



## 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 $\mu\text{M}$ )	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 from the sample in the Graph Pad Prism screen:

The screenshot shows the Graph Pad Prism software interface. The 'Table format' is set to 'XY'. The table contains the following data:

	X	Group A	
	Log (Concentration)	Fluorescence (Buffer Adjusted)	
	X	Y	
1	10 $\mu\text{M}$	1.00000	6924
2	5 $\mu\text{M}$	0.69897	5652
3	2.5 $\mu\text{M}$	0.39794	4216
4	1.25 $\mu\text{M}$	0.09691	2786
5	0.63 $\mu\text{M}$	-0.20066	1924
6	0.31 $\mu\text{M}$	-0.50864	1524
7	Title		
8	Sample 1-1		3496
9	Sample 1-2		3527
10	Sample 1-3		3543
11	Sample 2-1		3276
12	Sample 2-2		3304
13	Sample 2-3		3371
14	Sample 3-1		2264
15	Sample 3-2		2271
16	Sample 3-3		2269

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.



- 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.

		X	A
		Log (Concentration) (Interpolated)	Fluorescence (Buffer Adjusted) (Entered)
		X	Y
1	Sample 1-1	0.262	3496.000
2	Sample 1-2	0.268	3527.000
3	Sample 1-3	0.272	3543.000
4	Sample 2-1	0.213	3276.000
5	Sample 2-2	0.219	3304.000
6	Sample 2-3	0.234	3371.000
7	Sample 3-1	-0.071	2264.000
8	Sample 3-2	-0.068	2271.000
9	Sample 3-3	-0.069	2269.000

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

	Log (Concentration) (Interpolated)	Estimated concentration ( $\mu\text{M}$ )	Average ( $\mu\text{M}$ )
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	