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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.

											<u> </u>				
	Plate#1														
	1	2	3	4	5	6	7	8	9	10	11	12	1		
4	0.000												Endpoint		
вĮ		0.344	0.126	0.257	0.315	0.377	0.426	0.375	0.380	0.369	0.363		Lm1 450		
c		0.362	0.131	0.250	0.320	0.341	0.348	0.359	0.360	0.353	0.343		Automix: Once		
D		0.314	0.116	0.230	0.299	0.331	0.339	0.336	0.332	0.343	0.339		Calibrate: On		
E		0.357	0.115	0.243	0.311	0.367	0.381	0.382	0.349	0.371	0.367		Column Priority		
F		0.308	0.123	0.252	0.319	0.347	0.380	0.354	0.341	0.345	0.350		Plate Last Read:		
G		0.329	0.123	0.255	0.320	0.345	0.355	0.361	0.336	0.362	0.381		10:38 AM 3/17/2008		
н															

#### This is a standard readout using SoftMax Pro.

⊽Ш4	6-16 S	Saporin	-	╝╘┛╞╜	ו			
			_			,		
Sample	Wells	Values	R	Result	stddev	%control	CV%	Concentration
Sa01	B3	0.126	Εı	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	84	0.257	Εı	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	Εı	0.314	0.008	90.656	2.4	10000.000
	C5	0.320						
	D5	0.299						
	E5	0.311						
	F5	0.319						
High	ligh	t the	"	% Con	trol" a	nd "C	V%'	Copy.

# Preparing and Interpreting Cytotoxicity Data

## 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.

				_						%	Contro	bl
										1	CV	%
	Resu	ilts 🕇 Graphs 🕇 Lay	Data 1			<b>•</b>					1	
na	lyze 🥖	🔥 Change 🗸 👘 🕅		× 🗆 🗆 (	0	≥ B <i>I</i>	<u>u</u> x <sup>2</sup> x <u>y</u>	1 Α΄ α.	1	P /	/	
1		X Labels	А			8				C /		
Т		Concentration	Concentration 46-16 Saporin			21-168 OX7-8AP			64-80 Mab-7AP + OX7			
		X	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		17.285	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		
- 11											••••••	

- 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.

X Column Numbers (XY Graph) Numbers +/- Error bar Iext (bar graph) None (column graph) Series, Start at: 0.00 Interval: 1.00	Y Columns For each data set (condition) enter: A single column of values 3 - replicates to calculate error bars Mean, Standard Deviation, N
Defaults Make these choices defaults for future Automatic column widths	ure tables. Help Cancel OK

# Preparing and Interpreting Cytotoxicity Data

### 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.

Analyze Data	Parameters: Transforms				
Analysis               Eultrin analysis.	Function List Standard functions  Interchange X and Y (then transform as specified below).  Image: Interchange X and Y (then transform X values using X=Log(X)  K=  Transform X values using Y=Y/K  Different K for each data set  K=  When it is impossible to transform a SD or SEM  When it is impossible to transform a SD or SEM				
Data to analyze       Image: Concelent of the sets       Image: Concelent of the sets	© _ponvert to an asymmetric 95% confidence interval.   Replicates  © _Iransform individual Y values  © _Iransform the average of replicates  New graph  ✓ Create a new graph of the results  Help Me Decide				

- 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.





# Preparing and Interpreting Cytotoxicity Data

### 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.

_	Resu	Its Graphs Layouts Nonlin fit of T	ransform of Data 1	Table of results	3	
na	alyze 🛓	🔥 Change- 闇 🗹 T 🎞 🔨 🗴		$I \ \underline{U} \ X^2 \ X_2 \ \underline{A}^* \ \underline{A}^* \ \alpha^*$		
1			A	B	С	
٦			46-16 Saporin	21-168 OX7-SAP	54-80 Mab-ZAP + OX7	
			Y	Y	Y	
	1	Sigmoidal dose-response				
	2	Best-fit values				
4	3	BOTTOM	27.04	16.42	17.08	20.
4	4	TOP	102.5	102.3	108.0	101
	5	LOGEC50	-6.888	-10.86	-8.676	-8.6
	6	EC50	1.293e-007	1.368e-011	2.109e-009	2.1
	7	Std. Error				
r	8	BOTTOM	5.866	1.139	7.702	8.3
	9	TOP	1.651	0.9190	3.748	4.0
	10	LOGEC50	0.1268	0.03883	0.1938	0.2
	11	95% Confidence Intervals				
	12	BOTTOM	11.96 to 42.12	13.49 to 19.35	-2.725 to 36.88	-0.8
	13	TOP	98.28 to 106.8	99.98 to 104.7	98.36 to 117.6	91.
	14	LOGEC50	-7.214 to -6.562	-10.96 to -10.76	-9.174 to -8.178	-9.2
	15	EC50	6.107e-008 to 2.740e-007	1.087e-011 to 1.722e-011	6.696e-010 to 6.644e-009	5.3
	16	Goodness of Fit				
	17	Degrees of Freedom	5	5	5	5
	18	R <sup>2</sup>	0.9825	0.9987	0.9668	0.9
	19	Absolute Sum of Squares	74.48	14.92	327.5	379
1	20	Sy.x	3.860	1.727	8.094	8.7
	_					

- 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.

General Xaxis Left Yaxis Right Yaxis

Gaps and Direction: Standard

Appearance-

Range

E Auto

Axis title

Minimum: -15.0

Numbering or labeling

Commas: @ 10000 C 10.00

Maximum: 6.0



Help Cancel OK

Help Cancel OK