

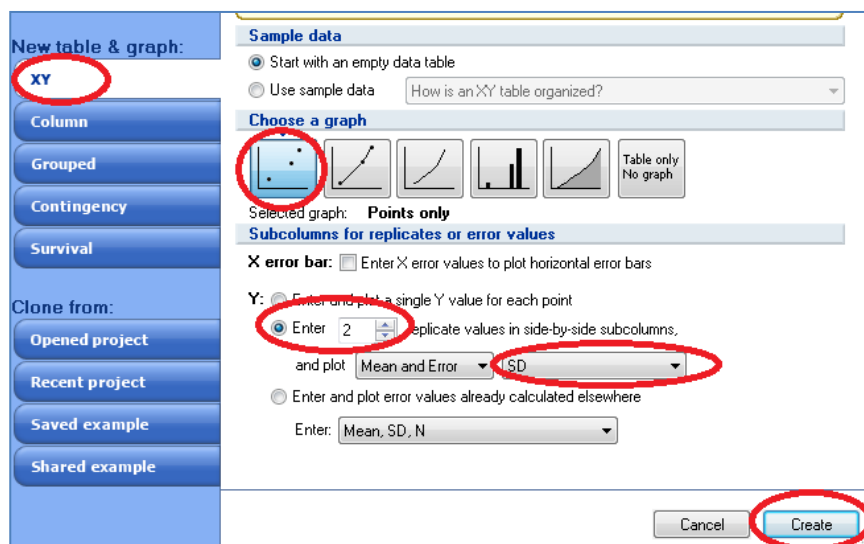
AlphaLISA immunoassay data processing using GraphPad Prism® version 5.

Introduction

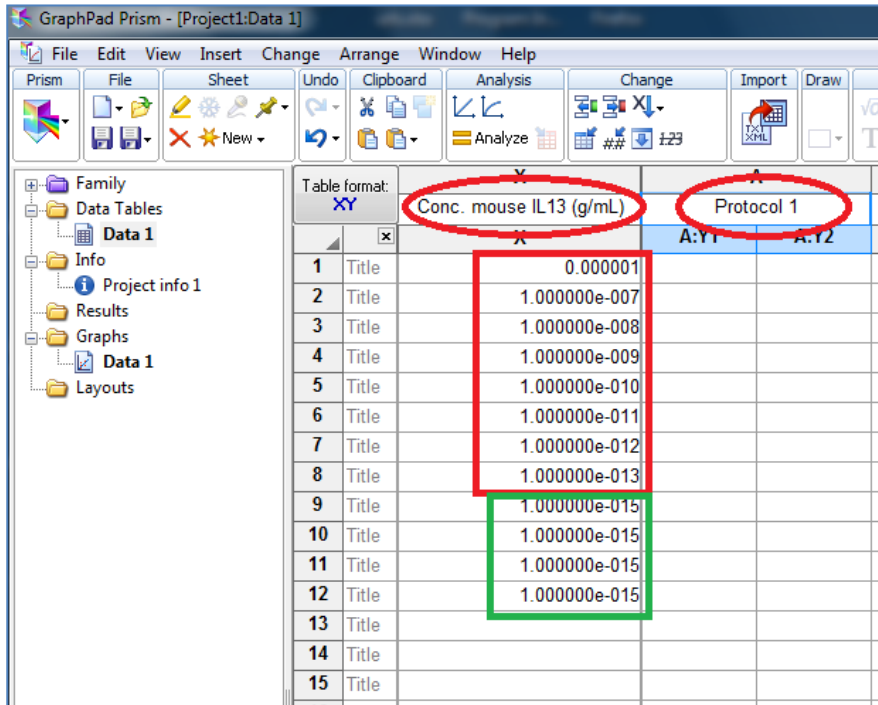
AlphaLISA® immunoassay data can be fit to either a linear curve (using just the linear portion of your data) or a dose-response curve. A dose response curve (or sigmoidal or 4PL curve) is typically used to process AlphaLISA immunoassay data in order to take advantage of the full dynamic range of the assay. These types of curves can be fit using standard statistical software, such as GraphPad Prism®. Step-by-step instructions for processing your data using GraphPad Prism® version 5 are presented below.

Step-by-step instructions

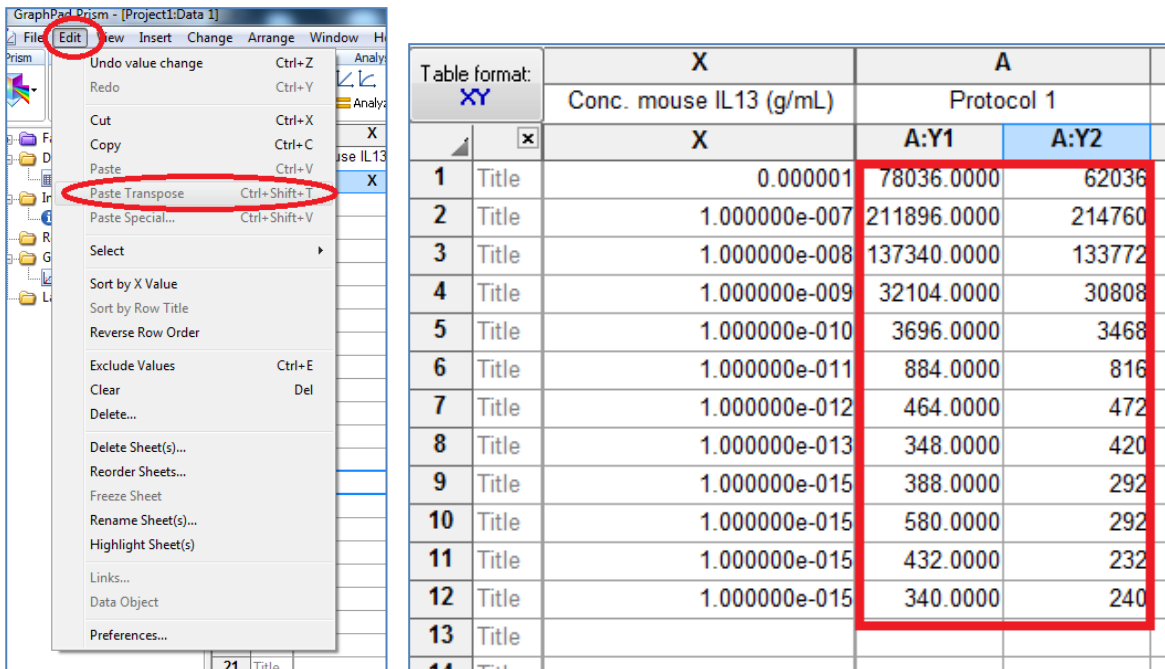
1. Open GraphPad Prism® version 5
2. Select XY graph – Points only, and enter the number of replicates (samples run in duplicate, triplicate, etc.)



- Enter the relevant information in the fields indicated below. The concentration of analyte is written in scientific notation. For your zero values of analyte, write a concentration that is two log below your lowest concentration of analyte tested – you will not be able to do your first transform if you enter zeroes instead.



- Paste your data from Microsoft Excel. You can use **Edit > Paste Transpose** to have GraphPad Prism® transpose your data (switching your data from being listed horizontally to vertically – rows vs. columns).



- If you have data points you need to exclude (for example, data points past the Hook Point, or any outliers), select the relevant data points, right click with your mouse, then choose "Exclude Values". The excluded values will now appear in blue italics. These data will not be used for the analysis.

Table format: XY		X	A		B	
		Conc. mouse IL13 (g/mL)	Protocol 1		Title	
	X		A:Y1	A:Y2	B:Y1	B:Y2
1	Title	0.000001	78036.0000*	62036*		
2	Title	1.000000e-007	211896.0000			
3	Title	1.000000e-008	137340.0000			
4	Title	1.000000e-009	32104.0000			
5	Title	1.000000e-010	3696.0000			
6	Title	1.000000e-011	884.0000			
7	Title	1.000000e-012	464.0000			
8	Title	1.000000e-013	348.0000			
9	Title	1.000000e-015	388.0000			
10	Title	1.000000e-015	580.0000			
11	Title	1.000000e-015	432.0000			
12	Title	1.000000e-015	340.0000			
13	Title					

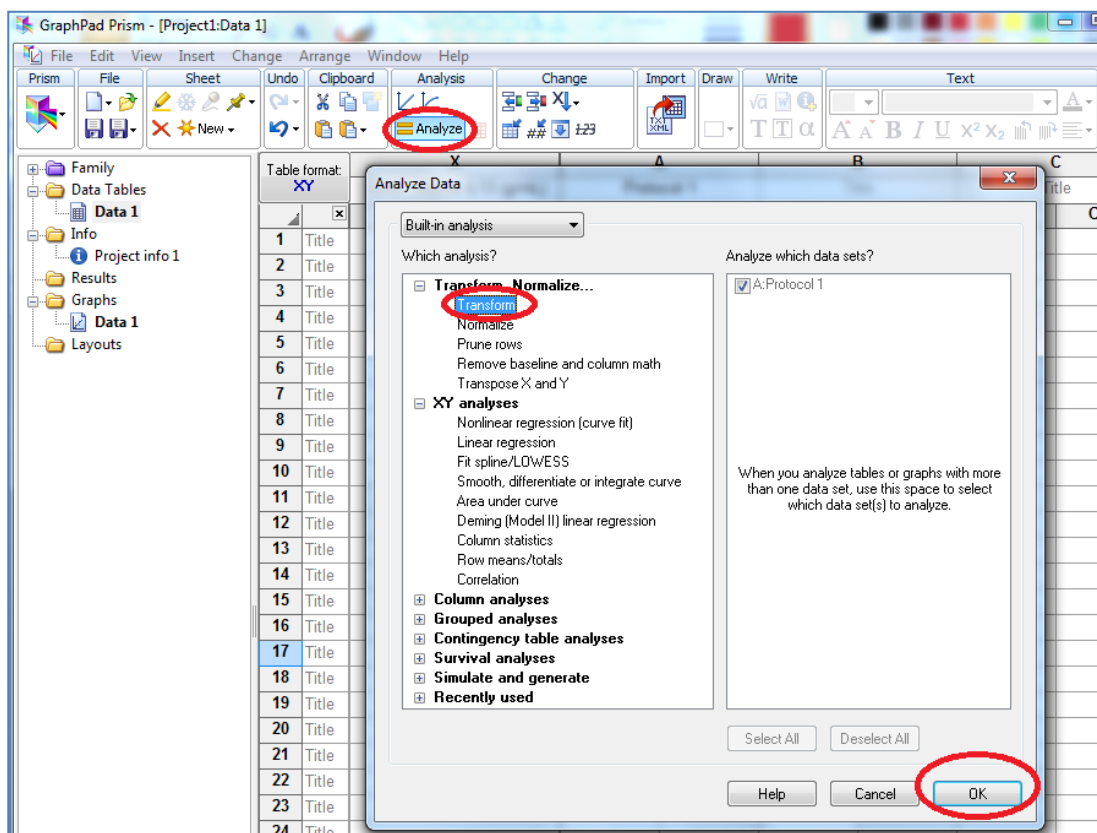
- Next, calculate the value corresponding to the signal of your blanks (zero analyte) + 3 SD. This can be done using Microsoft Excel® or a scientific calculator. Enter these in values directly underneath the relevant data set. This will be used to determine your sensitivity (LDL).

X	A	
Conc. mouse IL13 (g/mL)	Protocol 1	
X	A:Y1	A:Y2
0.000001	78036.0000*	62036*
1.000000e-007	211896.0000	214760
1.000000e-008	137340.0000	133772
1.000000e-009	32104.0000	30808
1.000000e-010	3696.0000	3468
1.000000e-011	884.0000	816
1.000000e-012	464.0000	472
1.000000e-013	348.0000	420
1.000000e-015	388.0000	292
1.000000e-015	580.0000	292
1.000000e-015	432.0000	232
1.000000e-015	340.0000	240
	696.9935	

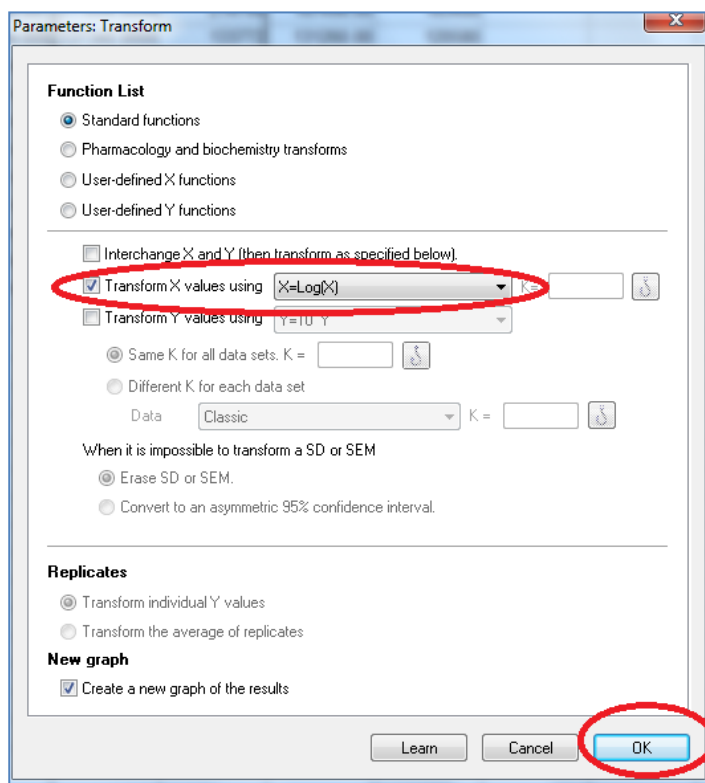
7. Enter the signal for all of your unknowns (in singlicate, duplicate, triplicate, etc.) underneath.

X	A	
Conc. mouse IL13 (g/mL)	Protocol 1	
X	A:Y1	A:Y2
0.000001	78036.0000*	62036*
1.000000e-007	211896.0000	214760
1.000000e-008	137340.0000	133772
1.000000e-009	32104.0000	30808
1.000000e-010	3696.0000	3468
1.000000e-011	884.0000	816
1.000000e-012	464.0000	472
1.000000e-013	348.0000	420
1.000000e-015	388.0000	292
1.000000e-015	580.0000	292
1.000000e-015	432.0000	232
1.000000e-015	340.0000	240
	696.9935	
	6565.0000	6407
	18001.0000	18772

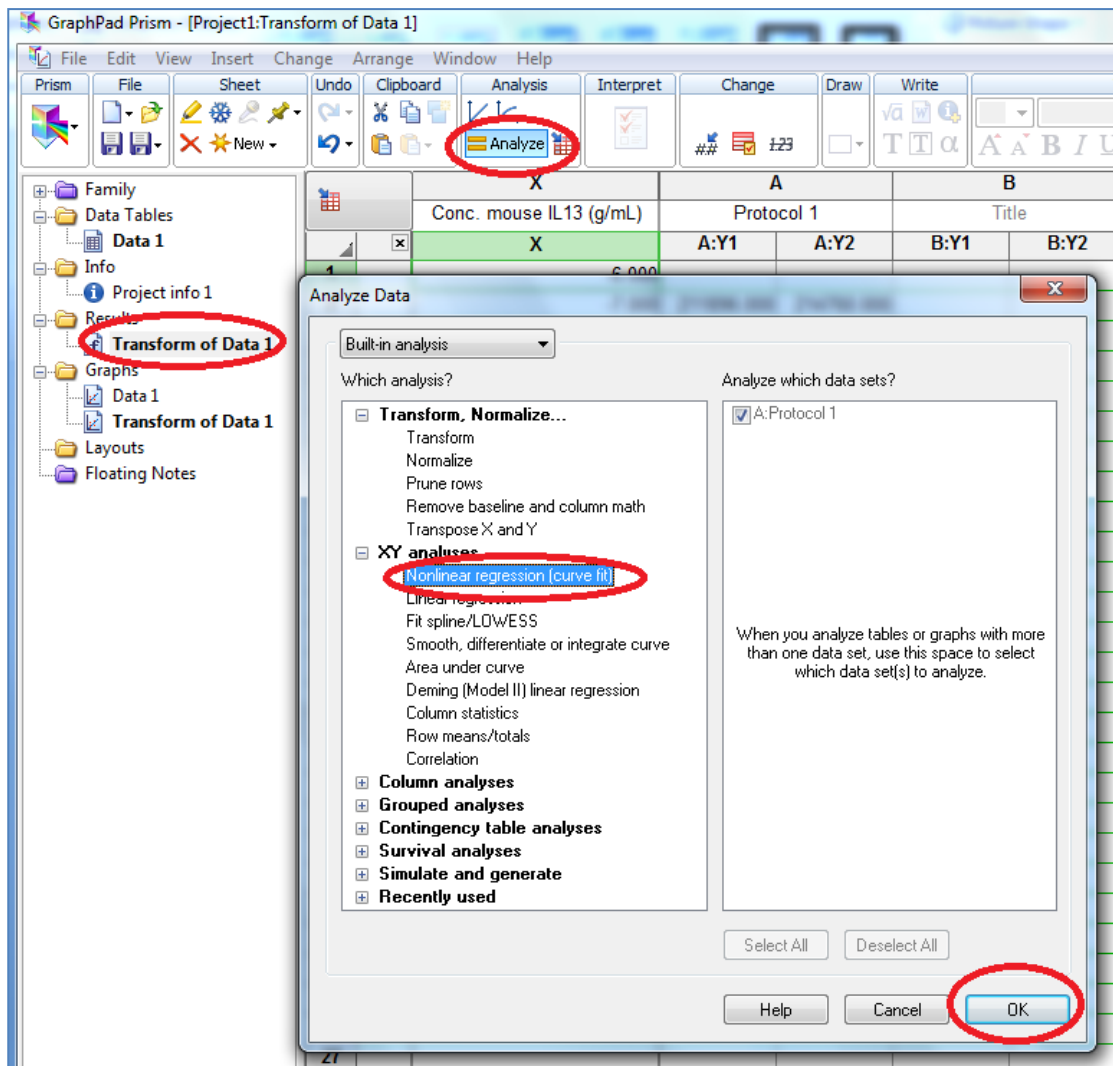
8. In the Analysis box, select Analyze, then click Transform.



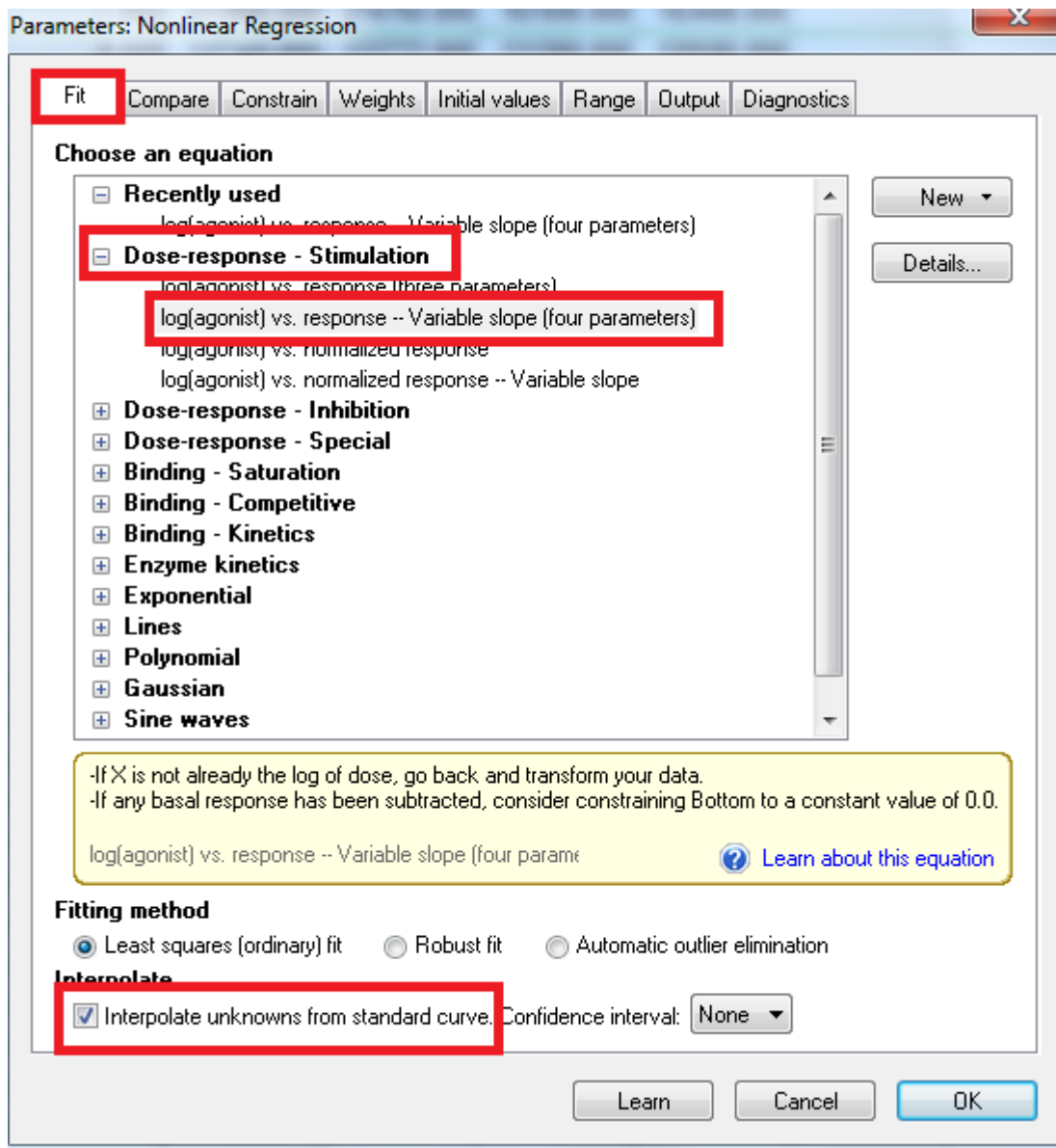
9. Transform X values using $X = \text{Log}(X)$.



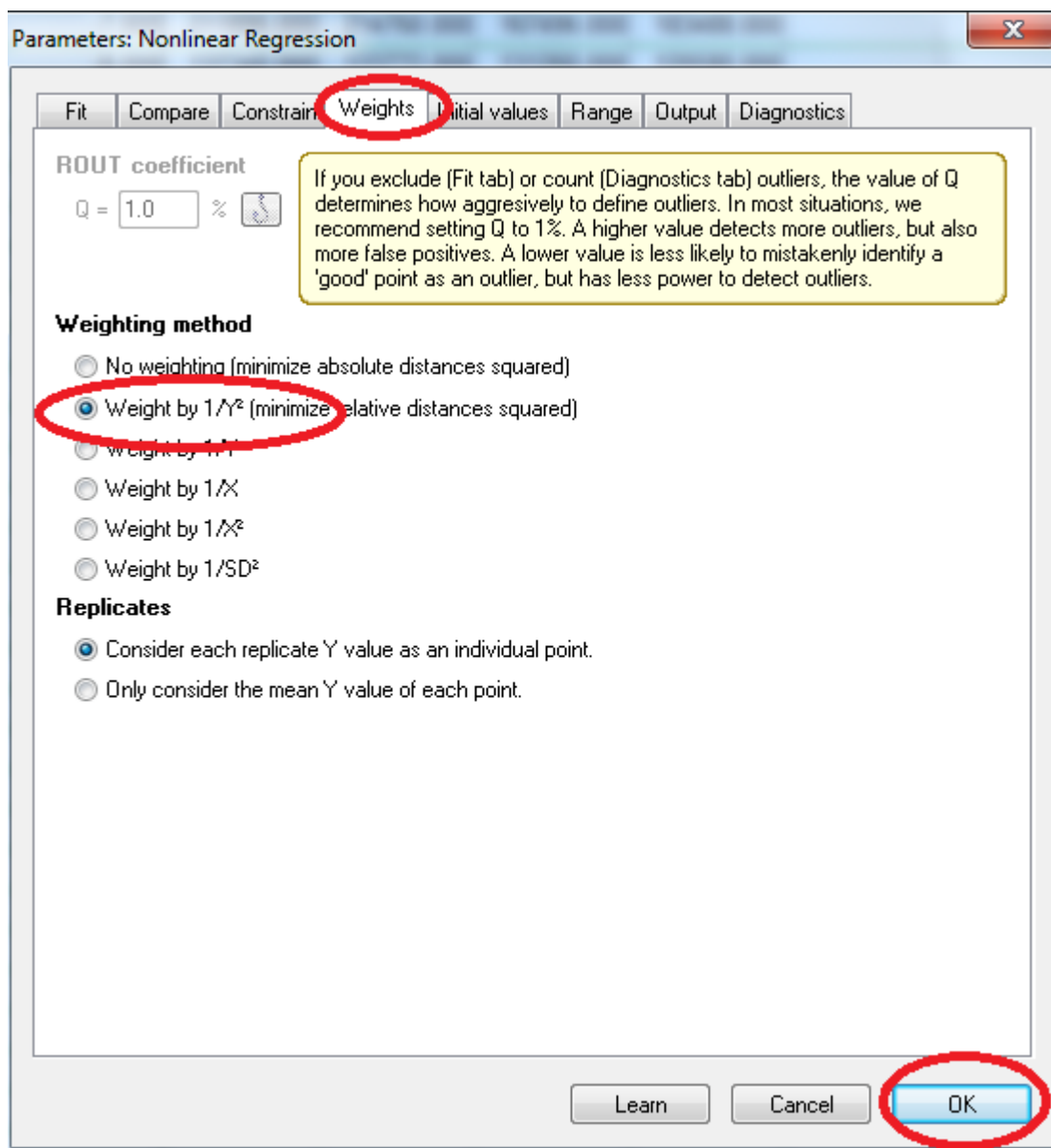
10. Make sure you have your Transformed data selected under Results in the left panel. In the Analysis window again, select Analyze > Nonlinear regression (Curve fit).



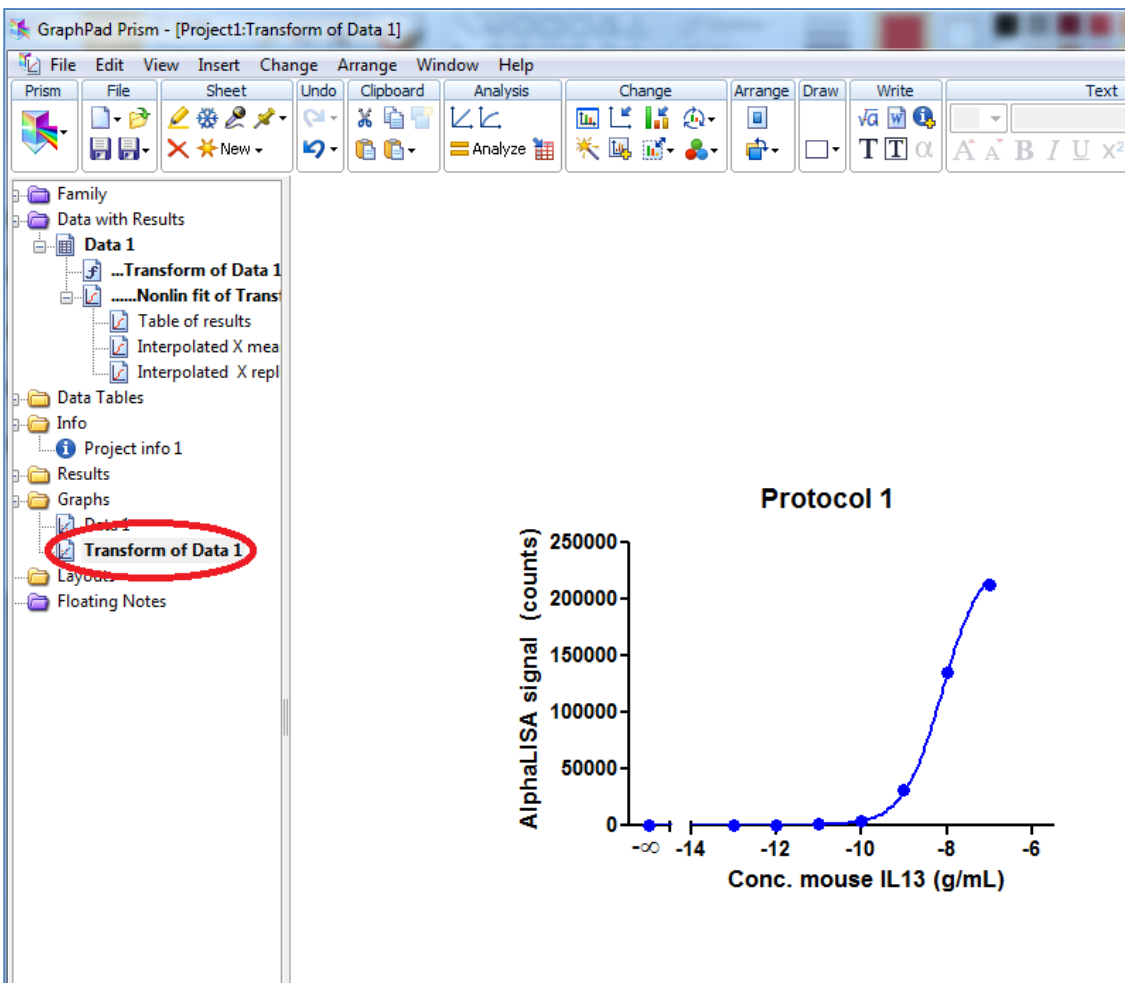
11. In the Parameters window, underneath the Fit tab, select Dose-response – Stimulation > Variable slope (four parameters). Make sure you also checkmark the box for “interpolate unknowns from standard curve”. KEEP THIS WINDOW OPEN.



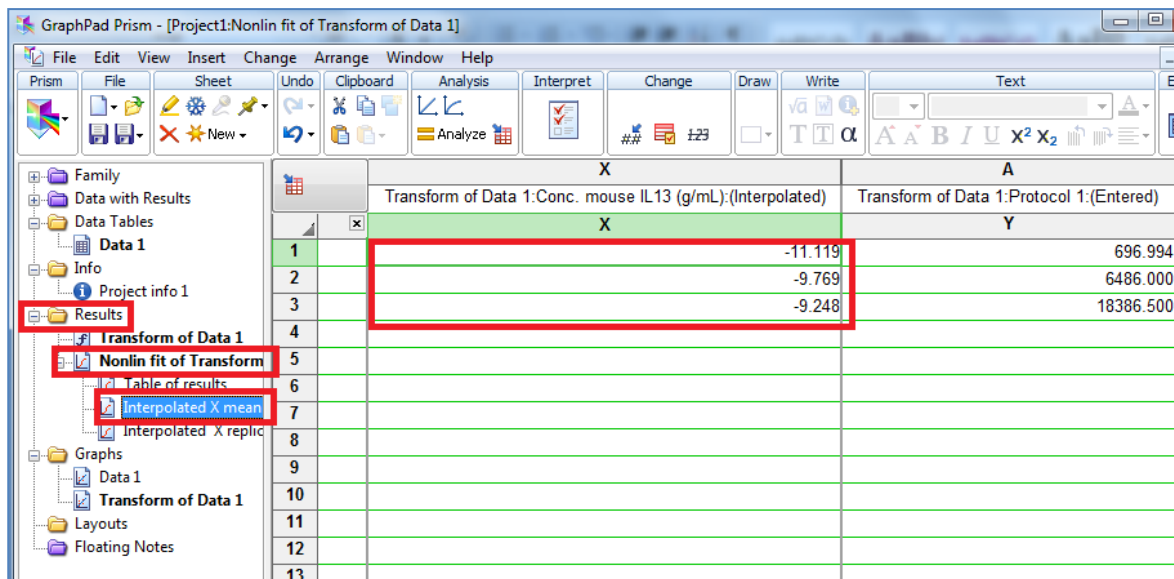
12. Go to the Weights tab, and select $1/Y^2$. Select OK.



13. In the left panel, underneath Graphs, select your Transformed data. Make your graph look pretty by double-clicking on anything you want to change (you may need to play with this a bit).



- To interpolate your unknowns (or derive your LDL), select Results > Nonlin fit of Transform > Interpolated X mean values.



- Your unknowns (or LDL) are given in the order in which they were entered (value for LDL/sensitivity, followed by your unknowns). They are still in Log X values. You can transform these values back to “g/mL” using $X = 10^X$.

