



## Standard Curves Analysis

### Introduction

A standard curve is used to calibrate an instrument or assay. The Standard Curves macro in SigmaPlot provides five equations that may be fit to your data.

These range from a straight line equation to two different five parameter logistic equations.

The X data may or may not be logarithmic and, if not, may still be graphed logarithmically. Multiple Y replicate columns may be used.

Predicted values may be obtained after the curve fit is performed: Y values from Xs, X values from Ys, and ECxx values from xx percentages.

These predicted values may be added to your graph as symbols with drop-lines to the X and Y axes.

The Dynamic Curve Fitting algorithm may be used to help solve difficult curve fitting problems involving local minima.

These are typically encountered with the five parameter logistic functions.

## Using the macro

The macro panel below shows the various standard curve options.

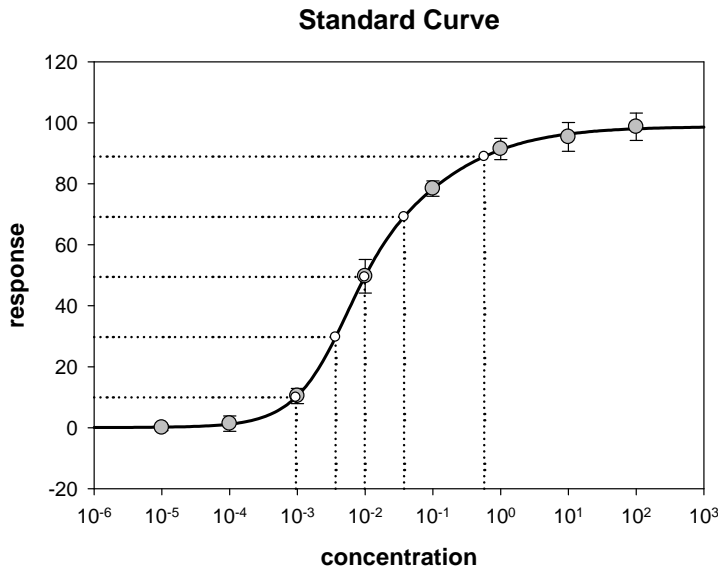
The screenshot shows the 'Standard Curve' dialog box with the following settings and annotations:

- Equation:** Four Parameter Logistic. *Annotation:* Use log X axis whether X data has been log transformed or not.
- Equation description:**  $y = \min + (\max - \min) / [1 + (x / EC50)^{-Hillslope}]$ . The four parameter logistic function is the classic dose-response "S" shaped curve, and is most often plotted with a log-x axis.
- Dynamic curve fit:** . *Annotation:* Perform 200 fits to find the best fit.
- Curve fit tolerance:** 1.0e-10. *Annotation:* Select curve fit tolerance and maximum number of iterations to assist achieving convergence.
- Maximum fit iterations:** 1000.
- Data:**
  - X data column:** log X. *Annotation:* Use if X data has already been log transformed.
  - Last Y replicate column:** Y3. *Annotation:* Select for replicate data and specify the last replicate column above.
  - Log format X data:** . *Annotation:* Select to predict values from unknowns and then select the type of prediction to the right.
  - Y replicates:** . *Annotation:* Y replicate columns must begin immediately to the right of the X column.
- Predict values:**
  - Predict unknowns:** . *Annotation:* Select variable to predict and possibly include in the graph.
  - Solve for EC% from column:** Percents.
  - Predict:**
    - Ys from Xs
    - Xs from Ys
    - EC% from %s
    - Plot predicted values
- Results placed in column 13**

## To use this macro

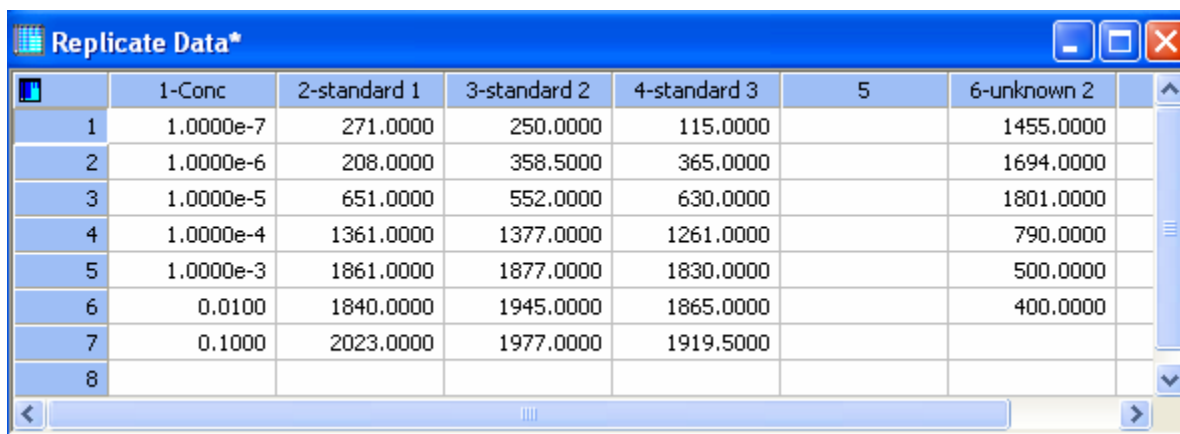
1. Put either X and Y columns or X and multiple replicate Y columns into your worksheet. These columns must be adjacent. See the example in the Y replicates section. If you plan to compute predicted Xs or Ys from the computed curve, you will also need to enter a column of the source values.
2. Select the equation from the Equation list to use to fit the curve. Your options are (see the equation descriptions in the Equations section):
  1. straight line,
  2. quadratic equation,
  3. Four parameter logistic equation
  4. five parameter logistic equation
  5. five parameter logistic equation – 2 slopes

3. Use the Log X axis scale checkbox to select whether or not to plot the X axis using a common log scale. This option will be automatically selected if you have X data in log format and you select the Log format X data checkbox.
4. If you find that your data is difficult to fit then select the Dynamic curve fit checkbox. In this case 200 curve fits will be performed using initial starting values that span the parameter ranges. The best fit will be selected from the 200 results.
5. If the curve fit does not converge then there is some bad relationship between the equation selected and the data being analyzed. But the goal of a standard curve is to obtain a smooth curve representation to the data so it may be important to achieve convergence. The best way to do this is to increase the curve fit tolerance from the standard  $1e-10$  to  $1e-3$ , say, or some value where convergence occurs. The maximum number of fit iterations may also be increased.
6. Select the columns to use for the X and Y data from the X data column and Y data column drop list boxes. If your X data is already in a log format, make sure you check the Log format X data option.
7. If you have replicate Y measurements for each X data point then select the Y replicates option and then select the Last Y replicate column.
8. Select the Predict unknowns option to compute results using the solution to the fit. You can compute a column of new Y values from given Xs, or Xs from given Y values. If you are using the four or five parameter logistic equations you can compute EC percent values for a specified range of percent values. You can also elect to plot the results of these on your standard curve by selecting the Plot predicted values checkbox.
9. When finished, click OK. A standard curve is created, and if you elected to compute additional values, they are also plotted using drop lines to indicate the X and Y values.



## Y replicates

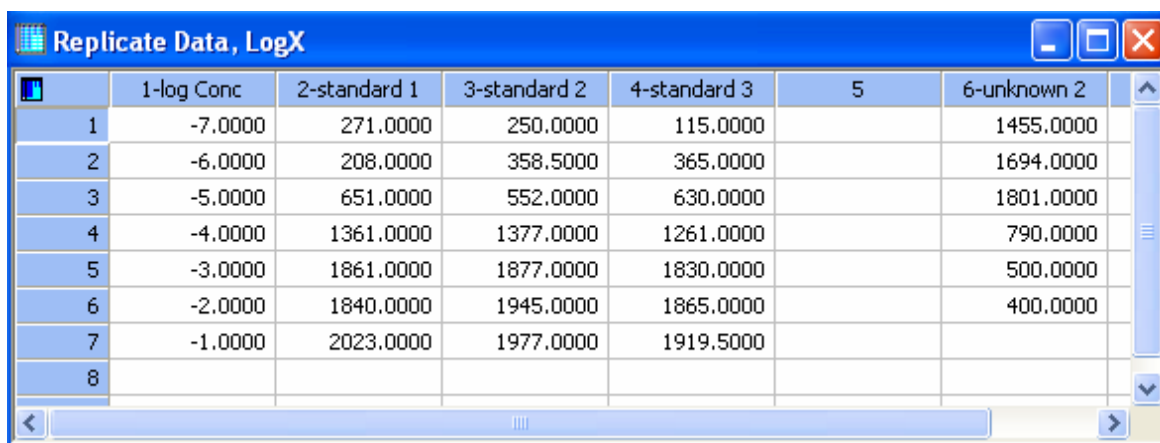
If you have multiple measurements for each X value, select the Y replicates option and then select the last replicate column. The Y replicate columns must be to the right of and adjacent to the X data column.



	1-Conc	2-standard 1	3-standard 2	4-standard 3	5	6-unknown 2
1	1.0000e-7	271.0000	250.0000	115.0000		1455.0000
2	1.0000e-6	208.0000	358.5000	365.0000		1694.0000
3	1.0000e-5	651.0000	552.0000	630.0000		1801.0000
4	1.0000e-4	1361.0000	1377.0000	1261.0000		790.0000
5	1.0000e-3	1861.0000	1877.0000	1830.0000		500.0000
6	0.0100	1840.0000	1945.0000	1865.0000		400.0000
7	0.1000	2023.0000	1977.0000	1919.5000		
8						

## Log data format

If your X data uses simple integers, especially negative numbers, it is already in log format and you should select the Log format X data option. The macro will automatically create a new column of equivalent numeric data, and automatically plot X on a log axis scale.



	1-log Conc	2-standard 1	3-standard 2	4-standard 3	5	6-unknown 2
1	-7.0000	271.0000	250.0000	115.0000		1455.0000
2	-6.0000	208.0000	358.5000	365.0000		1694.0000
3	-5.0000	651.0000	552.0000	630.0000		1801.0000
4	-4.0000	1361.0000	1377.0000	1261.0000		790.0000
5	-3.0000	1861.0000	1877.0000	1830.0000		500.0000
6	-2.0000	1840.0000	1945.0000	1865.0000		400.0000
7	-1.0000	2023.0000	1977.0000	1919.5000		
8						

## Equations

### Linear Equation

$$y = y_0 + ax$$

A straight line, characterized by the slope  $a$  and the  $y$ -intercept  $y_0$ .

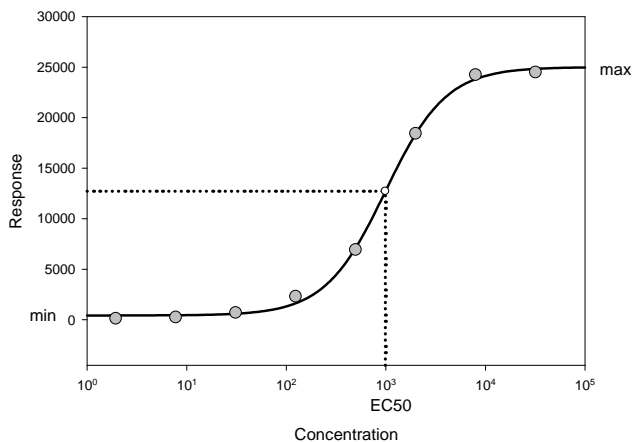
### Quadratic Equation

$$y = y_0 + ax + bx^2$$

The standard parabolic equation with quadratic coefficient  $b$ , slope  $a$  and intercept  $y_0$ .

### Four Parameter Logistic Equation

$$y = \min + \frac{\max - \min}{1 + \left(\frac{x}{EC50}\right)^{-Hillslope}}$$



This is a typical dose-response curve with a variable slope parameter. It is sometimes abbreviated as 4PL. The four parameters are:

Min - bottom of the curve

Max - top of the curve

EC50 – the x value for the curve point that is midway between the max and min parameters. It is called the half-maximal effective concentration. Equivalent definitions are ED50 (half-maximal effective dose) and for inhibition curves IC50 (half-maximal inhibitory concentration).

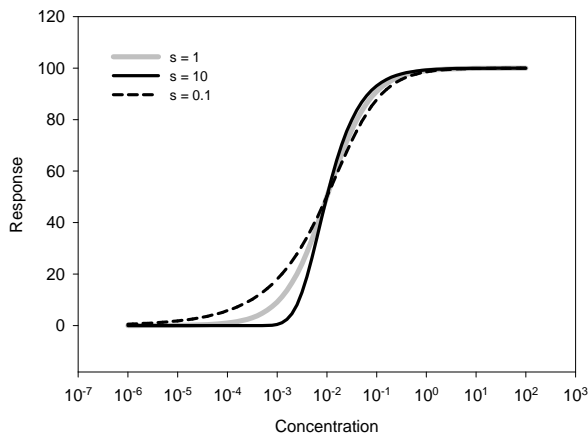
Hill slope – characterizes the slope of the curve at its midpoint. Large values result in a steep curve whereas small values a shallow curve. The 4PL curve will increase with x if the Hill slope is positive and decrease if it is negative.

### Five Parameter Logistic Curve

$$y = \min + \frac{(\max - \min)}{1 + \left(\frac{x}{x_b}\right)^{\text{Hill slope}}}$$

where

$$x_b = EC_{50} \left( \frac{-1}{\text{Hill slope}} \right) \log_2 \left( \frac{1}{s} \right)$$



This is the Richard’s formulation of the five parameter logistic. It adds an asymmetry parameter ‘s’ to the four parameter logistic. The asymmetry is shown above with large changes in curvature with changes in s in the lower curve but relatively small changes in the upper curve.

The additional algebraic equation for  $x_b$  maintains  $EC_{50}$  as the half-maximum y value. The equation has been written so that a positive Hill slope results in a curve that increases with x.

Four of the five parameters are the same as those in the four parameter logistic.

s – controls the asymmetry. If s = 1 then this function is the same as the four parameter logistic. s less than 1 decreases the overall slope of the curve whereas s greater than 1 increases the overall slope.

### Five Parameter Logistic – Two Slopes

The Ricketts and Head equation with two “slope” parameters (the parameters actually better describe the two different curvatures) is shown below. It has a different shape than Richard’s equation and will fit some data sets better.

$$y = \min + \frac{\max - \min}{1 + f_x \frac{EC_{50}}{EC_{50}} \frac{\ddot{O}}{\ddot{O}}^{-Slope_1} + (1 - f_x) \frac{EC_{50}}{EC_{50}} \frac{\ddot{O}}{\ddot{O}}^{-Slope_2}}$$

The equation can be written in terms of SlopeCon to force Slope1 and Slope2 to be the same sign. If this is not the case then in rare situations, error in the data will result in a fit with slopes of opposite sign. In this case, the function attempts to follow the error which results in an irregularly shaped curve.

$$y = \min + \frac{\max - \min}{1 + f_x \frac{EC_{50}}{EC_{50}} \frac{\ddot{O}}{\ddot{O}}^{-Slope} + (1 - f_x) \frac{EC_{50}}{EC_{50}} \frac{\ddot{O}}{\ddot{O}}^{-Slope SlopeCon}}$$

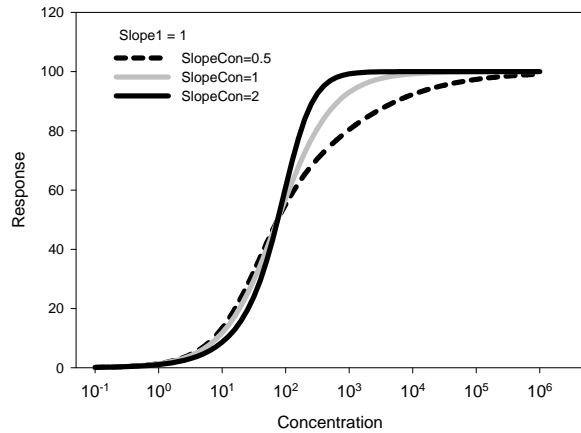
Where

$$Slope = Slope_1$$

$$Slope_2 = Slope SlopeCon, \quad SlopeCon > 0$$

$$f_x = \frac{1}{1 + \frac{EC_{50}}{EC_{50}} \frac{\ddot{O}}{\ddot{O}}^{AvSlope}}$$

$$AvSlope = \frac{2|Slope| SlopeCon}{(1 + SlopeCon)}$$



The graph shows the increasing asymmetry with increasing SlopeCon (and therefore increasing Slope2). If SlopeCon=1 then Slope1 = Slope2 and the curve is symmetric and identical to the four parameter logistic curve.

## References

1. Richards, F.J. A flexible growth function for empirical use. *J. Exp. Botany* 10. pp290-300.
2. Ricketts, J.H. and G. Head. A five-parameter logistic equation for investigating asymmetry of curvature in baroreflex studies. *Am. J. Physiol.* 277 (Regulatory Integrative Comp. Physiol. 46). R441-R454. 1999.