

Mediomics Application Note Data Analysis using Statistical Analysis Software

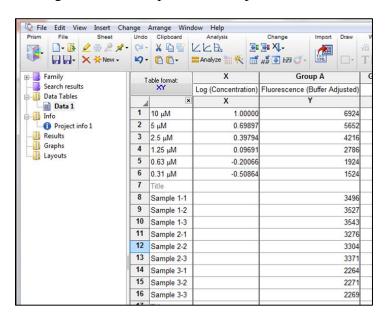
Data Analysis can be performed using software like Sigma Plot and Graph Pad Prism. A standard curve should be made to fit to a sigmoidal 4-parameter logistic equation (or an appropriate non-linear fitting equation). Then the curve can be used to interpolate the x-values (concentration) using the y-values (raw fluorescence readings).

For example, if you are using Graph Pad Prism for data analysis, follow these steps:

1. Convert the concentration for the standard curves to log values:

Well	1	2	3	4	5	6	7
Concentration (in µM)	10.00	5.00	2.50	1.25	0.63	0.31	0.00
Log values (Concentration)	1	0.69897	0.39794	0.09691	-0.20066	-0.50864	
Fluorescence (Buffer Adjusted)	6924	5652	4216	2786	1924	1524	1219

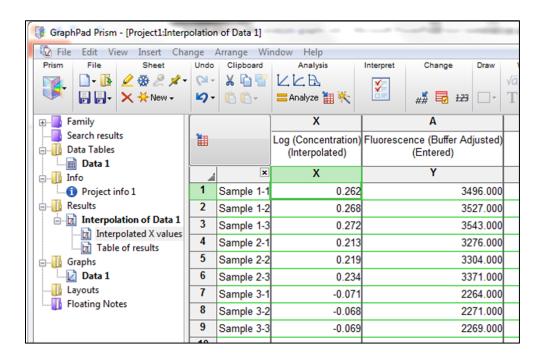
2. Input the converted log values, the corresponding fluorescence values, and the fluorescence readings form the sample in the Graph Pad Prism screen:



3. Under Analysis tab, click on the third icon (Interpolate a standard curve). Then select "Sigmoidal, 4PL, X is log (concentration)" and press OK. You may change other parameters depending on your requirements.



4. On the left hand side, under "Results" select "Interpolation of Data 1"> "Interpolated X values." These are the corresponding x values (log of concentration) based on the fluorescence readings of the samples.



5. Convert the log values to concentration. The average of the replicate values is the estimated concentration for each sample.

	Log (Concentration) (Interpolated)	Estimated concentration (μΜ)	Average (μM)
Sample 1-1	0.26172	1.83	1.85
Sample 1-2	0.26841	1.86	
Sample 1-3	0.27185	1.87	
Sample 2-1	0.21282	1.63	1.67
Sample 2-2	0.21919	1.66	
Sample 2-3	0.23426	1.71	
Sample 3-1	-0.0705	0.85	0.85
Sample 3-2	-0.068	0.86	
Sample 3-3	-0.0687	0.85	