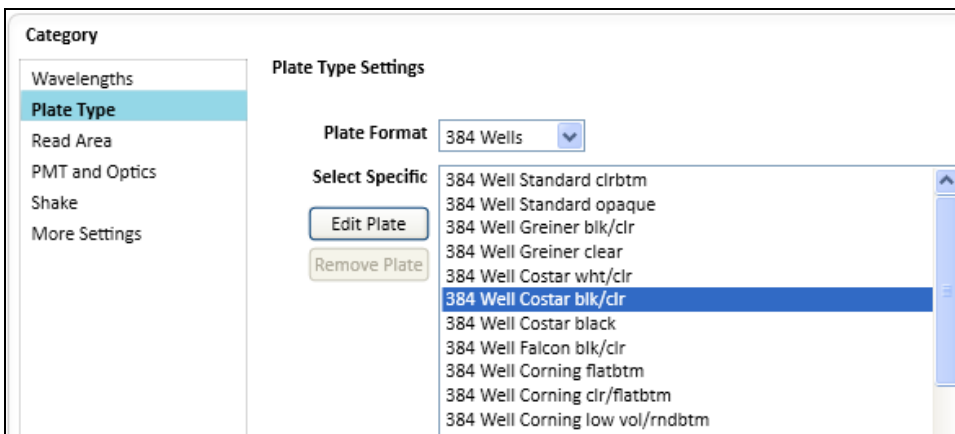


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3. This opens the Settings window. Fluorescence read mode and Endpoint read type are already selected in the pre-configured protocol. Enter wavelength settings as shown below:



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.



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

Category

- Wavelengths
- Plate Type
- Read Area
- PMT and Optics
- Shake
- More Settings

Read Area Settings

384 Well Costar blk/cfr Select All

	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	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
I	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
J	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
K	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
L	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
M	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
N	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
O	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
P	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

You can choose to read an entire plate or a subset of wells. Drag the cursor to select the wells to be read.

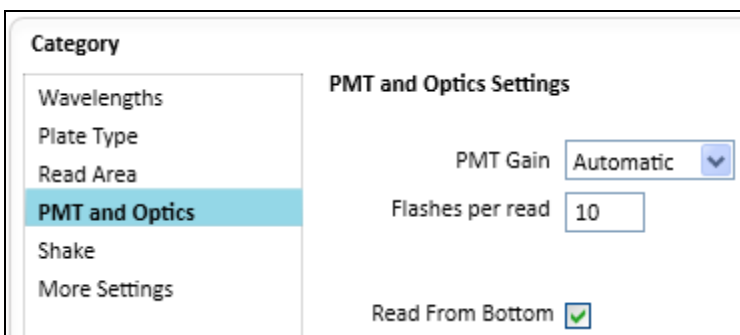
[More Information](#)

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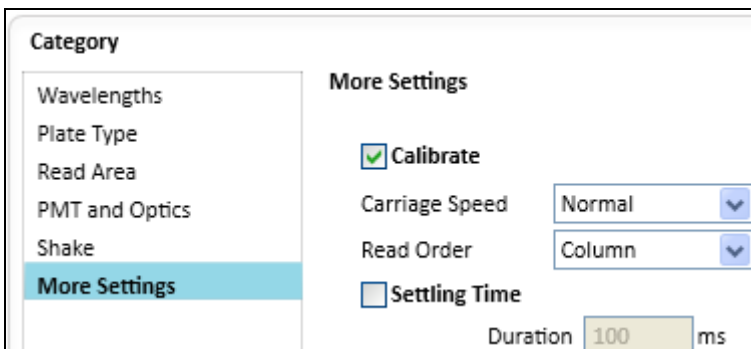
6. In the PMT and Optics category, the PMT Gain setting "Automatic" is recommended, as it enables the widest range of sample brightness to be detected in a single plate read without the need for manual PMT gain adjustment.

The number of Flashes per read may be adjusted. Fewer flashes enable faster plate reads, while more flashes enable higher performance.

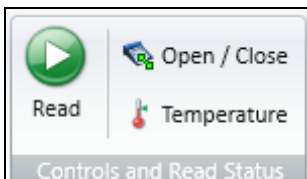
Make sure the "Read from Bottom" box is checked.



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



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

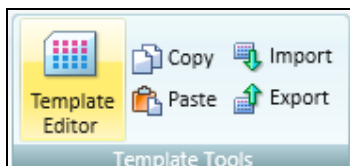


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9. After the plate is read, data will appear in the plate section:

		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		52.4	48.1	47.7	83.5	83.7	84.7	774	745	732	758	753	703	519	273	219	206	244	65.3	82.4	82.8	47.6	47.3	47.4	46.5
B		7.95	7.75	7.78	67.2	67.6	67.7	99.9	94.3	94.2	108	96.2	96.0	109	120	124	134	136	12.8	65.5	65.3	7.86	7.93	7.66	7.64
C		48.1	48.1	47.3	83.9	82.0	81.9	876	847	784	711	823	783	555	312	347	225	301	65.3	81.8	83.1	47.4	47.1	47.0	47.3
D		7.76	7.69	7.75	67.4	68.5	67.9	108	98.5	95.2	95.2	94.0	99.1	112	133	132	136	157	12.5	66.5	66.3	7.81	7.74	7.65	7.71
E		54.7	47.6	51.9	83.9	83.8	84.3	972	889	787	696	839	886	663	333	294	257	332	102	83.0	83.1	47.7	50.0	47.3	46.3
F		8.83	7.77	8.20	68.2	69.0	69.2	118	107	100	99.2	111	98.9	127	125	145	148	163	13.9	66.6	66.0	7.76	7.81	7.74	7.49
G		55.5	53.1	47.8	82.7	83.3	84.3	10...	871	965	968	10...	898	682	380	337	274	315	65.6	82.7	82.4	47.7	47.5	47.7	47.5
H		8.05	8.09	7.76	68.0	68.1	68.7	118	109	109	118	114	113	128	136	164	158	163	12.8	66.9	67.3	7.79	7.80	7.67	7.54
I		51.2	47.6	47.7	83.4	83.1	83.5	10...	984	10...	10...	986	939	727	351	295	276	349	67.3	82.4	83.0	48.7	47.6	46.9	46.8
J		7.87	7.85	7.93	67.7	67.8	68.6	121	110	117	116	113	107	132	141	146	151	175	12.8	66.5	67.3	7.83	7.77	7.70	7.78
K		61.9	47.5	47.9	82.0	82.6	82.9	10...	10...	993	914	953	988	668	355	285	347	342	68.1	82.2	82.7	47.2	47.9	48.1	50.7
L		8.12	7.79	7.75	66.8	67.9	67.6	126	117	113	101	100	112	106	136	142	156	168	13.0	66.0	65.5	7.72	7.82	7.93	7.96
M		46.5	48.1	53.9	81.9	82.2	83.2	10...	995	10...	973	990	941	713	409	301	313	372	67.5	82.8	81.4	47.1	47.1	46.8	46.8
N		7.53	7.75	7.96	66.8	67.4	67.3	129	113	122	114	118	111	132	148	144	150	176	12.7	66.7	65.4	7.46	7.62	7.55	7.61
O		46.2	48.1	51.0	84.8	86.0	82.9	10...	962	10...	855	892	939	745	426	349	314	352	66.2	83.0	81.6	46.7	47.1	46.4	47.5
P		7.64	7.72	7.89	66.8	67.2	66.9	122	109	116	124	121	109	128	156	158	154	170	12.7	66.1	65.4	7.59	7.77	7.69	7.76
		46.0	52.8	47.8	83.3	81.2	89.0	10...	976	10...	10...	10...	10...	754	424	393	333	493	66.6	83.6	81.9	46.4	46.8	47.3	46.9
		7.57	8.19	7.86	66.8	67.4	67.0	125	110	113	110	103	111	133	146	173	168	187	12.7	66.9	65.4	7.53	7.73	7.50	7.60
		46.1	49.8	47.8	82.1	81.8	81.3	10...	10...	10...	990	10...	801	409	364	347	395	65.5	82.3	81.6	48.1	46.4	46.6	47.4	
		7.33	8.09	7.90	67.1	67.1	66.7	123	118	122	116	104	110	136	147	159	168	171	12.7	65.9	65.4	7.66	7.59	7.70	7.43
		46.1	47.6	47.8	80.5	82.8	80.4	10...	901	10...	960	10...	10...	723	418	408	352	393	63.2	82.9	86.9	46.3	191	54.1	45.6
		7.42	7.60	7.51	65.3	67.5	66.0	122	98.7	119	104	114	112	125	139	170	162	172	12.2	66.1	65.5	7.52	13.1	7.69	7.49
		46.1	103	53.6	85.8	81.7	82.8	10...	942	10...	10...	10...	10...	734	455	358	352	391	62.7	83.0	80.1	46.6	45.5	46.7	45.5
		7.65	9.05	8.15	66.4	66.8	66.3	120	110	121	119	113	114	127	156	156	165	175	12.2	66.7	64.4	7.50	7.44	7.55	7.34
		46.6	85.6	54.3	81.9	81.4	80.4	10...	983	10...	986	10...	997	802	409	393	349	393	108	80.8	80.6	46.0	45.9	135	45.3
		7.51	8.43	8.07	66.0	67.7	66.2	117	117	111	111	116	116	141	145	167	167	176	13.5	64.4	65.0	7.62	7.49	10.7	7.42
		46.5	46.8	57.4	81.8	82.0	81.1	10...	983	10...	10...	10...	997	828	467	408	374	412	63.7	81.6	80.8	46.9	45.6	46.8	45.7
		7.33	7.77	8.44	67.2	67.5	66.8	124	106	120	115	110	111	134	157	165	157	164	12.2	66.4	64.9	7.66	7.55	7.61	7.44
		85.5	92.6	47.4	81.2	82.6	82.5	11...	10...	11...	10...	11...	10...	820	529	430	390	403	63.2	87.5	81.6	48.6	46.1	46.3	46.1
		8.69	9.80	7.62	66.8	68.8	67.6	133	124	122	128	129	123	138	183	193	189	207	12.3	67.2	65.2	7.95	7.47	7.38	7.39
		46.1	50.9	46.9	82.5	83.0	86.3	11...	10...	10...	11...	10...	11...	806	457	384	365	395	63.6	83.0	82.2	47.3	46.1	61.2	46.8
		7.61	7.91	7.71	67.7	67.6	67.5	127	120	110	125	103	114	147	144	156	164	175	12.7	66.8	65.3	7.71	7.71	8.08	7.74

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

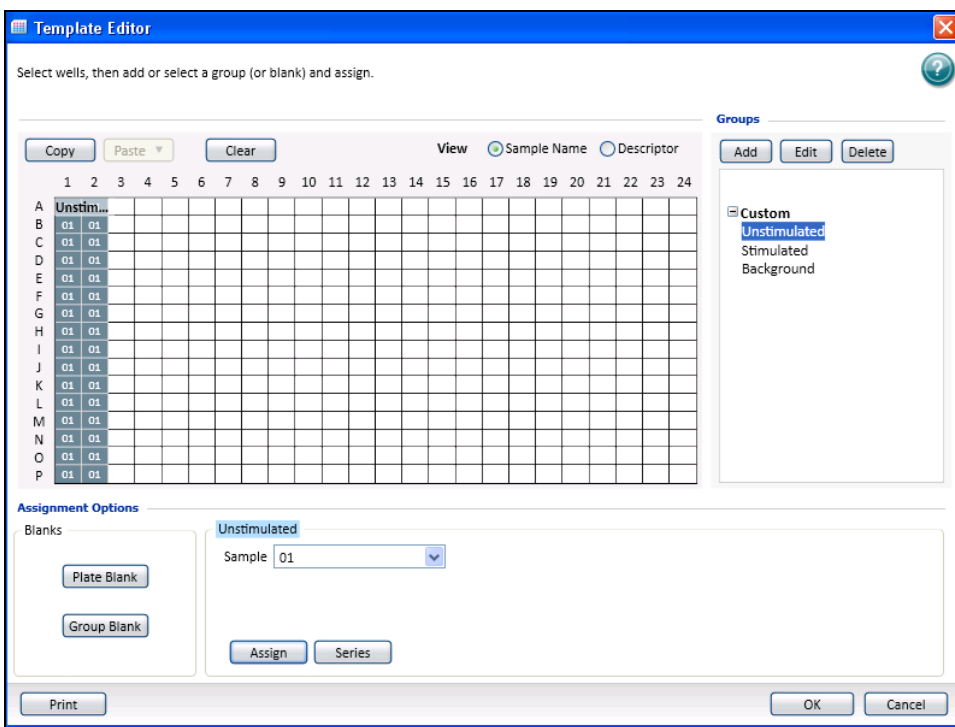


...or on the plate section header.

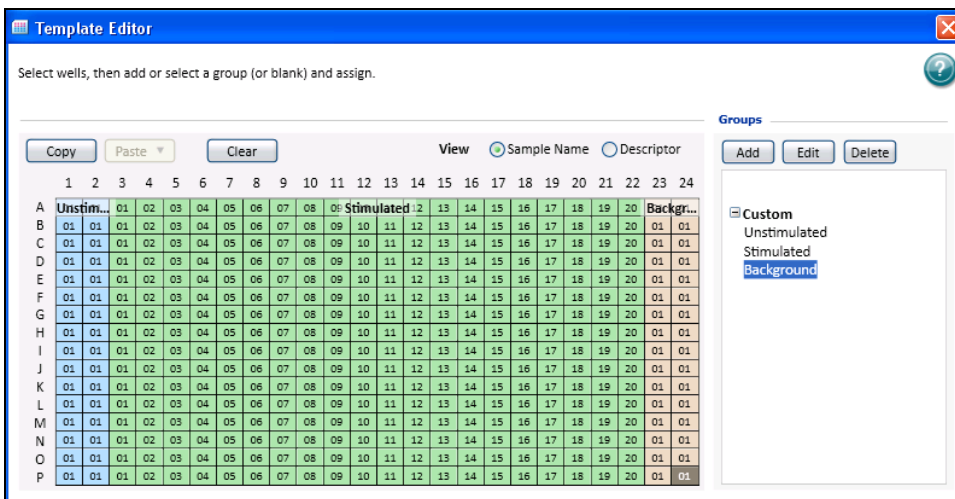


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11. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.

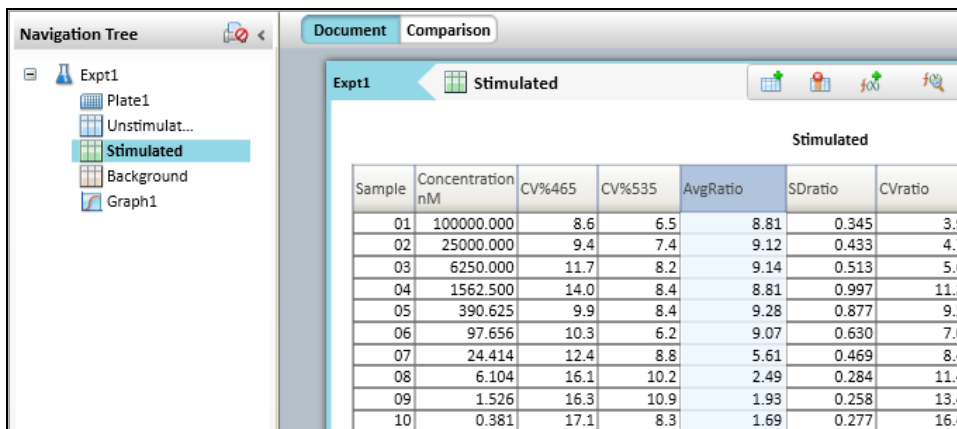


Template with wells assigned to different template groups:



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



Sample	Concentration nM	CV%465	CV%535	AvgRatio	SDratio	CVratio
01	100000.000	8.6	6.5	8.81	0.345	3.9
02	25000.000	9.4	7.4	9.12	0.433	4.7
03	6250.000	11.7	8.2	9.14	0.513	5.6
04	1562.500	14.0	8.4	8.81	0.997	11.3
05	390.625	9.9	8.4	9.28	0.877	9.5
06	97.656	10.3	6.2	9.07	0.630	7.0
07	24.414	12.4	8.8	5.61	0.469	8.4
08	6.104	16.1	10.2	2.49	0.284	11.4
09	1.526	16.3	10.9	1.93	0.258	13.4
10	0.381	17.1	8.3	1.69	0.277	16.4

Have a question?

NA: 800-955-6288 or INTL: 760-603-7200 ext. 40266

Contact our Technical Support Team

Email: drugdiscoverytech@lifetech.com

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C. Results

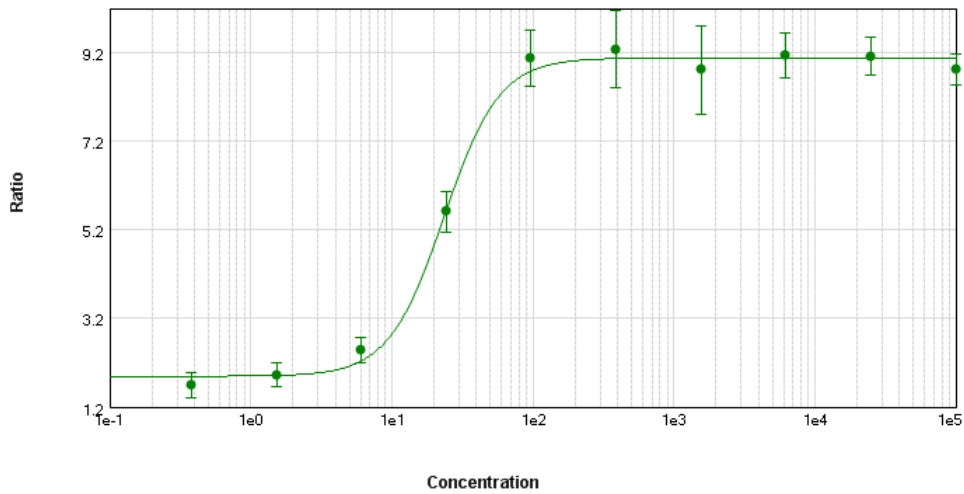


Figure 1: GeneBLAzer® Assay. GeneBLAzer® assay performed using the Molecular Devices SpectraMax® M5 microplate reader and GeneBLAzer® MC3R CRE-*bla* CHO-K1 cell line stimulated with NDP- α -MSH. $Z' = 0.74$.