



Version 4.0

Step-by-Step Examples

Nonlinear Standard Curves: RIA and ELISA

Analyzing radioimmunoassay (RIA) or an enzyme-linked immunosorbent assay (ELISA) data is a two-step process:

- Prepare and assay a set of "known" samples, i.e., samples that contain the substance of interest in amounts that you choose and which span the range of concentrations that you expect to be present in your "unknown" samples. The quantity that you measure (radioactivity, optical density, or luminescence, for example) is related graphically to sample concentration to produce a standard curve.
- Assay the "unknown" samples. Then, for each measurement obtained, use the standard curve to find the corresponding substance concentration. Graphically, this amounts to finding the X-axis value (concentration) for a given Y-axis value (measured quantity).

Prism automates creation of a standard curve and interpolating unknown concentrations from it. For an example of a linear standard curve, see the Step-by-Step Example "Linear Standard Curves".

This article includes the following techniques:

- Fitting data to a three- or four-parameter sigmoidal (logistic) model
- Log transforming data
- Finding "unknown" values using a standard curve and displaying them on the graph
- Showing X values on a graph in the "Power of 10" format
- Placing logarithmically spaced tick marks on a graph axis

Entering Standard-Curve and "Unknown" Data

In the Welcome dialog, choose **Create a new project** and **Type of graph**. Under the **XY** tab, choose the leftmost thumbnail, for "Points only".

RIA/ELISA data often span such large concentration ranges that concentrations are plotted as their logarithms. We'll follow that convention here and let Prism compute the logarithms for us.

¹ Adapted from: Miller, J.R., *GraphPad Prism Version 4.0 Step-by-Step Examples*, GraphPad Software Inc., San Diego CA, 2003. *Step-by-Step Examples* is one of four manuals included with Prism 4. All are available for download as PDF files at www.graphpad.com. While the directions and figures match the Windows version of Prism 4, all examples can be applied to Apple Macintosh systems with little adaptation. We encourage you to print this article and read it at your computer, trying each step as you go. Before you start, use Prism's **View** menu to make sure that the Navigator and all optional toolbars are displayed on your computer.

Enter data for the standard curve. For our example, enter "known" sample concentrations into the X column, rows 1-6, and the corresponding assay results into the Y column. Sometimes you'll want to include data from a zero-concentration sample. Since our data will be graphed on a semi-log plot and it's impossible to plot the log of 0, we approximate the zero-concentration point in row 1 with an X coordinate of 1.0e-014, two log units below the lowest non-zero X value. Including this data point will help to define the top of the fitted curve more accurately.

Just below the standard curve values, enter the assay results (Y values, rows 7-10) for the "unknown" samples, leaving the corresponding X cells blank. Later, Prism will fit the standard curve and then report the unknown concentrations using that curve.

	X Title	Data Set-A
	X	Y
1	1e-014	100.0
2	1e-012	97.2
3	1e-011	93.1
4	5e-011	67.9
5	1e-010	30.7
6	5e-010	4.8
7		35.0
8		53.0
9		78.0
10		90.0

Log Transforming the X Values

Click **Analyze**. From the **Data manipulations** category, select **Transforms**.

In the **Parameters: Transforms** dialog, choose to **Transform X values using X=Log(X)**. At the bottom of this same dialog box, choose to **Create a new graph of the results**. When you click **OK** to exit this dialog, Prism displays a new Results sheet with the log-transformed X values.

	X	Y
1	-14.000	100.000
2	-12.000	97.200
3	-11.000	93.100
4	-10.301	67.900
5	-10.000	30.700
6	-9.301	4.800
7		35.000
8		53.000
9		78.000
10		90.000

Click on the yellow **Graphs** tab to view the plot of the log-transformed data.

Analysis and Curve Fitting

Click the **Analyze** button. From the **Curves & regression** category, select **Nonlinear regression (curve fit)**.

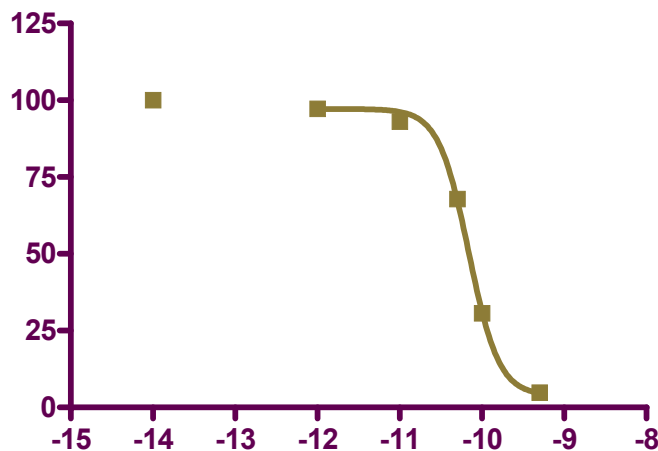
In the **Parameters: Nonlinear Regression (Curve Fit)** dialog box (**Equation** tab), choose **Classic equations**. Select **Sigmoidal dose-response (variable slope)**.

The sigmoidal model should work well for a one-site competitive binding system following mass-action kinetics. You have other options: choose another equation, or enter a user-defined equation (see the step-by-step example “Fitting Data to User-Defined Equations”). Or consider a cubic spline or LOWESS, which provides a smoothed curve without fitting the data to a specific equation.

Since we want our unknown concentrations to be provided, check the box to **Also calculate...Unknowns from standard curve**. Select the **Range** tab, and under **Minimum X value**, choose to **Start the curve at X = -12** (we are truncating the curve at the lowest non-zero X coordinate).

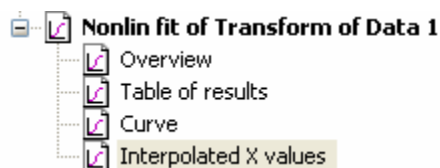
We will format the graph so as not to show the 100-percent-binding control point at X = -14. Many users would prefer to include that point and to substitute a value of “o” at X = -14, with or without breaking (segmenting) the X axis. For an example of this technique, see the Step-by-Step Example “Analyzing Dose-Response Data”.

When you click **OK** to leave the parameters dialog, Prism performs the fit and creates a Results sheet.



Finding “Unknown” Values

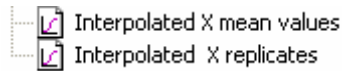
Prism displays the results of the curve fit on the **Table of results** subpage of the **Results-x:Nonlinear regression (curve fit)** sheet. But for a routine RIA, you may not care too much about the information contained there. Switch to the **Interpolated X values** subpage.



Prism reports the corresponding X value for each unpaired Y value on your Data sheet. These values are expressed as logarithms of concentration.

X	Y
-10.036	35.000
-10.182	53.000
-10.410	78.000
-10.617	90.000

If your data table contains replicate Y values, Prism will report interpolated X values both on the basis of the mean of replicates and of each individual replicate.



Although it's generally easiest to plot and analyze semi-logarithmic RIA/ELISA data, we'd like to read our unknown concentrations directly, not as logarithms. Click **Analyze**. From the **Data Manipulations** category, select **Transforms**.

In the **Parameters: Transforms** dialog, check the box for **Transform X values using....** Select the equation **X=10^X** from the drop-down list.

Click on **OK**. Prism creates another Results sheet, on which the X values are now expressed as concentration. In this figure, column headings were added and the number format was changed (**Change... Number Format...**).

Concentration	Binding
X	Y
9.209e-011	35
6.580e-011	53
3.892e-011	78
2.414e-011	90

Showing "Unknowns" on the Graph

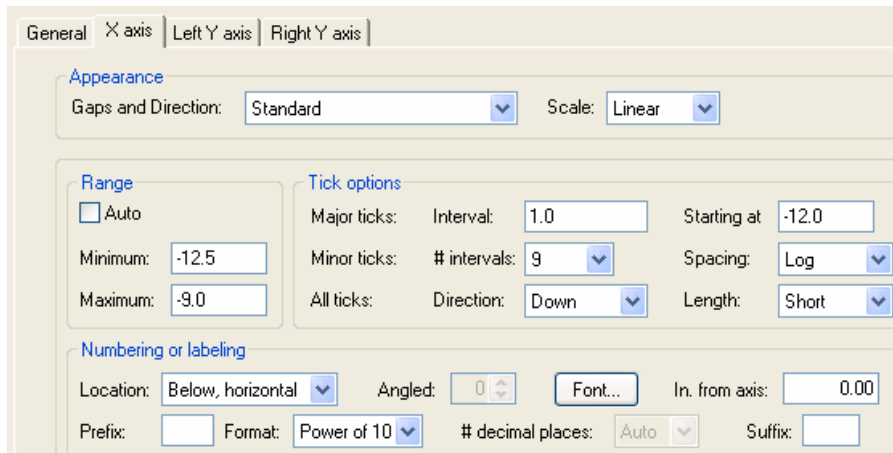
Prism's automatic graph includes the data from the Data sheet and the curve. To add the "unknowns" to the graph:

Switch to the graph **Transform of Data 1 graph**. Choose **Change... Add Data Sets....** From the top drop-down box in the **Add Data Sets to Graph** dialog, select the data set containing the **...Interpolated X values**. Click **OK** to return to the graph, which now includes the unknown values plotted using a different symbol.

If you want the "unknowns" represented as spikes projected to the X axis (rather than data points), click **Change** and select **Symbols & Lines....** From the **Data set** drop-down list, select **...Interpolated X values**. Change the symbol **Shape** to one of the last four options (spikes), and set the **Size** to **0** or **1**.

Final Graph Formatting

Double-click on the X axis, make sure the X axis tab is selected, and make the following settings. The axis scale remains linear, so that the horizontal positions of the data points are still determined by their logarithms. But as an aid to the viewer, the ticks labels are changed to the corresponding **Power of 10** values, and the tick marks themselves are spaced logarithmically.



The final graph, with some formatting changes not discussed here, is shown in the next figure. The table has been paste-embedded from the last Results sheet. To that, switch to the Results sheet and choose **Edit... Copy**. Then switch to the graph, choose **Edit... Paste Table**, and position the copy of the table where you want it.

