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.

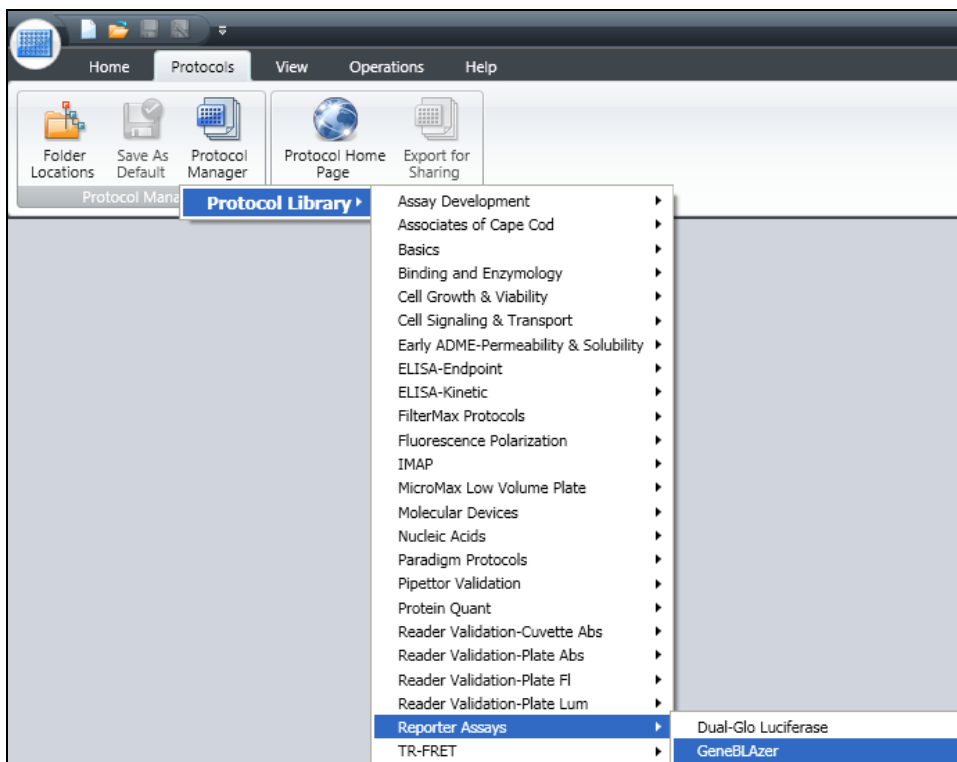**Setup Guide on the Molecular Devices SpectraMax® M5/M5e Microplate Reader**

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

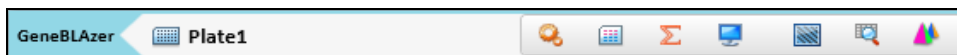**Setup Guide on the Molecular Devices SpectraMax® M5/M5e Microplate Reader**

B. Instrument Setup:

1. Open SoftMax® Pro 6 software. Click on "Protocol Manager" to open the Protocol Library. Within the "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.

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:
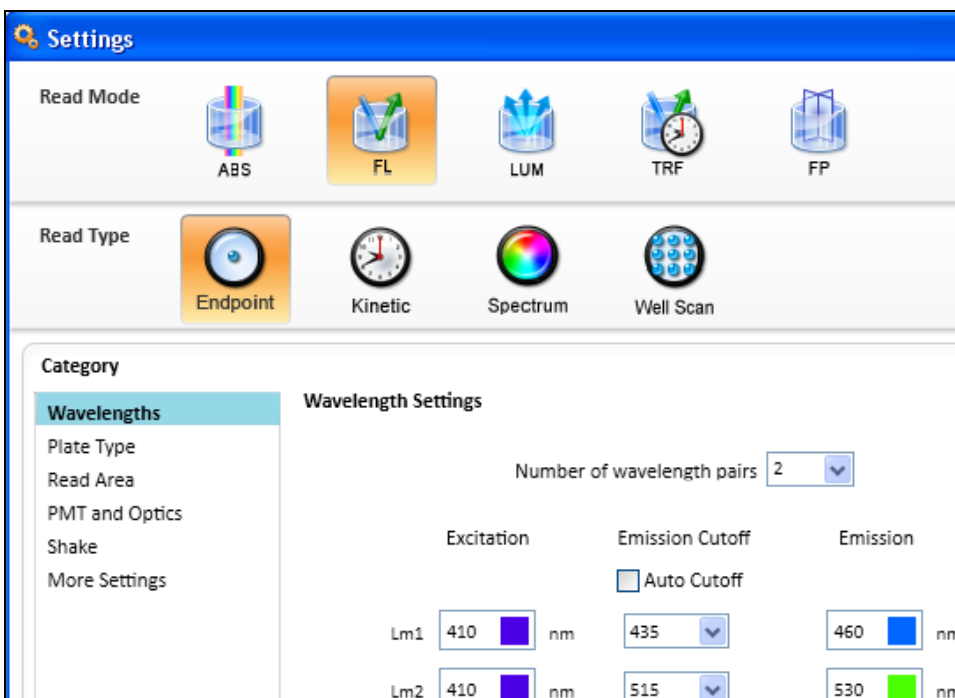
4.   Choose the desired plate type, using the upper dropdown menu to choose plate format (96 or 384 wells) and the "Select Specific" menu to choose the specific plate type.
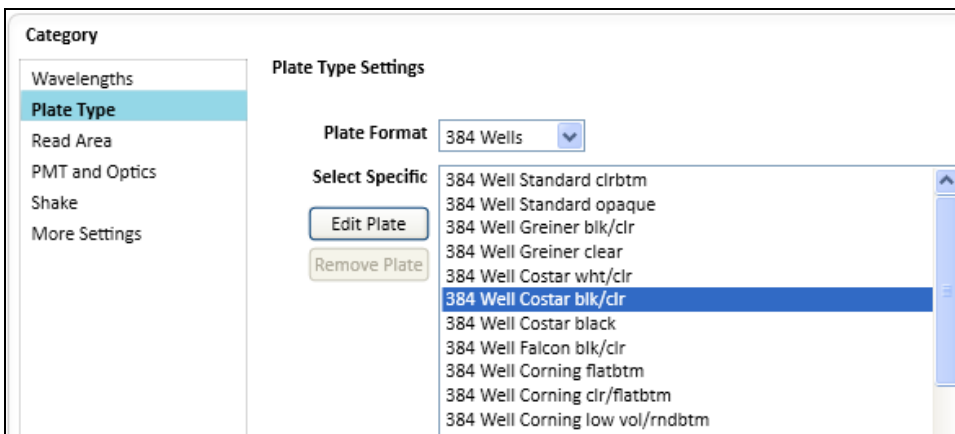
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5. Now select the area of the plate to read.

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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.
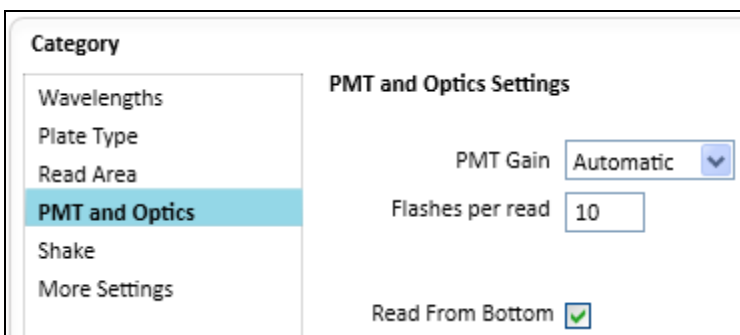
7. In the category "More Settings", the settings shown below should be used.
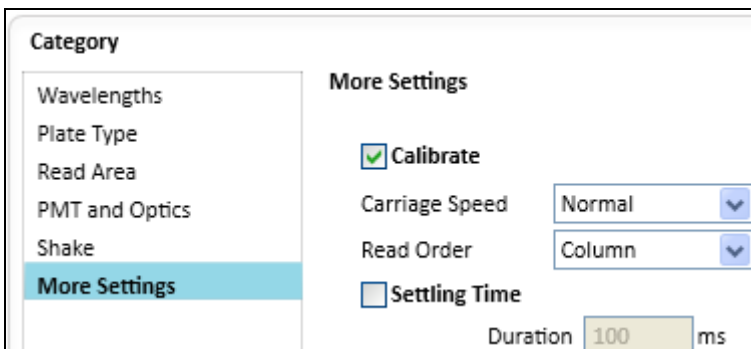
8. To read the plate, click the green "Read" button at the top of the screen.
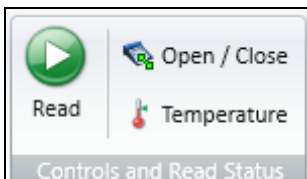
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9. After the plate is read, data will appear in the plate section:

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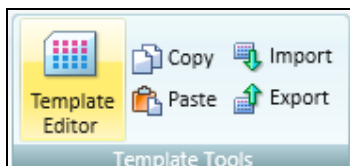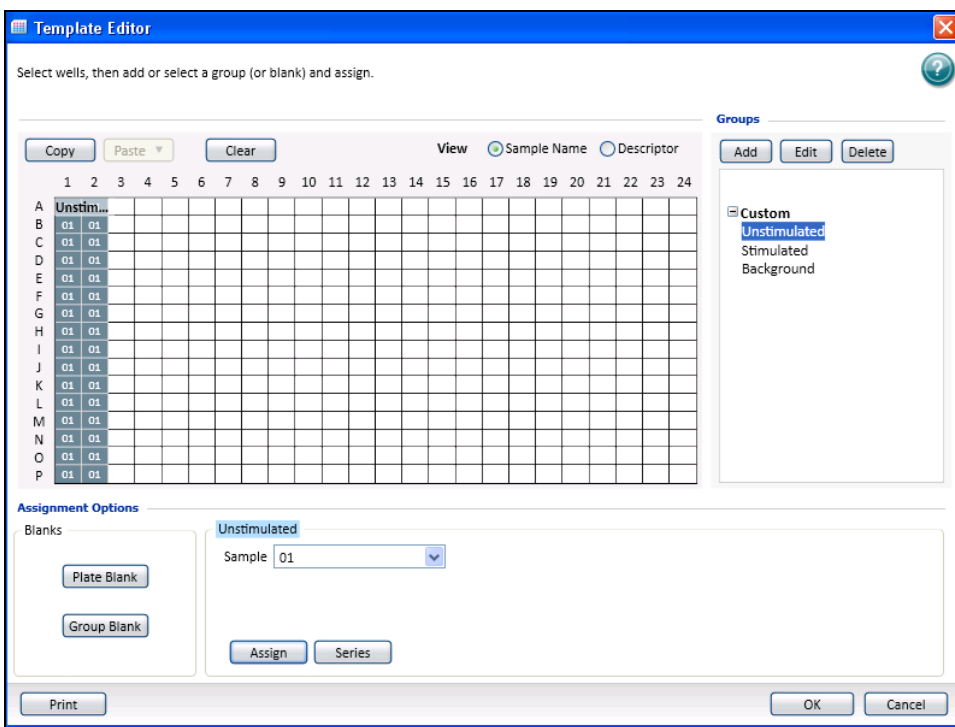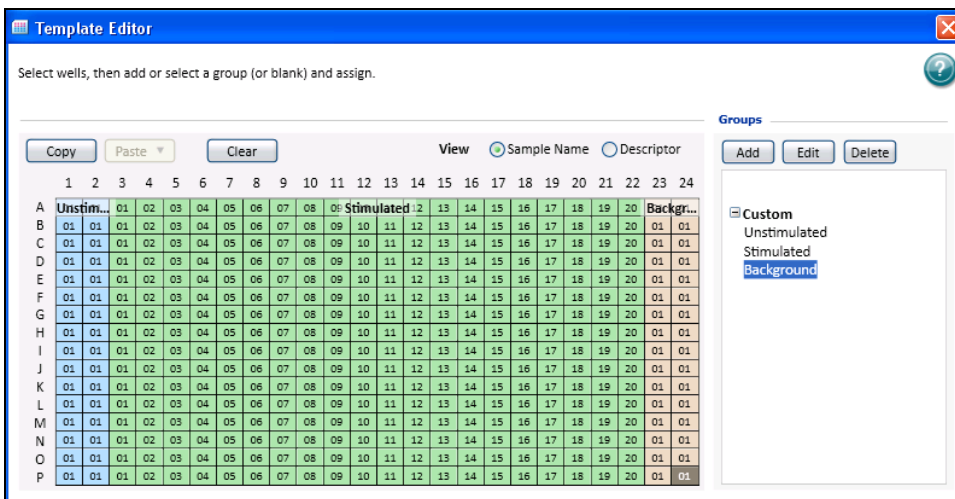

…or on the plate section header.

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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.
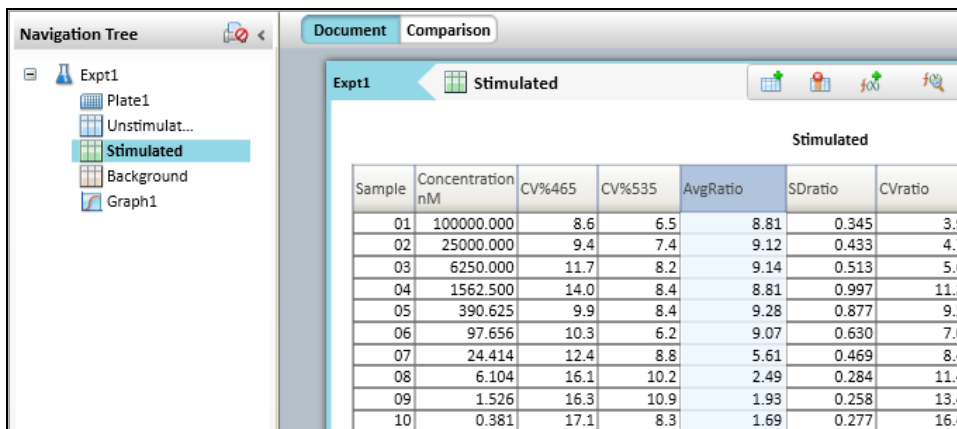

Template with wells assigned to different template groups:

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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

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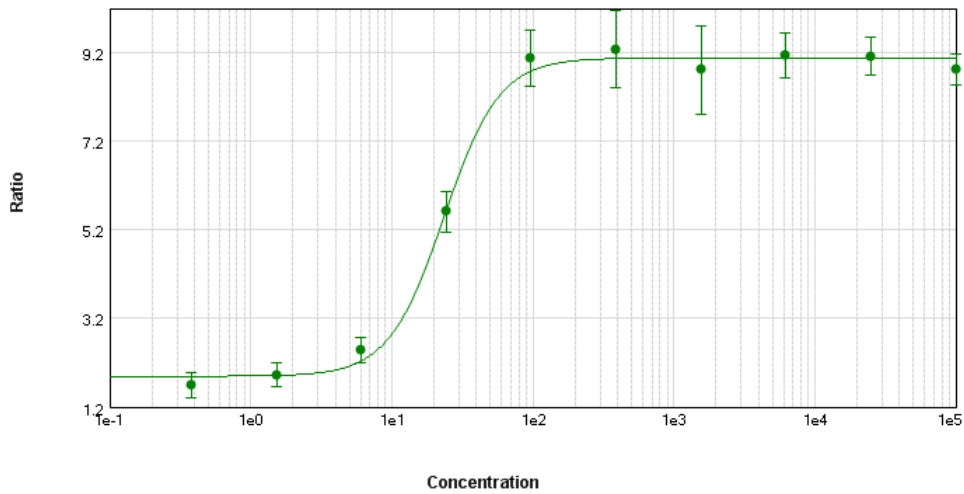
C. Results

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.