

Linear Standard Curves

This article includes the following techniques:

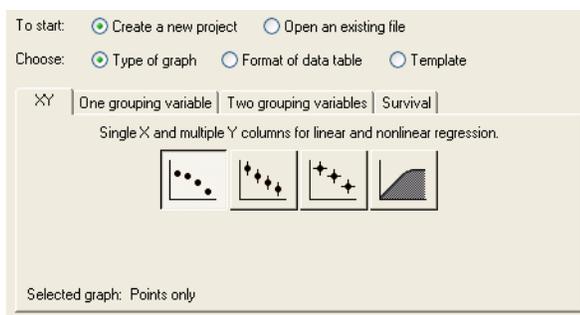
- Linear regression
- Finding “unknown” values using a standard curve and displaying them on the graph

A standard curve is a graph relating a measured quantity (radioactivity, fluorescence, or optical density, for example) to concentration of the substance of interest in "known" samples. You prepare and assay "known" samples containing the substance in amounts chosen to span the range of concentrations that you expect to find in the "unknown" samples. You then draw the standard curve by plotting assayed quantity (on the Y axis) vs. concentration (on the X axis). Such a curve can be used to determine concentrations of the substance in "unknown" samples. Prism automates this process.

Prism can fit standard curves using nonlinear regression (curve fitting), linear regression, or a cubic spline (or LOWESS) curve. This article shows how to find "unknown" concentrations using a standard curve obtained using linear regression.

Entering Linear Standard Curve and “Unknown” Data

In the Welcome dialog, make the settings shown below.



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.

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Enter data for the standard curve. In our example, shown below, we entered concentrations for the "known" samples into the X column, rows 1-5, and the corresponding assay results into the Y column. Just below the standard curve values, starting in row 6, enter the assay results for the "unknown" samples into the Y column, leaving the corresponding X cells blank.

	Protein (mg/mL)	Optical Density
	X	Y
1	0.05	0.097
2	0.10	0.183
3	0.20	0.290
4	0.30	0.371
5	0.40	0.462
6		0.141
7		0.190
8		0.258
9		0.423

Click on the default table name (drop-down list on the toolbar). Type a new name for the table. That name will be used as the title for your graph.

Linear Regression with "Unknown" Interpolation

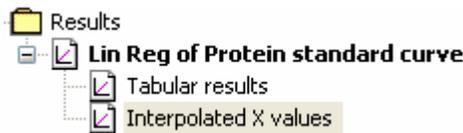
Click **Analyze**. From the **Curves & regression** category, select **Linear regression**.

In the **Parameters: Linear Regression** dialog, check the box labeled **Unknowns from standard curve**, because we want our unknown concentrations to be provided. Prism's default is to draw a linear regression line from the first to the last X coordinate on the data table. In our case, the line would therefore start at X = 0.05. To extend the line leftward to the Y axis, choose to **Start regression line at X= 0.0** in the **Output options** frame. Set the number of **Significant Digits** (to appear on the Results sheet) to **3**.

When you click **OK** to leave the linear regression parameters dialog, Prism performs the fit and displays the best-fit values.

Best-fit values	
Slope	1.01 ± 0.0686
Y-intercept	0.0690 ± 0.0169
X-intercept	-0.0685
1/slope	0.992
95% Confidence Intervals	
Slope	0.789 to 1.23
Goodness of Fit	
r ²	0.986
Sy.x	0.0196
Is slope significantly non-zero?	
F	216
DFn, DFd	1.00, 3.00
P value	0.0007
Deviation from zero?	
	Significant

Switch to the **Interpolated X values** subpage of the Results sheet to view the “unknown” values computed on the basis of the linear regression.

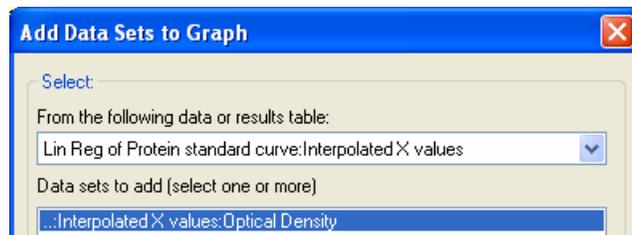


Prism reports the corresponding X value for each unpaired Y value on your Data sheet.

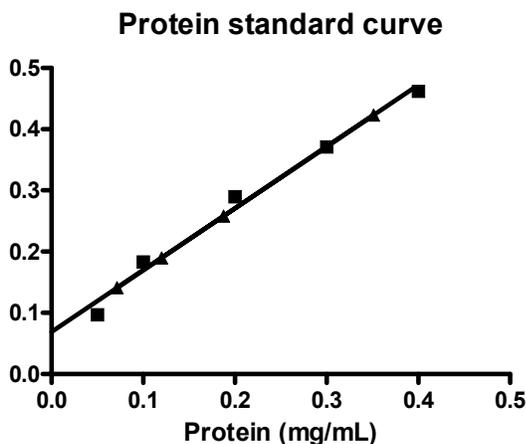
Protein (mg/mL)	Optical Density
X	Y
0.071	0.141
0.120	0.190
0.188	0.258
0.351	0.423

Adding “Unknown” Data Points to the Graph

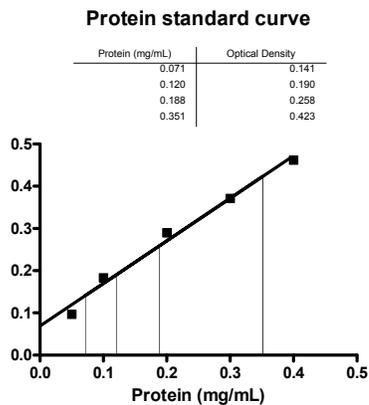
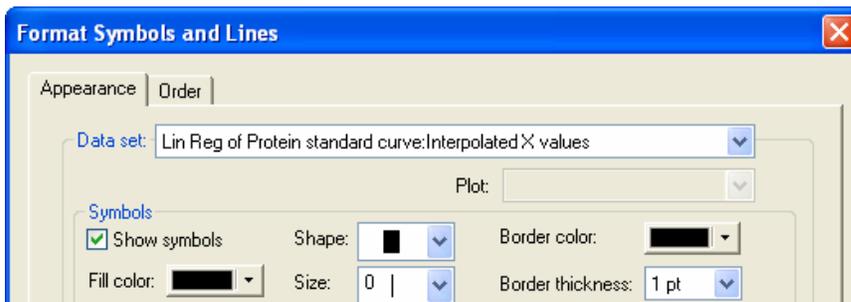
Prism's automatic graph includes the data from the Data sheet and the curve. To add the "unknowns" to the graph, click the yellow **Graphs** tab on the toolbar. Click the **Change** button and then select **Add Data Sets...**



Using this dialog, you can add any appropriate data to any graph. Verify the settings shown above and click **OK** to return to the graph. The graph now includes the “unknown” values plotted in distinctive symbols (here, triangles).



If you want the "unknowns" represented as spikes projected to the X axis (rather than data points), click on the **Change** button and select **Symbols & Lines....** Verify that the **Appearance** tab is selected, and from the **Data set** drop-down list, select **Lin Reg of Protein standard curve: Interpolated X values**. Change the symbol **Shape** to one of the last four options (spikes) and set the **Size** to **0**.



Standard Curve

“Unknowns” are represented as spikes on the graph; their numerical values are shown on an embedded table pasted from the **Interpolated X values** subpage of the Results sheet.