ADVANCED TARGETING SYSTEMS

10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

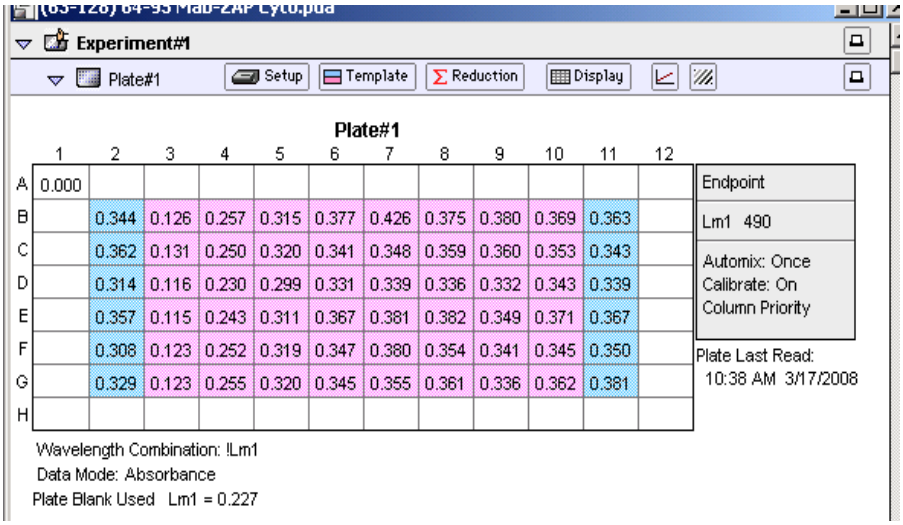
PREPARING AND INTEPRETING CYTOTOXICITY DATA

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.

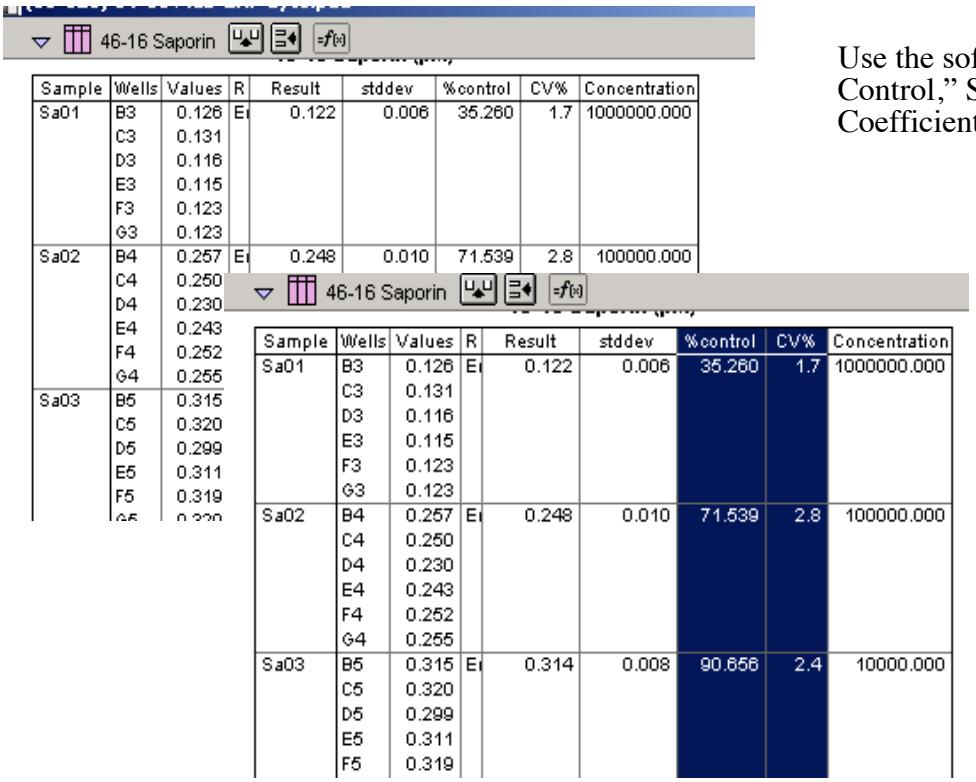
If the mean A490 of the control wells on plate is <0.250, return the plates to the incubator for another 30 minutes, then read again.

(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.



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

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

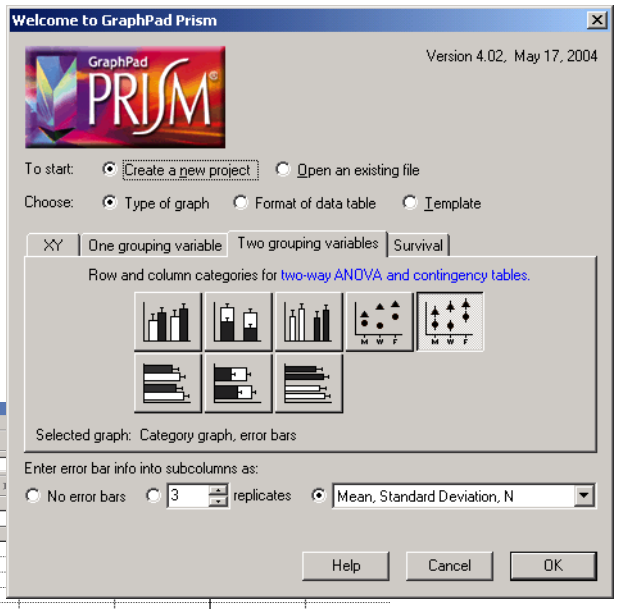
ADVANCED TARGETING SYSTEMS

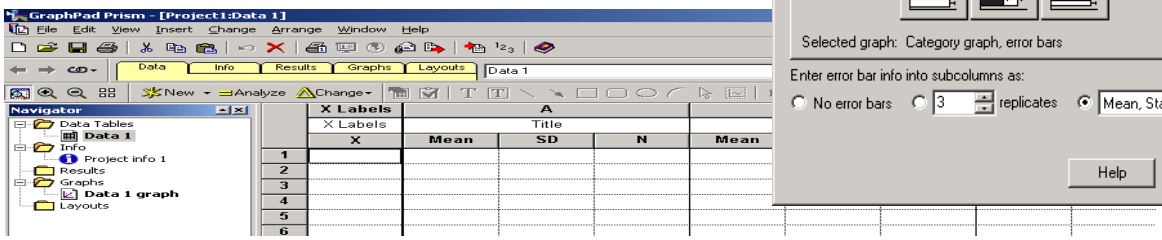
10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

PREPARING AND INTEPRETING CYTOTOXICITY DATA

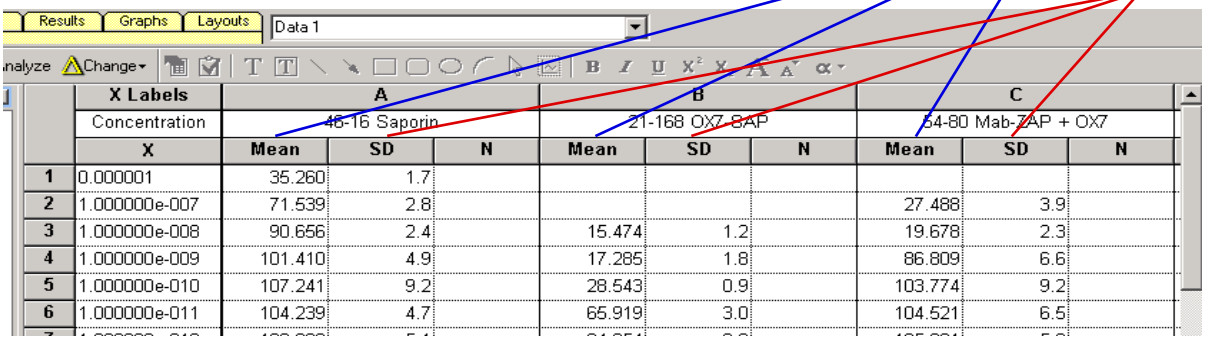
You can import this data into any spreadsheet/graph analytic software. Here is an example using Prism GraphPad.

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.

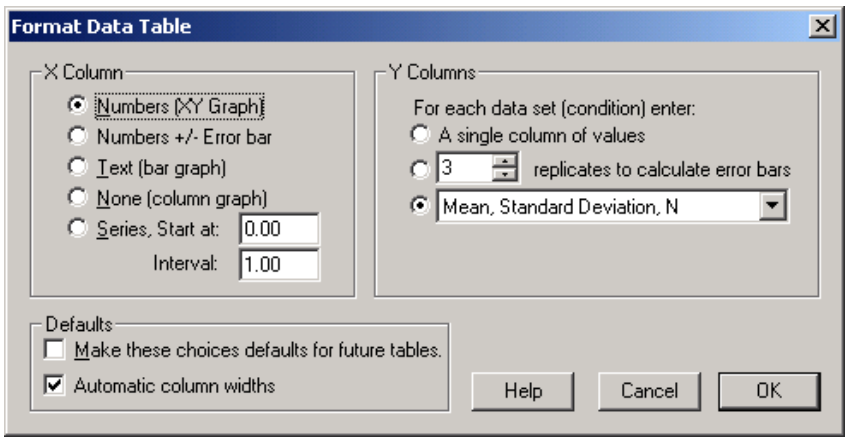


	X	A Mean	A SD	A N	B Mean	B SD	B N	C Mean	C SD	C N
1	0.000001	35.260	1.7							
2	1.000000e-007	71.539	2.8					27.488	3.9	
3	1.000000e-008	90.656	2.4		15.474	1.2		19.678	2.3	
4	1.000000e-009	101.410	4.9		17.285	1.8		86.809	6.6	
5	1.000000e-010	107.241	9.2		28.543	0.9		103.774	9.2	
6	1.000000e-011	104.239	4.7		65.919	3.0		104.521	6.5	

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." 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.

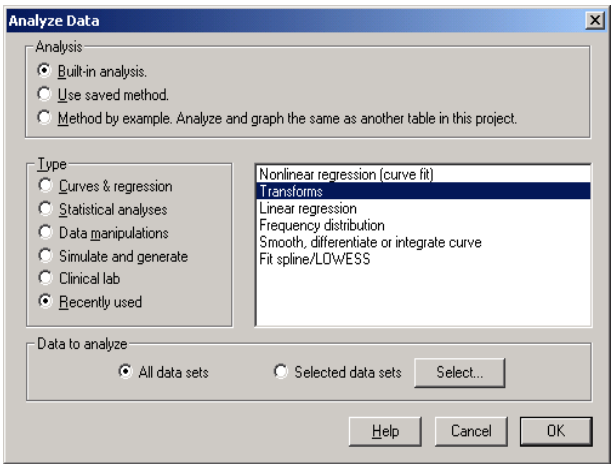


ADVANCED TARGETING SYSTEMS

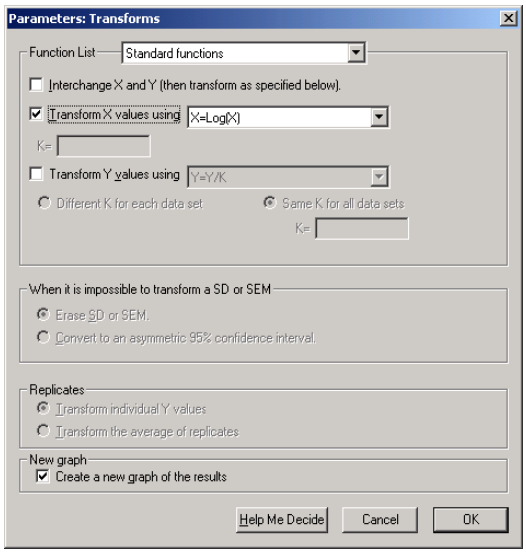
10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

PREPARING AND INTEPRETING CYTOTOXICITY DATA

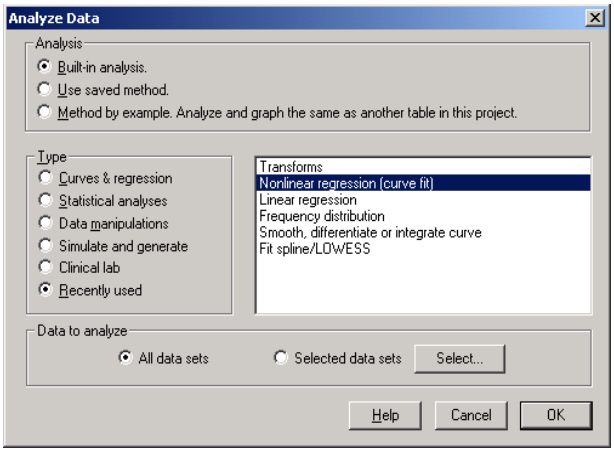
Open the "Analyze" menu from the toolbar and this window pops up. Use "Built-in analysis" and highlight "Transforms" from the list of options and select "All data sets" for data to analyze. Click OK.



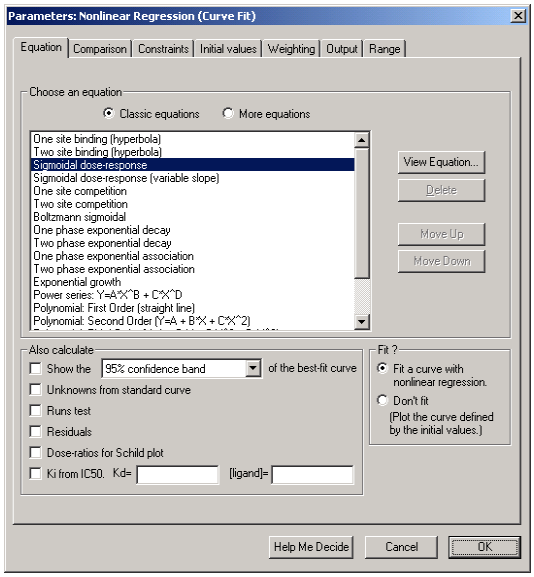
This window pops up. Choose "Standard functions" from function list and select Transform X values using "X=Log(X)." Click OK.



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.



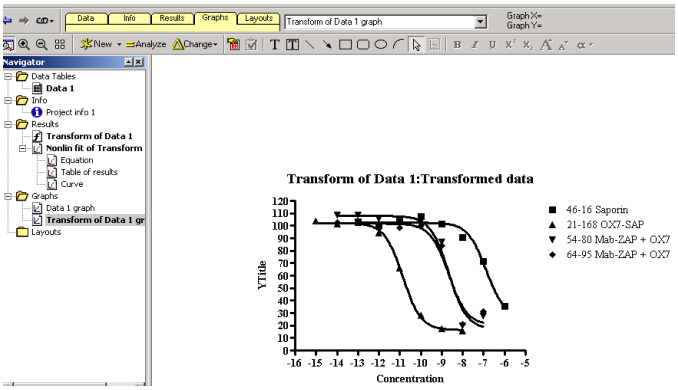
ADVANCED TARGETING SYSTEMS

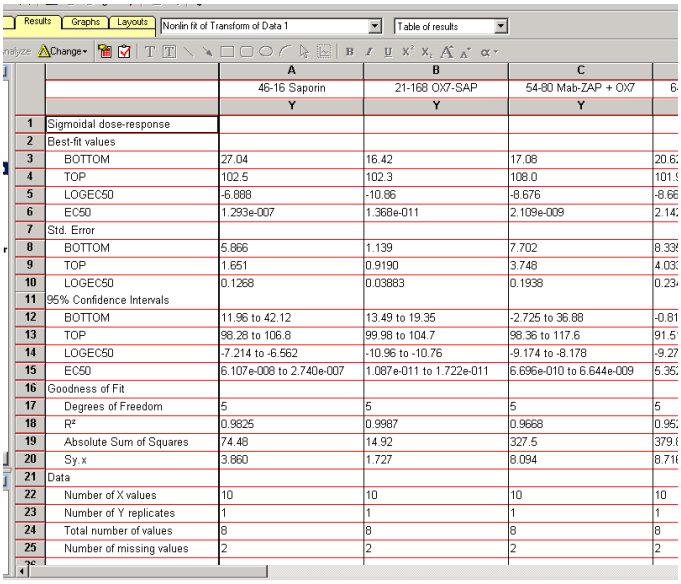
10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

PREPARING AND INTEPRETING CYTOTOXICITY DATA

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.

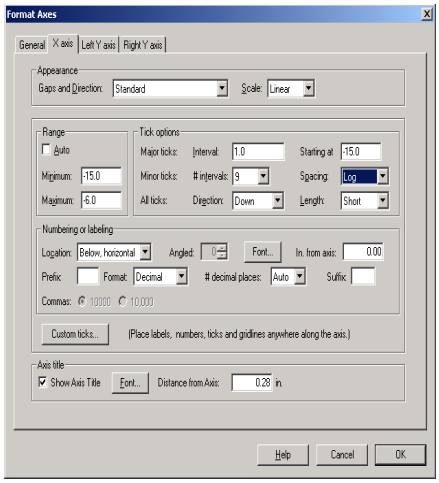
Click on the "Graphs" folder and this appears.



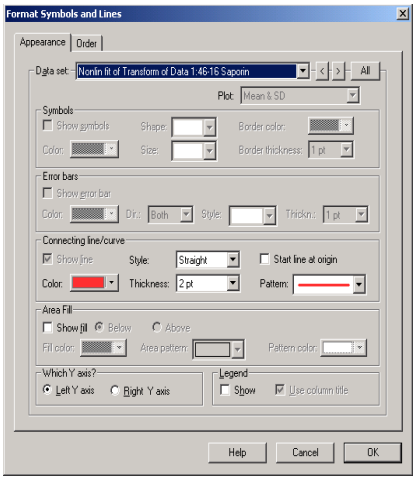


		A	B	C
		46-16 Saporin	21-168 OX7-SAP	54-80 Mab-ZAP + OX7
		Y	Y	Y
1	Sigmoidal dose-response			
2	Best-fit values			
3	BOTTOM	27.04	16.42	17.08
4	TOP	102.5	102.3	108.0
5	LOGEC50	-6.888	-10.86	-8.676
6	EC50	1.293e-007	1.368e-011	2.109e-009
7	Std. Error			
8	BOTTOM	5.866	1.139	7.702
9	TOP	1.651	0.9190	3.748
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
21	Data			
22	Number of X values	10	10	10
23	Number of Y replicates	1	1	1
24	Total number of values	8	8	8
25	Number of missing values	2	2	2

Click on any of the graph axes and this window pops up. You can adjust the parameters here for all the axes.



Click on any point/symbol from one of the lines on the graph and this window pops up. Here you can adjust what color or symbol you want the lines and points to be.



Here is the final graph. You can add the text with the EC50/ED50* values from the results page to the graph and color code them.

*EC50 is the concentration at which 50% of maximal effect is observed.

ED50 is the Median Effective Dose (produces desired effect in 50% percent of population).