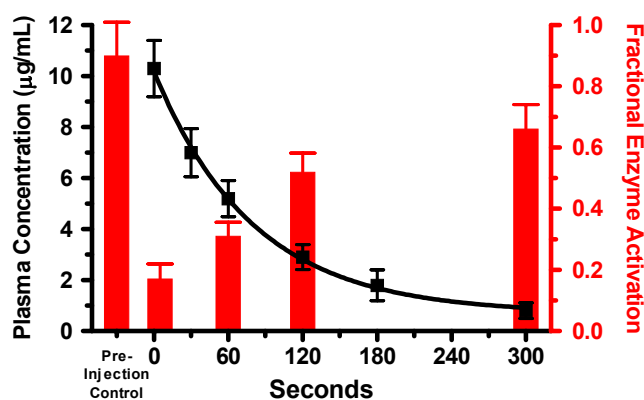GraphPad
PRISM®

Version 4.0

*Step-by-Step Examples*

# Combining a Bar Graph with a Line Graph[1]

Occasionally, you may wish to use both bars and lines on the same plot.



In this example, we'll use point symbols and a fitted exponential decay curve to show the time course for plasma concentration of an injected drug. On the same graph, we'll graph periodic chemical measurements (activity of a tissue enzyme), shown using vertical bars.

This article includes the following special techniques:

- Bar graphs created from XY graphs by converting point symbols
- Fitting an exponential decay curve
- Adding a second(right-hand) Y axis

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[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.

# Creating an XY Graph with Fitted Curve

In the Prism Welcome dialog, select **Create a new project** and indicate that you will create the data table and its linked graph by choosing the **Type of graph**.

To get "bars" on this graph, we will replace the point symbols with spikes—lines projected from each X,Y coordinate to the X axis—widened to look like bars. So our graph is entirely an XY plot. Choose the **XY** tab. Prism displays the four possible graph types.
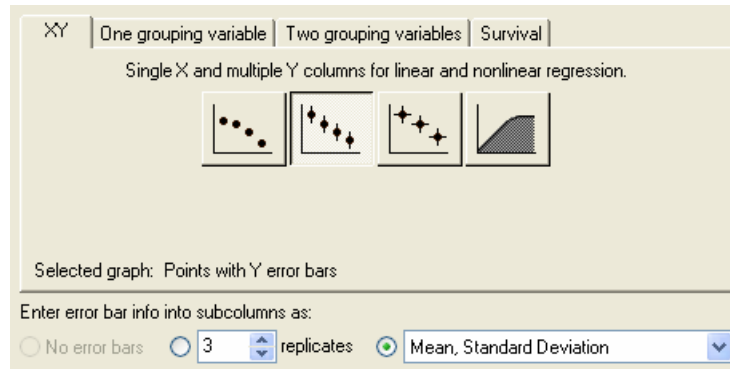


Choose the second graph-type thumbnail, because we want to graph points combined with Y, but not X, error bars. Then indicate that you will enter data into subcolumns as **Mean, Standard Deviation**. Click **OK** to exit the Welcome dialog. Prism displays an appropriately formatted data table.

Enter the data below, including column headings. The X values are the times after drug injection, the first Y column contains plasma drug levels, and the second Y column contains the enzyme activity measurement. Measurements in this example don't always coincide—the first enzyme activity measurement is made 30 seconds before drug treatment, for example—creating some blank cells. Prism has no problem with that.

| | X Values | A | | B | |
|---|---|---|---|---|---|
| | Seconds | Plasma drug level | | Enzyme activity | |
| | X | Mean | SD | Mean | SD |
| 1 | -30 | | | 0.90 | 0.110 |
| 2 | 0 | 10.3 | 1.10 | | |
| 3 | 5 | | | 0.17 | 0.050 |
| 4 | 30 | 7.0 | 0.94 | | |
| 5 | 60 | 5.2 | 0.71 | 0.31 | 0.046 |
| 6 | 120 | 2.9 | 0.49 | 0.52 | 0.062 |
| 7 | 180 | 1.8 | 0.60 | | |
| 8 | 300 | 0.8 | 0.31 | 0.66 | 0.080 |

To view the default graph, click the **Graphs** tab on the toolbar.
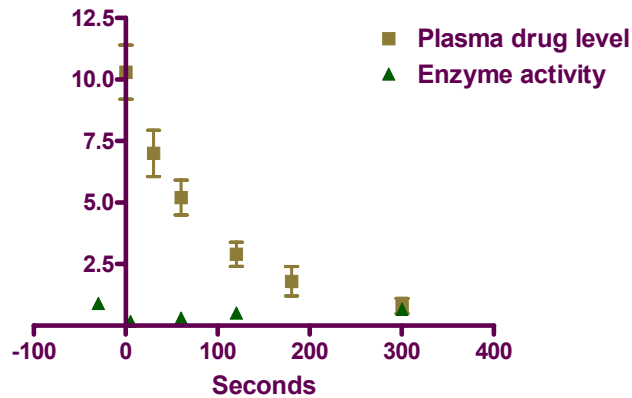
Move the Y axis so that it intersects the X axis at its leftmost point: Double click on the X axis, select the **General** tab, and set **Origin** to **Lower Left**.

Click the **Analyze** button to open the **Analyze Data** dialog box. Under **Data to analyze**, choose **Selected data sets**, then click the **Select** button to open the **Select Data Sets** dialog box. Select **Data Table-1:Plasma drug level** and deselect **Data Table-2:Enzyme activity**. Click **OK** to return to the **Analyze Data** dialog. Finally, choose **Nonlinear regression (curve fit)** from the list of **Curves & regression** analyses.

Click **OK** to move to the **Parameters: Nonlinear Regression (Curve Fit)** dialog. With the **Equation** tab selected, choose **One phase exponential decay** from the **Classic equations** list. When you click **OK** to exit this box, Prism adds the fitted curve to your graph.

We won't use the legend in this example, so we can get rid of it. Select the two elements of the legend (click on one element, hold the **Shift** key, and click on the other). Press **Delete**.

## Adding a Second Y Axis

The activity data are shown as triangular point symbols. They are barely visible because the Y axis is scaled to accommodate the plasma concentration values, which are larger numbers. This is a good time to use dual Y axes, giving the enzyme activity data a separate, right-hand scale. Double-click on symbol for "Enzyme Activity" (triangle). In the **Format Symbols and Lines** dialog, select the **Right Y axis** radio button to assign this data to a right-hand axis, which Prism will add to the graph automatically.
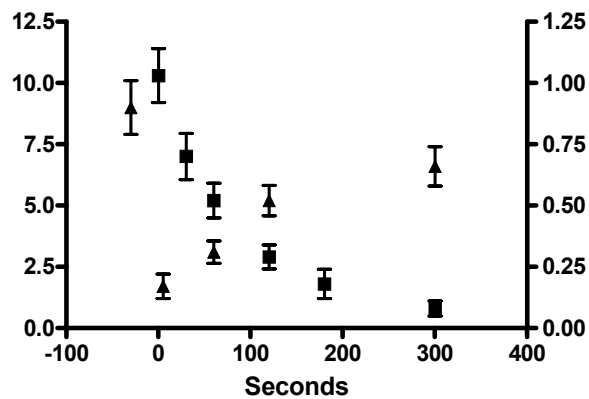
## Changing Point Symbols to "Bars"

Replacing the triangular point symbols with bars is merely a matter of choosing a different "symbol". Return, if necessary, to the **Format Symbols and Lines** dialog (**Change... Symbols & Lines**). Under the **Appearance** tab, make sure the data set chosen is **...Enzyme activity**, then choose the fourth shape from the bottom from the **Shape** drop-down list. Adjust the **Size** to **8** and assign this "bar" a color. Assign a **Color** and **Border**, if desired. Here are the selections:
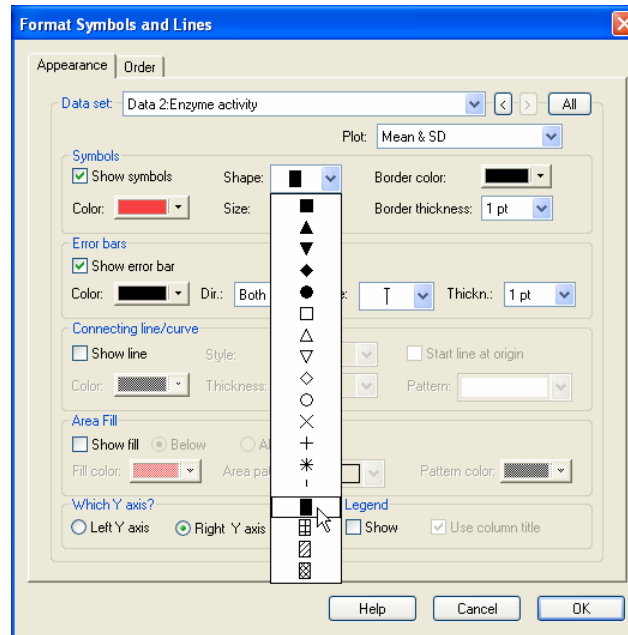


When you click **OK** to return to the graph, you may notice that the square symbol at X=120 is obscured by the newly created bar. Bring the symbol toward the "front" by choosing **Change... Symbols & Lines**. Select the **Order** tab, choose the data set **...Plasma drug level**, and click **Down**.

## Final Graph Formatting

Refer to the finished graph on page 1 as you go through the changes in this section:

Edit each axis title by selecting the default title and then typing a meaningful name. To insert Greek symbols, such as the *mu* in the left-hand Y axis title, click where you want the character, then use the α button on the toolbar.

The label "Pre-Injection Control" is simply a text object. Click the text tool, then click where you want to place the label and type. Click elsewhere to leave the text-editing mode. Select the label and then use the text editing buttons or **Format...Text** to change font size, center-justify, etc., and use your arrow keys to make fine adjustments in the position of the label.

If you are using colored bars, you may wish to color the right-hand Y axis to match. To do that, click once on that axis to select it, then choose **Change... Selected Object(s)...**. Assign a color under **Lines, Arrows & Arcs**. With the axis still selected, choose **Change... Selected Text...** to color the axis title.

To adjust tick spacing and labeling, double-click on the appropriate axis to open the **Format Axes** dialog. Verify the selection of the proper tab, then change the **Range**  and **Tick options** settings as show in the following chart:

|                        | X axis | Left Y axis | Right Y axis |
|------------------------|--------|-------------|--------------|
| Range settings:        |        |             |              |
|   Minimum    | -50    | 0           | 0            |
|   Maximum    | 320    | 12          | 1            |
| Tick option settings:  |        |             |              |
|   Interval   | 60     | 2           | 0.2          |
|   Starting at | 0     | 0           | 0            |
|   Minor intervals | 2 | 2           | 2            |

While you're in the **Format Axes** dialog, if you would like to move the tick labels further away from the axis, change the setting for **Numbering or labeling… In. from axis**.

To reposition an axis title, click on the title (avoiding tick labels) to display a two-headed-arrow cursor. Drag title away from, or closer to, the axis.