

**PREPARING AND INTERPRETING CYTOTOXICITY DATA**

After 30 minutes in the incubator, read your plates on a plate reader using SoftMax Pro (or equivalent) software.

If the mean A490 of the control wells on plate is >0.250, the assay is complete.

If the mean A490 of the control wells on plate is <0.250, return the plates to the incubator for another 30 minutes, then read again.

*(You should notice a color change in the wells when the assay is complete. See "Cytotoxicity Assay for Targeted Toxins In Vitro")*

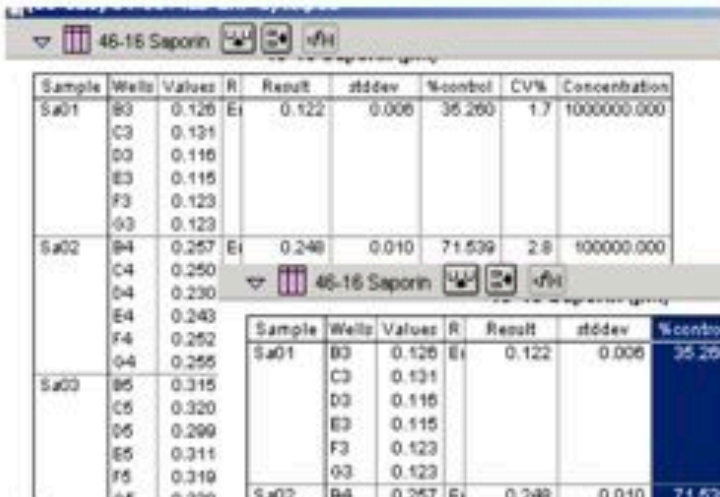
Continue reading the plates every 25-35 minutes until the mean A490 of the control wells on plate is >0.250.

This is a standard readout using SOFTmax Pro.



	1	2	3	4	5	6	7	8	9	10	11	12	Endpoint
A	0.000												
B		0.344	0.126	0.257	0.315	0.377	0.426	0.375	0.380	0.369	0.363		Left: 490
C		0.362	0.131	0.250	0.320	0.341	0.348	0.359	0.360	0.353	0.343		Automatic: Once Calibrate: On Column Priority
D		0.314	0.118	0.230	0.299	0.331	0.339	0.336	0.332	0.343	0.339		Plate Last Read 10:30 AM 3/17/2008
E		0.357	0.115	0.243	0.311	0.367	0.381	0.382	0.349	0.371	0.367		
F		0.308	0.123	0.252	0.319	0.347	0.380	0.354	0.341	0.345	0.350		
G		0.329	0.123	0.255	0.320	0.345	0.355	0.381	0.336	0.362	0.381		
H													

Wavelength Combination: Lml  
 Data Mode: Absorbance  
 Plate Blank Used: Lml = 0.227



Sample	Wells	Values	R	Result	stddev	%control	CV%	Concentration
Sa01	B3	0.126	E1	0.122	0.006	35.260	1.7	1000000.000
	C3	0.131						
	D3	0.116						
	E3	0.115						
	F3	0.123						
	G3	0.123						
Sa02	B4	0.257	E1	0.248	0.010	71.539	2.8	100000.000
	C4	0.250						
	D4	0.230						
	E4	0.243						
	F4	0.252						
	G4	0.255						
Sa03	B5	0.315	E1	0.314	0.008	90.656	2.4	100000.000
	C5	0.320						
	D5	0.299						
	E5	0.311						
	F5	0.319						
	G5	0.315						

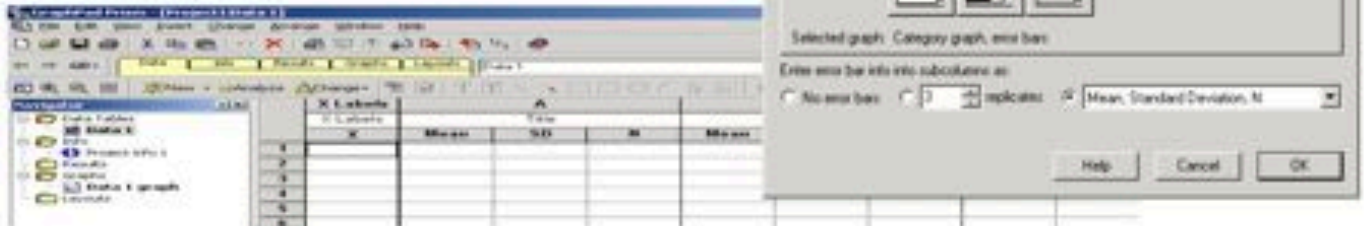
Use the software to calculate "Result," "% Control," Standard deviation "stddev," and Coefficient of Variation "CV%."

Highlight the "% Control" and "CV%" columns and "Copy."

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You can import this data into any spreadsheet/graph analytic software. Here is an example using Prism GraphPad.

1. Open the "File" menu on the toolbar and click on "New Project". This window opens up. Select "Create a new project" and click the "Two grouping variables" folder and select the "Category Graph with error bars" option. Have the software enter error bar info into subcolumns as "Mean, Standard Deviation, N." Click OK.
2. After completing step one, this is the window that should come up.



"Paste" the columns you copied from SOFTmax into the "Mean" and "SD" columns. The % Control column from SOFTmax will be pasted into the Mean column in Prism and the CV% column from SOFTmax will be pasted in the SD column in Prism.

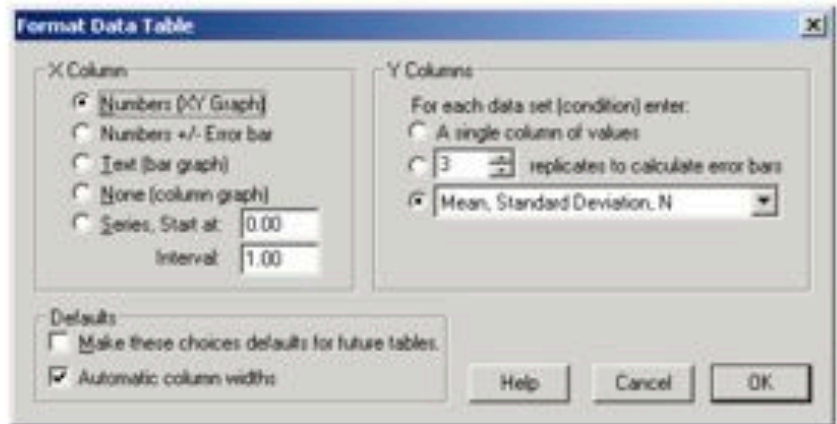
Enter in the Molar concentration corresponding to each point in the "X" axis column.



	X Labels Concentration	A 40-16 Saprop			B 21-168 Cox-2-66P			C 4-80 Mab-3AP + Cox2		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
1	0.000001	35.260	1.7							
2	1.000000e+007	71.539	2.8				27.488	3.9		
3	1.000000e+008	90.656	2.4	15.474	1.2		19.678	2.3		
4	1.000000e+009	101.410	4.9	17.205	1.8		86.809	6.6		
5	1.000000e+010	107.241	9.2	26.543	0.9		103.774	9.2		
6	1.000000e+011	104.239	4.7	65.919	3.0		104.521	6.5		

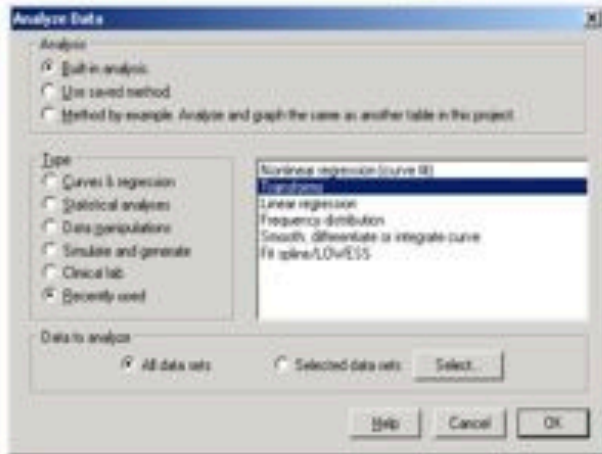
Open the "Change" menu on the toolbar and click on "Format Data Table." This window pops up.

Select the "Numbers (XY Graph)" option for the X column and make sure that "Mean, Standard Deviation, N" is also selected for the Y columns. "Automatic column widths" should be selected as the default. Click OK.

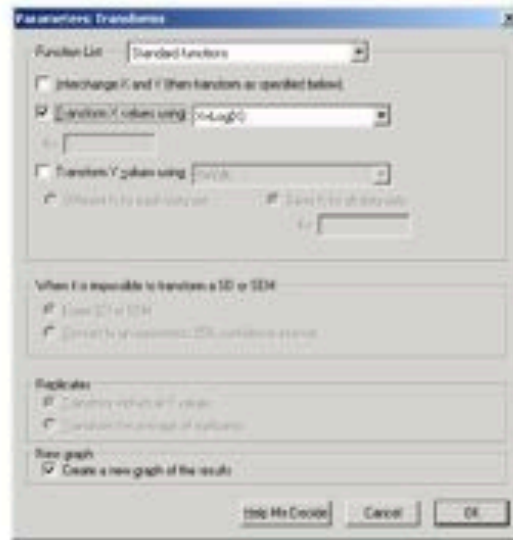


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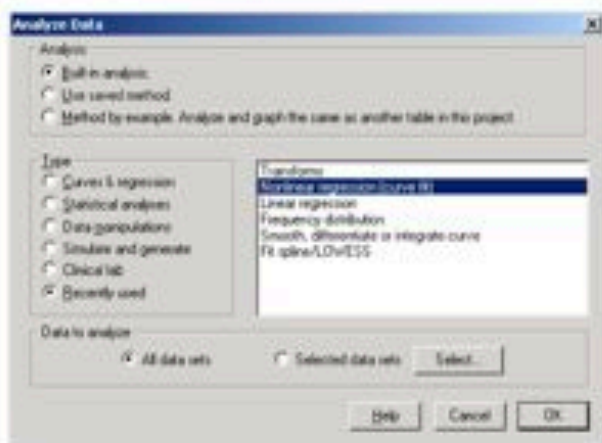
Open the "Analyze" menu from the toolbar and this window pops up. Use "Built-in analysis" and highlight "Transforms" from the list of options and select "All data sets" for data to analyze. Click OK.



This window pops up. Choose "Standard functions" from function list and select Transform X values using "X=Log(X)." Click OK.



Open the "Analyze" menu *again* from the toolbar and highlight "Nonlinear regression (curve fit)" from the list of options. Built-in analysis should already be selected as well as all data sets selected for data to analyze. Click OK.



This window pops up. From the Classic Equation selections, highlight "Sigmoidal dose response." Select "Fit a curve with nonlinear regression." Click OK.

