Mediomics Application Note
Data Analysis using Microsoft Excel- Relative Fluorescence
Creating the Standard Curve and Calculating Sample Concentrations

If analytical software is not available, data analysis can be performed using Microsoft Excel. A standard curve should be made to fit a linear curve. Then the curve can be used to interpolate the x-values (concentration) using the y-values (raw fluorescence readings).

Creating the Standard Curve

1. Paste the raw data obtained from reading the microplate with the plate reader, setting at 485/20,665/34; sensitivity 80

Well	1	2	3	4	5	6	7	8
Concentration (in µM)	10.00	5.00	2.50	1.25	0.63	0.31	0.00	Buffer Only
Fluorescence (Sen. 80)	7055	5783	4347	2917	2055	1655	1350	131

2. Subtract the reading in well 8 (F buffer) from step 1 from the fluorescence reading at each data point (F sample) from step 2 so that the equation is: F sample – F buffer

Well	1	2	3	4	5	6	7	8
F sample – F buffer	6924	5652	4216	2786	1924	1524	1219	0

3. Subtract the reading from well 7 in step 2 (F0) from the fluorescence at each data point from step 2 (F), then divide by F0 so that the equation is: (F-F0)/F0

Well	1	2	3	4	5	6	7
Relative Fluorescence (F-F0)/F0	4.68	3.64	2.46	1.29	0.58	0.25	0.00

4. Hold down the keyboard control button and select the “Concentration” points (10 through 0) in step 1 and the “Relative Fluorescence (F-F0)/F0” points from step 3

5. Go to the Insert Tab at the top of the Excel sheet and insert a scatter chart by selecting the first option "Scatter chart with only markers"

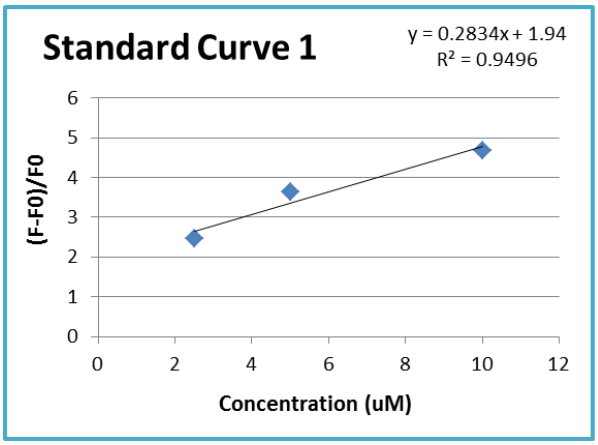
6. Right click the data points on the chart and choose "Add Trendline". In the pop up window, check mark "Display Equation on Chart" and "Display R-squared on chart"

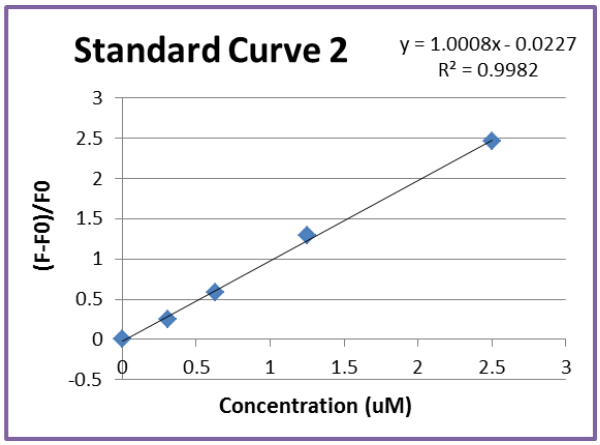
7. Use the x = (y-b)/m equation to calculate the µM of the standards (x) where y= the fluorescence at each data point in step 3, and b and m are obtained from the chart equation

Well	1	2	3	4	5	6	7
Concentration (in µM)	6.17	4.74	3.13	1.53	0.56	0.11	-0.23

Note:

If standard curve is not completely linear throughout the entire concentration range (0 µM to 10 µM), it can still be accurately analyzed by breaking it into two standard curves with high end and low end points, respectively. This can be done by going back to step 3 and making Standard Curve 1 with "Concentration" points 10, 5, 2.5 and Standard Curve 2 with points 2.5 through 0, or whichever range provides the most linear curve (R^2 value as close to 1 as possible). Points on each graph may be adjusted based on which points give the best fit line.





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
Standard Curve 1 Concentration (in µM)	9.67	6.00	1.83	-2.29	-4.80	-5.96	-6.85
Standard Curve 2 Concentration (in µM)	4.65	3.61	2.44	1.27	0.56	0.23	-0.02

Estimate the concentration with both standard curves, as shown above, to see which is more accurate. Based on the data above, samples with a concentration of 5-10 µM (fluorescence values between 5652-6924) are more accurately analyzed with Standard Curve 1; whereas samples with a concentration of 2.5 µM or below (fluorescence values of 4216 or less) are more accurately analyzed with Standard Curve 2.

Calculating the Sample Concentrations

8. After the standard curve has been created, it is time to analyze the samples.

9. Paste the raw data obtained from reading the microplate with the plate reader, setting at 485/20,665/34; sensitivity 80 (sensitivity may vary depending on plate reader)

Run in triplicates	Sample 1	Sample 2	Sample 3
Fluorescence: 1	3627	3407	2395
Fluorescence: 2	3658	3435	2402
Fluorescence: 3	3674	3502	2400

10. Subtract the reading in well 8 (F buffer) from step 1 from the fluorescence reading at each data point (F sample) from step 1 so that the equation is: F sample – F buffer

	Sample 1	Sample 2	Sample 3
F sample- F Buffer: 1	3496	3276	2264
F sample- F Buffer: 1	3527	3304	2271
F sample- F Buffer: 3	3543	3371	2269

11. Subtract the reading from well 7 of your standard curve (in step 2, F0, in this example, is 1219) from the fluorescence at each data point from step 10 (F), then divide by F0 so that the equation is: (F-F0)/F0

	Sample 1	Sample 2	Sample 3
(F-F0)/F0: 1	1.87	1.69	0.86
(F-F0)/F0: 2	1.89	1.71	0.86
(F-F0)/F0: 3	1.91	1.77	0.86

12. The average of the replicate values is the estimated concentration for each sample.

	Sample 1	Sample 2	Sample 3
Average uM	1.89	1.72	0.86