ADVANCED TARGETING SYSTEMS
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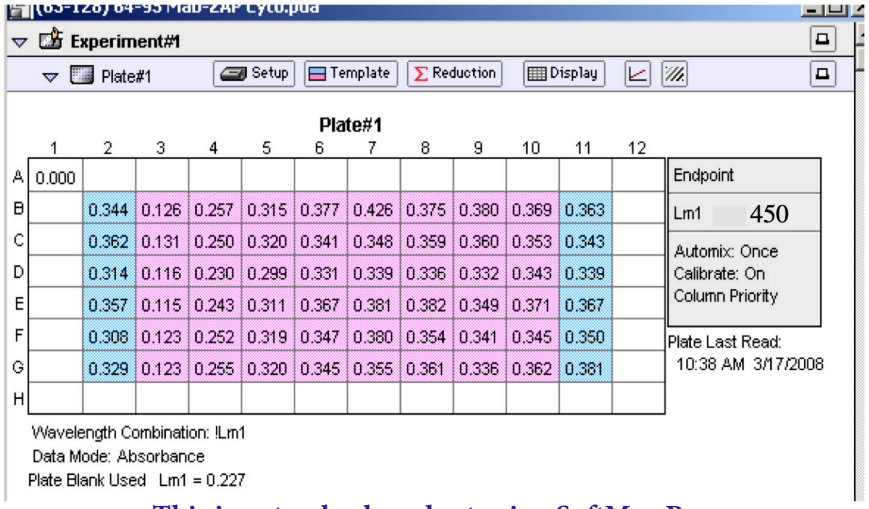
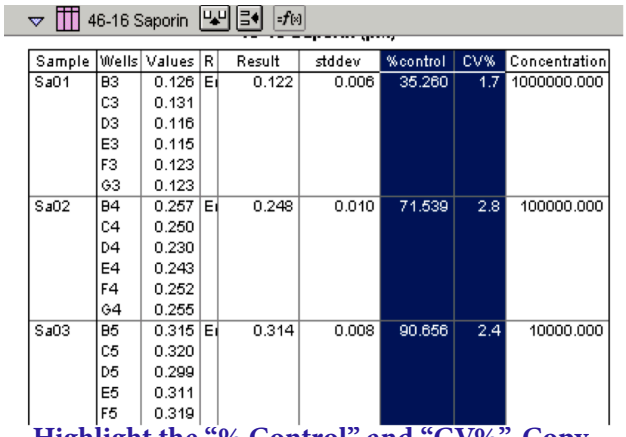
Preparing and Interpreting Cytotoxicity Data

SOFTMAX PRO

- After 30 minutes in the incubator, read your plates on a plate reader using SoftMax Pro (or equivalent) software.
- If the mean A450 of the control wells on plate is >0.3, the assay is complete. If the mean A450 of the control wells on plate is <0.3, return the plates to the incubator for another 30 minutes, then read again. Continue reading the plates every 25-35 minutes until the mean A450 of the control wells on plate is >0.3.
- You should notice a color change in the wells when the assay is complete.
- Use the software to calculate "Result," "% Control," Standard deviation "stddev," and Coefficient of Variation "CV%."
- Highlight the "% Control" and "CV%" columns and COPY the contents.



This is a standard readout using SoftMax Pro.

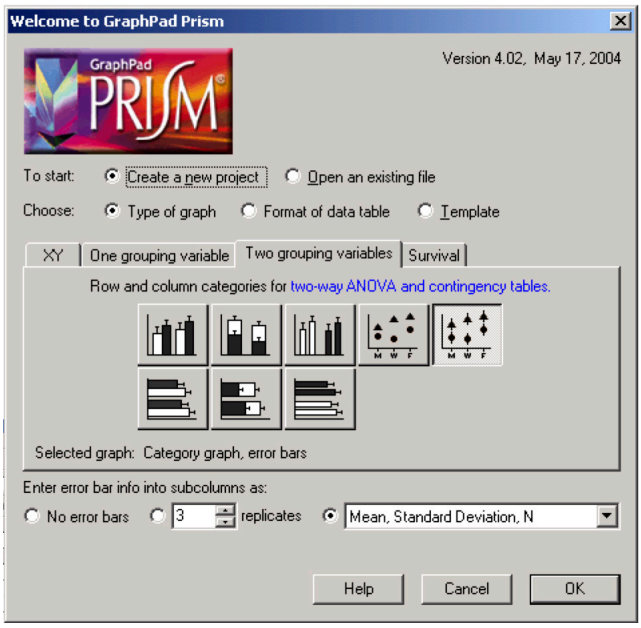


Sample	Wells	Values	R	Result	stddev	%control	CV%	Concentration
Sa01	B3	0.126	E	0.122	0.006	35.260	1.7	1000000.000
	C3	0.131						
	D3	0.116						
	E3	0.115						
	F3	0.123						
	G3	0.123						
Sa02	B4	0.257	E	0.248	0.010	71.539	2.8	100000.000
	C4	0.250						
	D4	0.230						
	E4	0.243						
	F4	0.252						
	G4	0.255						
Sa03	B5	0.315	E	0.314	0.008	90.656	2.4	10000.000
	C5	0.320						
	D5	0.299						
	E5	0.311						
	F5	0.319						

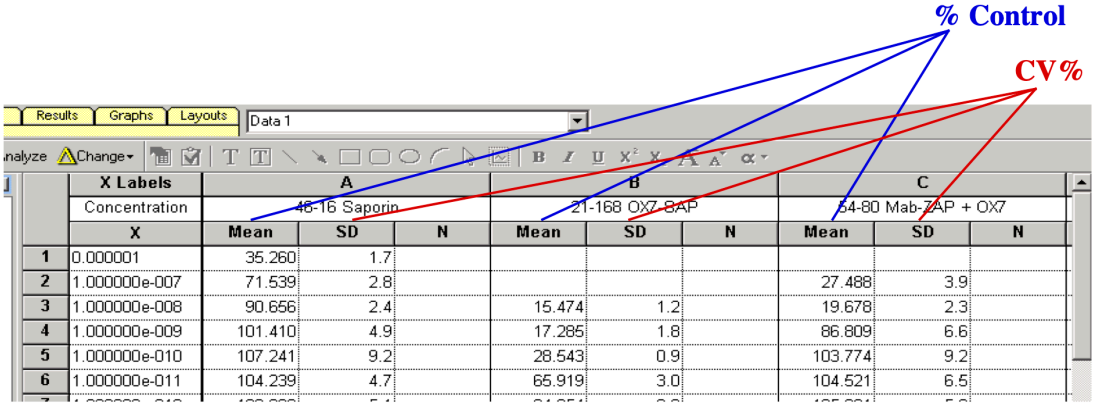
Highlight the "% Control" and "CV%". Copy.

Prism GraphPad

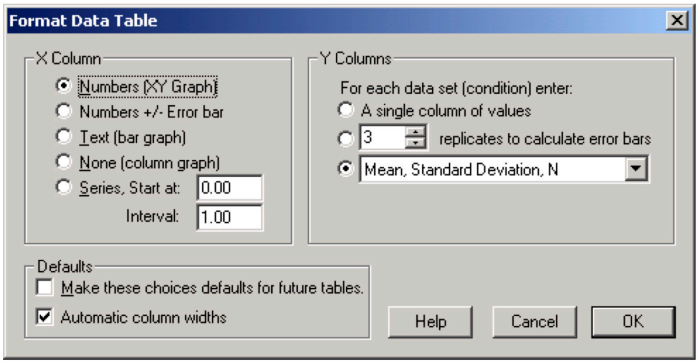
- You can import this data into any spreadsheet/graph analytic software such as Prism GraphPad.
- Open the "File" menu on the toolbar and click on "New Project".
- Select "Create a new project" and click the "Two grouping variables" folder and select the "Category Graph with error bars" option.
- Have the software enter error bar info into subcolumns as "Mean, Standard Deviation, N." Click OK.



- 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.
- The CV% column from SoftMax will be pasted in the SD column.
- Enter in the Molar concentration corresponding to each point in the "X" axis column.

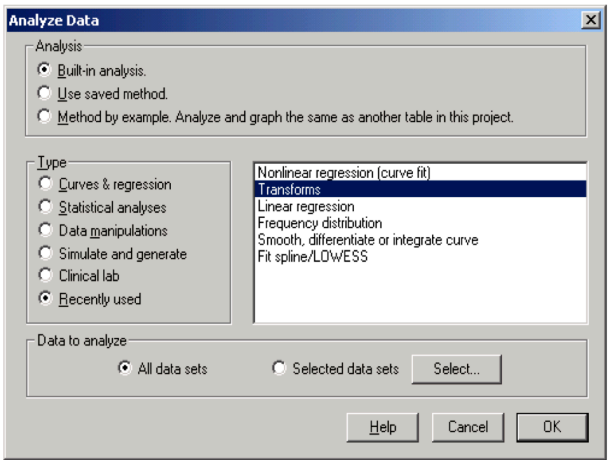


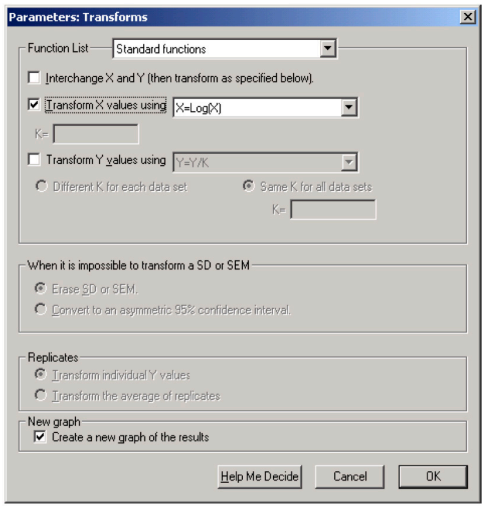
- Open the "Change" menu on the toolbar and click on "Format Data Table."
- Select the "Numbers (XY Graph)" option for the X column and make sure that "Mean, Standard Deviation, N" is also selected for the Y columns. "Automatic column widths" should be selected as the default. Click OK.



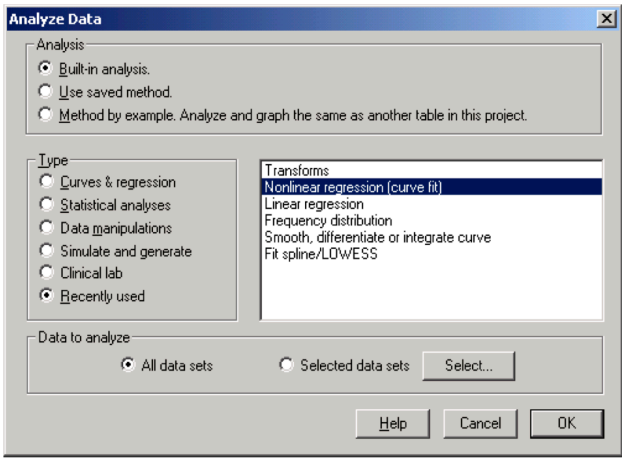
Prism GraphPad (continued)

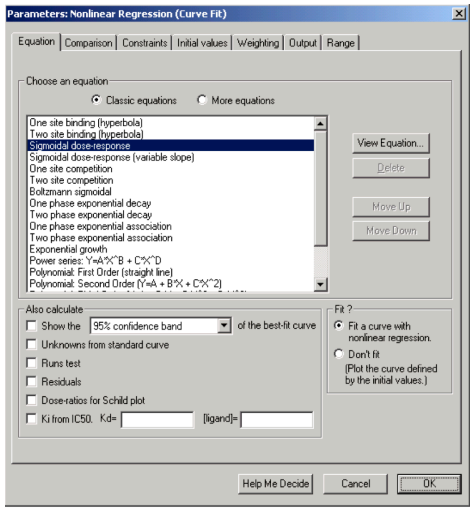
- Open the "Analyze" menu from the toolbar. Use "Built-in analysis" and highlight "Transforms" from the list of options and select "All data sets" for data to analyze. Click OK.
- Choose "Standard functions" from function list and select Transform X values using "X=Log(X)." Click OK.





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





Prism GraphPad (continued)

- After completing all the transformation steps, the software calculates the results. The calculation of major importance is the "EC50" listed on line #6 for each sample.

		A	B	C
		46-16 Saporin	21-168 OX7-SAP	54-80 Mab-ZAP + OX7
		Y	Y	Y
1	Sigmoidal dose-response			
2	Best-fit values			
3	BOTTOM	27.04	16.42	17.08
4	TOP	102.5	102.3	108.0
5	LOGEC50	-6.888	-10.86	-8.676
6	EC50	1.293e-007	1.368e-011	2.109e-009
7	Std. Error			
8	BOTTOM	5.866	1.139	7.702
9	TOP	1.651	0.9190	3.748
10	LOGEC50	0.1268	0.03883	0.1938
11	95% Confidence Intervals			
12	BOTTOM	11.96 to 42.12	13.49 to 19.35	-2.725 to 36.88
13	TOP	98.28 to 106.8	99.98 to 104.7	98.36 to 117.6
14	LOGEC50	-7.214 to -6.562	-10.96 to -10.76	-9.174 to -8.178
15	EC50	6.107e-008 to 2.740e-007	1.087e-011 to 1.722e-011	6.696e-010 to 6.644e-009
16	Goodness of Fit			
17	Degrees of Freedom	5	5	5
18	R^2	0.9825	0.9987	0.9668
19	Absolute Sum of Squares	74.48	14.92	327.5
20	Sy.x	3.860	1.727	8.094

- Click on the "Graphs" folder.
- Click on any of the graph axes. Adjust the parameters for all the axes.
- Click on any point/symbol from one of the lines on the graph. Adjust what color or symbol you want the lines and points to be.