Setup for Z'-LYTE® 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 Z'-LYTE® Assay using the Z'-LYTE® Tyr6 kit (PV4122) against JAK2 JH1/JH2 kinase. The following document is intended to demonstrate setup of this instrument. **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, 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.

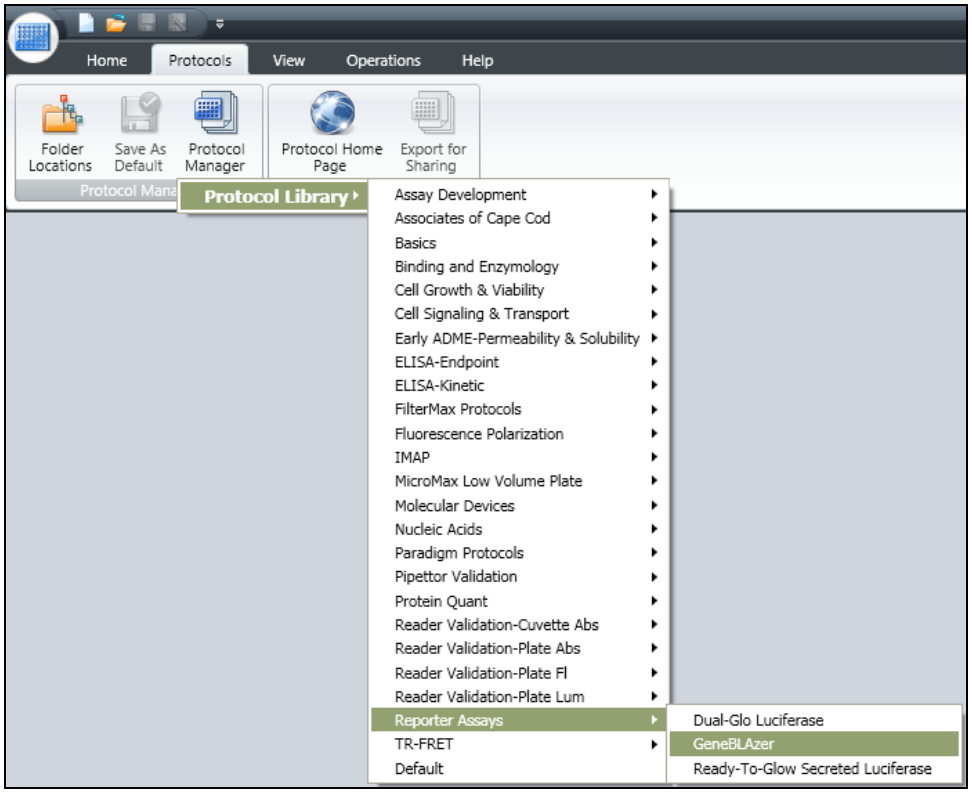
A. Recommended Optics

	Wavelength (nm)	Wavelength selection
Excitation	405/9	Monochromator
Emission 1	450/15	Monochromator
Emission 2	530/15	Monochromator
Emission 1 Cutoff	435	Filter
Emission 2 Cutoff	515	Filter

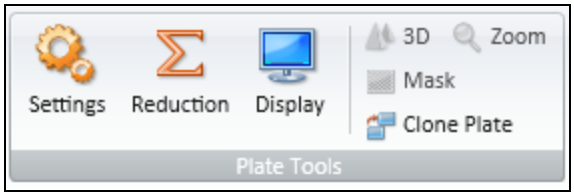
B. Instrument Setup:

1. Open SoftMax® Pro 6 software.

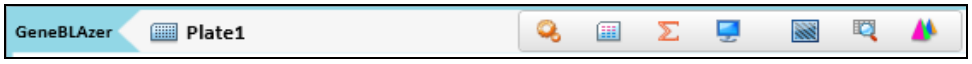
2. Click on the "Protocols" tab. Click on "Protocol Manager" to open the Protocol Library, and then open the "GeneBLAzer" protocol located in the "Reporter Assays" folder as shown below:



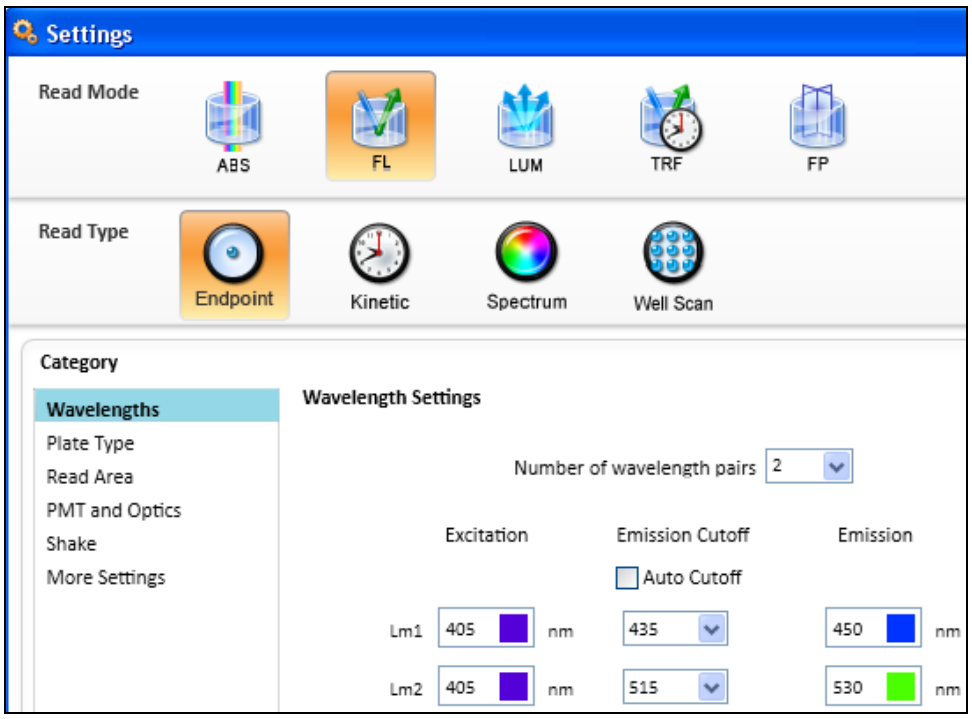
3. Click on the Settings icon either in the toolbar at the top of the screen…



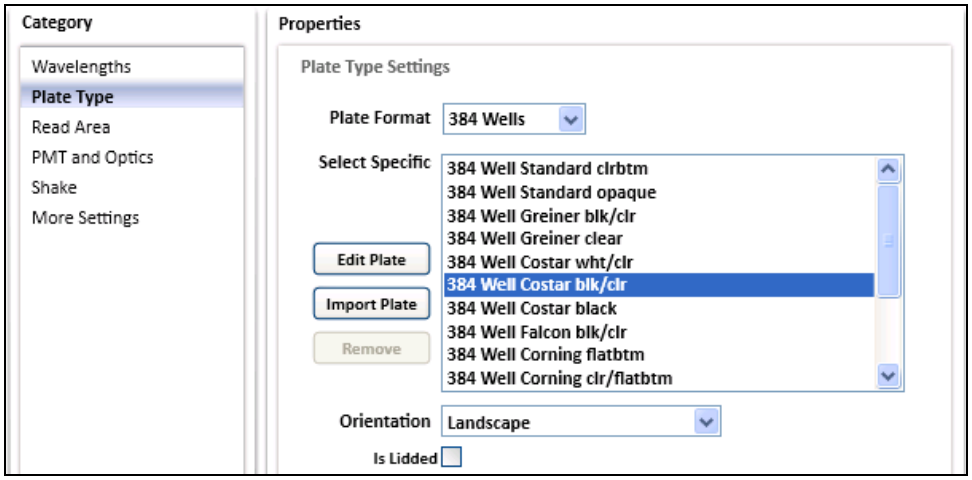
…or in the plate section header.



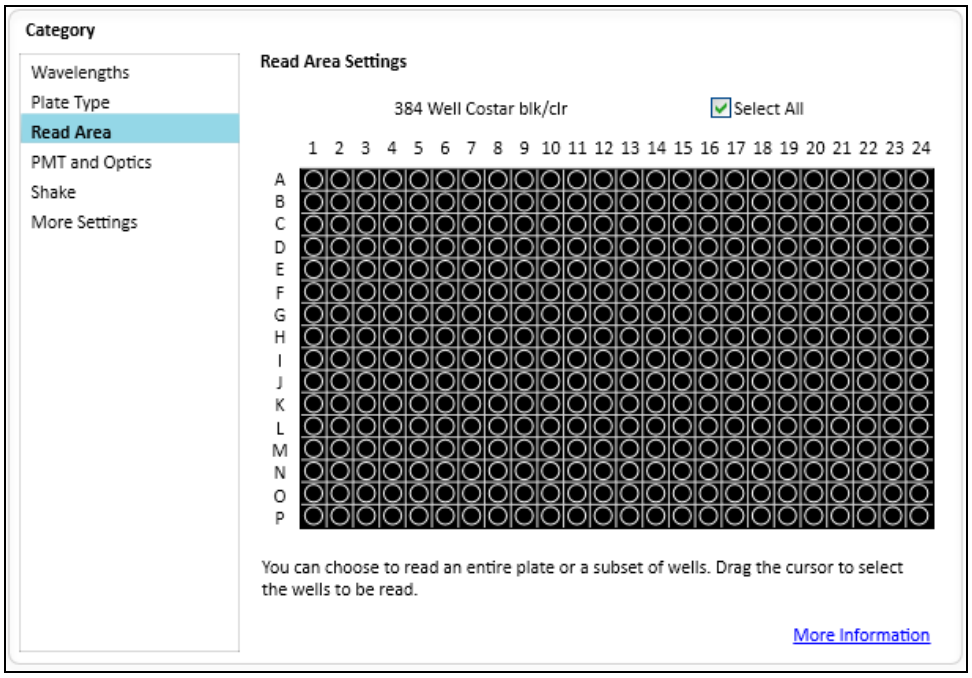
4. This opens the Settings window. Select Fluorescence read mode and Endpoint read type. Enter the optimized wavelength settings shown below:



5. 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.



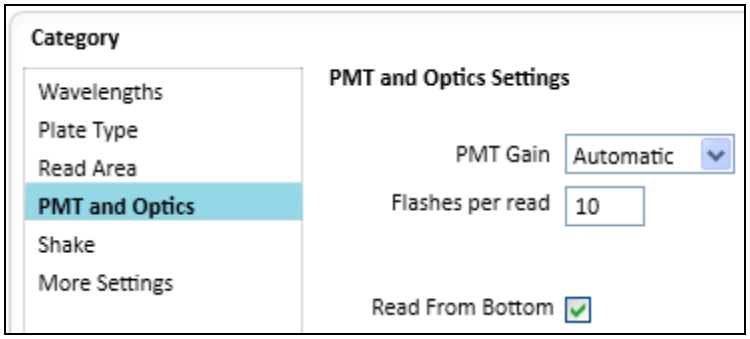
6. Now select the area of the plate to read.



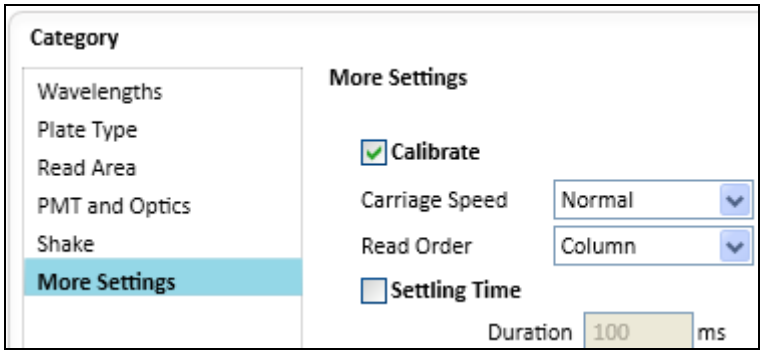
7. In the PMT and Optics category, the PMT Gain setting "Automatic" is recommended, as it enables the widest range of sample brightness to be detected in a single plate read without the need for manual PMT gain adjustment.

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

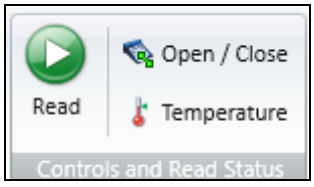
Make sure the "Read from Bottom" box is checked.



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:

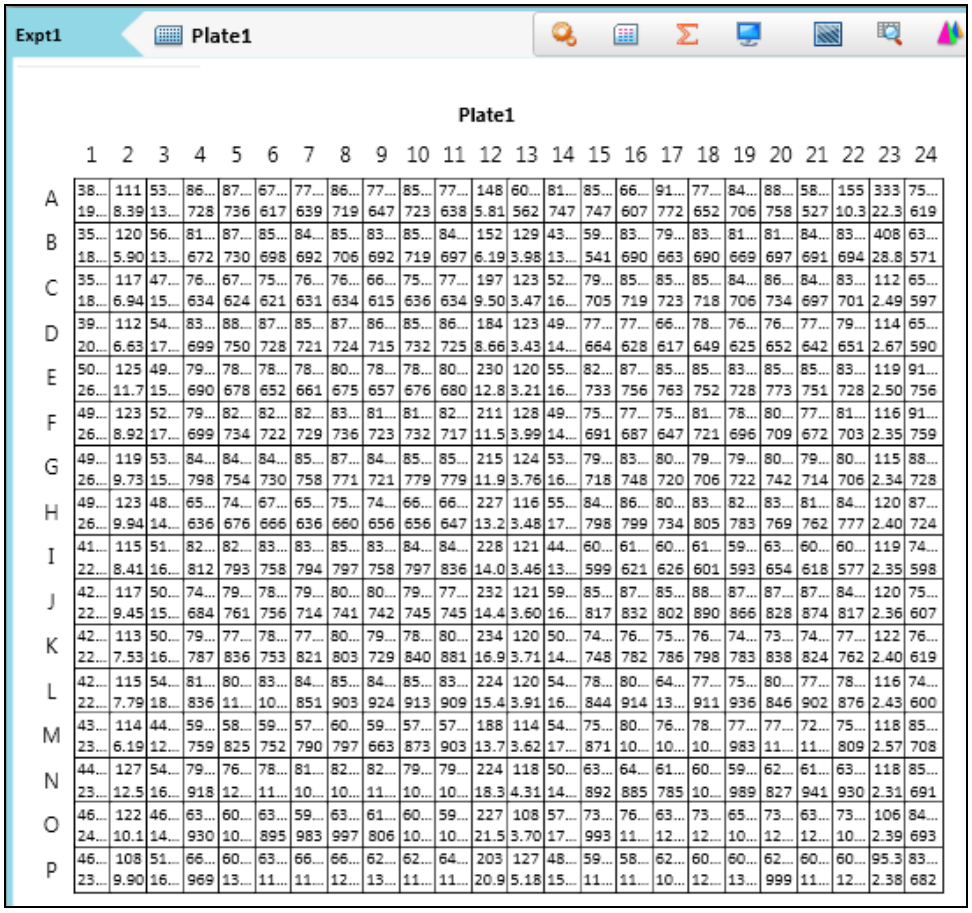
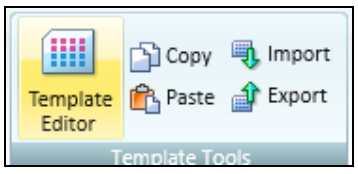


Plate1

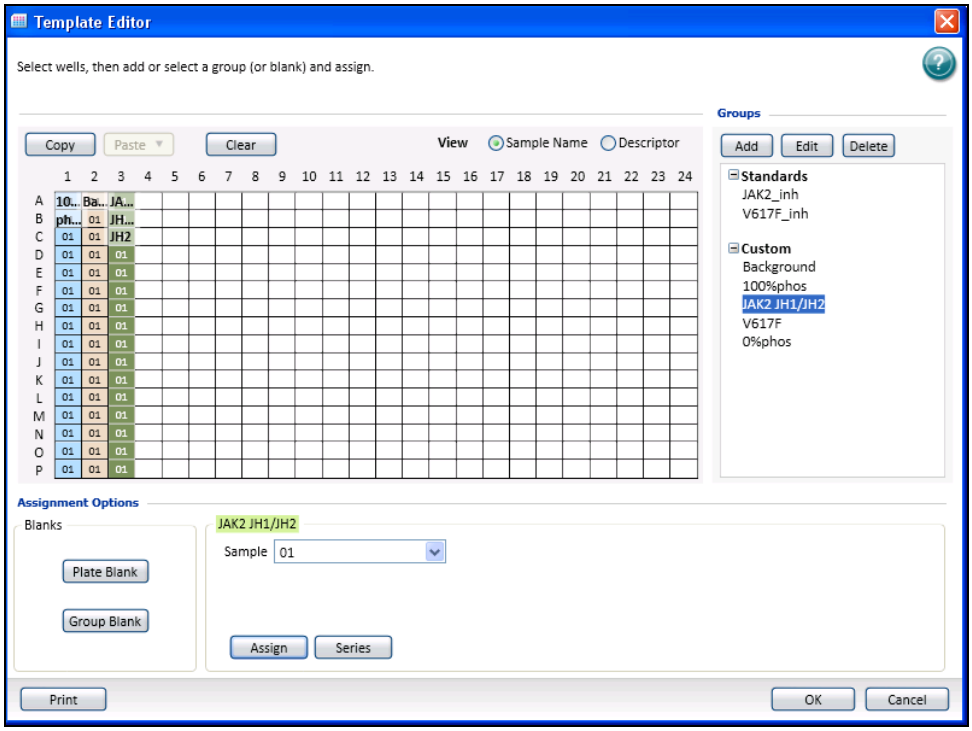
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	38...	111	53...	86...	87...	67...	77...	86...	77...	85...	77...	148	60...	81...	85...	66...	91...	77...	84...	88...	58...	155	333	75...
	19...	8.39	13...	728	736	617	639	719	647	723	638	5.81	562	747	747	607	772	652	706	758	527	10.3	22.3	619
B	35...	120	56...	81...	87...	85...	84...	85...	83...	85...	84...	152	129	43...	59...	83...	79...	83...	81...	81...	84...	83...	408	63...
	18...	5.90	13...	672	730	698	692	706	692	719	697	6.19	3.98	13...	541	690	663	690	669	697	691	694	28.8	571
C	35...	117	47...	76...	67...	75...	76...	76...	66...	75...	77...	197	123	52...	79...	85...	85...	85...	84...	86...	84...	83...	112	65...
	18...	6.94	15...	634	624	621	631	634	615	636	634	9.50	3.47	16...	705	719	723	718	706	734	697	701	2.49	597
D	39...	112	54...	83...	88...	87...	85...	87...	86...	85...	86...	184	123	49...	77...	77...	66...	78...	76...	76...	77...	79...	114	65...
	20...	6.63	17...	699	750	728	721	724	715	732	725	8.66	3.43	14...	664	628	617	649	625	652	642	651	2.67	590
E	50...	125	49...	79...	78...	78...	78...	80...	78...	78...	80...	230	120	55...	82...	87...	85...	85...	83...	85...	85...	83...	119	91...
	26...	11.7	15...	690	678	652	661	675	657	676	680	12.8	3.21	16...	733	756	763	752	728	773	751	728	2.50	756
F	49...	123	52...	79...	82...	82...	82...	83...	81...	81...	82...	211	128	49...	75...	77...	75...	81...	78...	80...	77...	81...	116	91...
	26...	8.92	17...	699	734	722	729	736	723	732	717	11.5	3.99	14...	691	687	647	721	696	709	672	703	2.35	759
G	49...	119	53...	84...	84...	84...	85...	87...	84...	85...	85...	215	124	53...	79...	83...	80...	79...	79...	80...	79...	80...	115	88...
	26...	9.73	15...	798	754	730	758	771	721	779	779	11.9	3.76	16...	718	748	720	706	722	742	714	706	2.34	728
H	49...	123	48...	65...	74...	67...	65...	75...	74...	66...	66...	227	116	55...	84...	86...	80...	83...	82...	83...	81...	84...	120	87...
	26...	9.94	14...	636	676	666	636	660	656	656	647	13.2	3.48	17...	798	799	734	805	783	769	762	777	2.40	724
I	41...	115	51...	82...	82...	83...	83...	85...	83...	84...	84...	228	121	44...	60...	61...	60...	61...	59...	63...	60...	60...	119	74...
	22...	8.41	16...	812	793	758	794	797	758	797	836	14.0	3.46	13...	599	621	626	601	593	654	618	577	2.35	598
J	42...	117	50...	74...	79...	78...	79...	80...	80...	79...	77...	232	121	59...	85...	87...	85...	88...	87...	87...	87...	84...	120	75...
	22...	9.45	15...	684	761	756	714	741	742	745	745	14.4	3.60	16...	817	832	802	890	866	828	874	817	2.36	607
K	42...	113	50...	79...	77...	78...	77...	80...	79...	78...	80...	234	120	50...	74...	76...	75...	76...	74...	73...	74...	77...	122	76...
	22...	7.53	16...	787	836	753	821	803	729	840	881	16.9	3.71	14...	748	782	786	798	783	838	824	762	2.40	619
L	42...	115	54...	81...	80...	83...	84...	85...	84...	85...	83...	224	120	54...	78...	80...	64...	77...	75...	80...	77...	78...	116	74...
	22...	7.79	18...	836	11...	10...	851	903	924	913	909	15.4	3.91	16...	844	914	13...	911	936	846	902	876	2.43	600
M	43...	114	44...	59...	58...	59...	57...	60...	59...	57...	57...	188	114	54...	75...	80...	76...	78...	77...	77...	72...	75...	118	85...
	23...	6.19	12...	759	825	752	790	797	663	873	903	13.7	3.62	17...	871	10...	10...	10...	983	11...	11...	809	2.57	708
N	44...	127	54...	79...	76...	78...	81...	82...	82...	79...	79...	224	118	50...	63...	64...	61...	60...	59...	62...	61...	63...	118	85...
	23...	12.5	16...	918	12...	11...	10...	10...	11...	10...	10...	18.3	4.31	14...	892	885	785	10...	989	827	941	930	2.31	691
O	46...	122	46...	63...	60...	63...	59...	63...	61...	60...	59...	227	108	57...	73...	76...	63...	73...	65...	73...	63...	73...	106	84...
	24...	10.1	14...	930	10...	895	983	997	806	10...	10...	21.5	3.70	17...	993	11...	12...	12...	10...	12...	12...	10...	2.39	693
P	46...	108	51...	66...	60...	63...	66...	66...	62...	62...	64...	203	127	48...	59...	58...	62...	60...	60...	62...	60...	60...	95.3	83...
	23...	9.90	16...	969	13...	11...	11...	12...	13...	11...	11...	20.9	5.18	15...	11...	11...	10...	12...	13...	999	11...	12...	2.38	682

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

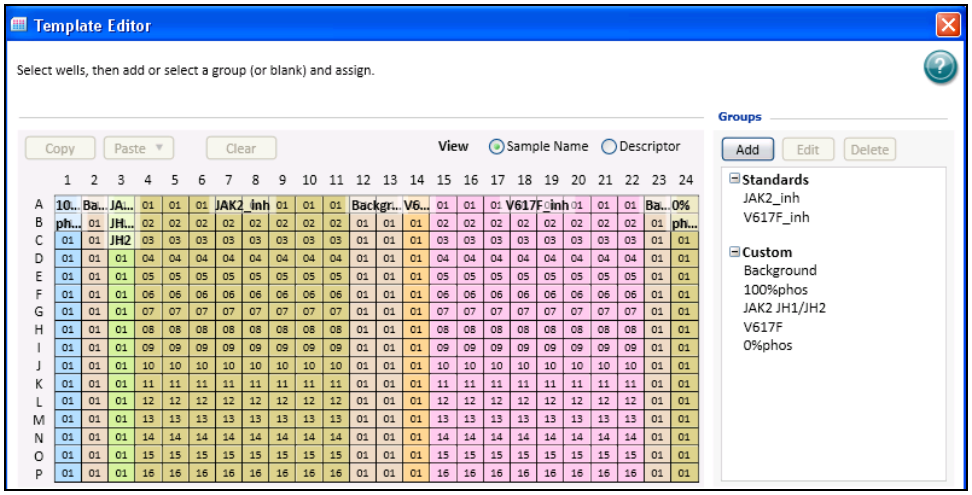


…or on the plate section header.

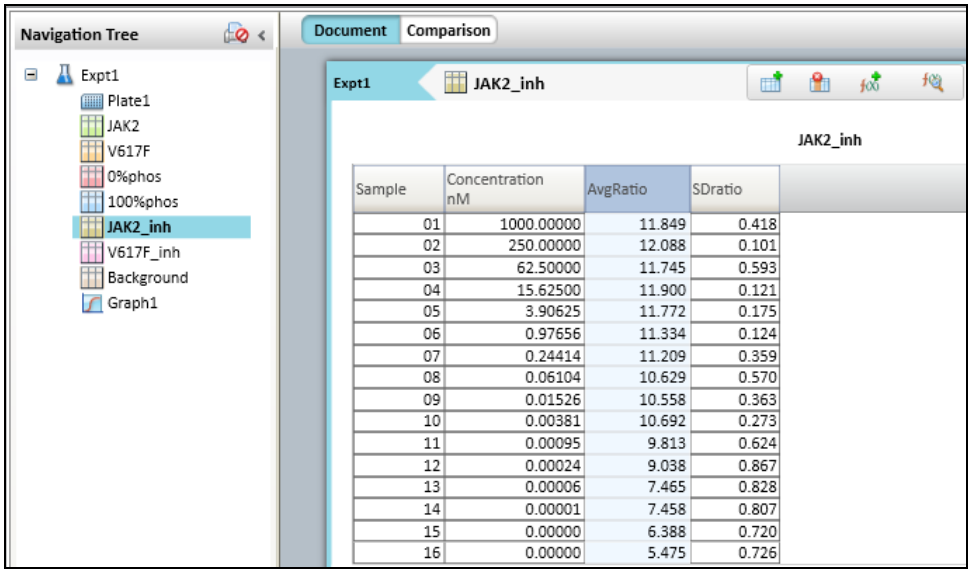
12. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.



Template with wells assigned to different template groups:



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



Sample	Concentration nM	AvgRatio	SDratio
01	1000.00000	11.849	0.418
02	250.00000	12.088	0.101
03	62.50000	11.745	0.593
04	15.62500	11.900	0.121
05	3.90625	11.772	0.175
06	0.97656	11.334	0.124
07	0.24414	11.209	0.359
08	0.06104	10.629	0.570
09	0.01526	10.558	0.363
10	0.00381	10.692	0.273
11	0.00095	9.813	0.624
12	0.00024	9.038	0.867
13	0.00006	7.465	0.828
14	0.00001	7.458	0.807
15	0.00000	6.388	0.720
16	0.00000	5.475	0.726

C. Results

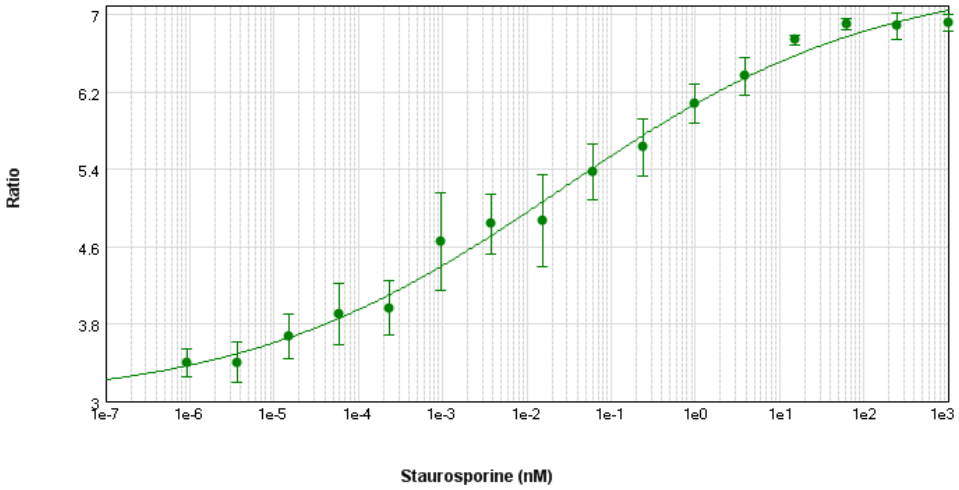


Figure 1: Z'-LYTE® Assay. JAK1 JH1/JH2 Dose-Response Curve read on the Molecular Devices SpectraMax® M5/M5e Multi-Mode Plate Reader. Z' = 0.81.