Prism 6 Step-by-Step Example

Analyzing dose-response data

In pharmacology experiments, a drug’s effect on a receptor is typically investigated by constructing a dose-response curve. A dose-response curve describes the relationship between increasing the dose (or concentration) of the drug and the change in response that results from this increase in concentration. A typical dose-response curve will span a large concentration range. For this reason, the dose in these curves is usually represented using a semi-logarithmic plot. On a semi-logarithmic plot, the amount of drug is plotted (on the X axis) as the log of drug concentration and response is plotted (on the Y axis) using a linear scale.

This step-by-step example is designed to guide beginning Prism users through constructing sigmoidal curves from dose–response data. The steps in this example are outlined here:

**Step 1: Enter data** – Create a data table for dose-response data. For more information on constructing XY data tables with Prism 6, please refer to the help section: [XY data tables](#)

**Step 2: Transform X values to log form** – Alter the number format of the X values. For more information on transforming data with Prism 6, please refer to the help section: [Transform data](#)

**Step 3: Normalize the Y values** – Express Y values from different data sets on a common scale. For more information on normalizing data with Prism 6, please refer to the help section: [Normalize](#)

**Step 4: Nonlinear regression** – Use an equation defined in Prism to determine specific parameter values for your data. For more information on analyzing data with nonlinear regression using Prism 6, please refer to the help section: [Nonlinear regression with Prism](#)

**Step 5: Compare the curves statistically** – Use Prism to determine whether two curves are statistically different. For more information on understanding the statistical analysis of nonlinear regression using Prism 6, please refer to the help section: [Compare tab](#)

**Step 6: Review the graph** – Check that the data and the analysis agree.

**Step 7: Review the results** – View and interpret the results of the analysis.

**Step 8: Review the analysis check list** – Access the Prism help to learn what assumptions the statistical analysis is based.

The graph that is produced by this example will look like this:

![Graph of dose vs. response](image-url)
Step 1: Enter data
In the Welcome to GraphPad Prism dialog box, begin work on a new XY table and graph with your own data by clicking the **Enter replicate values** (indicated by the red arrow). To work through this example, select **Dose-response - X is dose** indicated by the green arrow:

Click **Create** to begin working with the data table. On the data table, the X column defines the dose of agonist. In this example, dose is expressed as the decimal form of the molar concentration.

Prism automatically converts the numbers to a standard form of scientific notation when it is very small. For example, the X value in row 2 is 0.000001. Prism converts this number to 1.00e-008. To access the dialog box to change the number format, select the column that you would like to change and click the Change Decimal Format button in the toolbar.

The Y values (response, in arbitrary units) are arranged in groups (Group A, B, etc.); each group contains three subcolumns (named A:Y1-A:Y3, B:Y1-B:Y3, etc) and each replicate is entered in a subcolumn. To access the dialog box to change the number of subcolumns on a data table, click the Change Data Table button in the toolbar.
View the graph that is produced from the data by clicking **dose vs. response** under Graphs in the navigator bar. In the Change Graph Type dialog box that appears select **Points with error bars**, Plot **Mean and error** and **SEM** (highlighted by the red arrow below):

Click **OK**. The graph should look like this:
Step 2: Transform X values to log form
To convert the X values to their respective logarithms, begin by clicking Analyze. Choose Built-in analysis. From the Transform, Normalize... category, select Transform (as indicated by the red arrows in the image below). Click OK.

In the Parameters: Transforms dialog box, choose Transform X values using X=Log(X). At the bottom of this dialog box, there is an option to Create a new graph of the results. It should not be checked at this point.

Click OK. Prism displays a new Results sheet (illustrated below) with the X values transformed into logarithms.
Step 3: Normalize the Y values

The Y values in data sets A and B will be normalized to a common scale. This is useful when you want to compare the shape or position (EC50) of two or more curves and don't want the magnitude of the curve to be a factor in the comparison. With the log-transformed Results sheet in view, click the Analyze button (as indicated in Step 2). In the Analyze Data dialog, select the Transform, Normalize... category, then choose Normalize. Click OK. Note that Prism will be chaining two analyses – it will analyze (with the Normalize analysis) the results of another (Transform) analysis.

In the Parameters: Normalize dialog, specify how Prism will normalize the Y values. For this example, we will accept the default settings, which define 0% and 100% as the smallest and the highest values, respectively, for each of the data sets. Choose to present the results as Percentages. Since the results of this “analysis” are the values that we will graph, check the box to Create a new graph of the results. The selections are shown here:

The results of the normalization are displayed below. The Y values are replicates and 0% and 100% are defined by the mean of the replicates. It is neither possible nor desirable to normalize each subcolumn separately. For this reason, none of the upper Y values in the table are exactly 100.
Step 4: Nonlinear regression

With the normalized results sheet displayed, click on the Analyze button. From the XY analyses heading, select Nonlinear regression (curve fit) indicated by the red arrows (or click the Fit a curve with nonlinear regression button indicated by the green arrow):

In the Parameters: Nonlinear Regression dialog box, under the heading Dose Response - Stimulation, select log(agonist) vs. normalized response -- Variable slope (red arrow). Be sure to choose the equation with the word “normalized” in it, which forces the curve to go from 0 to 100.

Resist the urge to click OK! Read on...
Step 5: Compare the curves statistically
Instruct Prism to compare the fitted midpoints (logEC$_{50}$) of the two curves statistically. Click the Compare tab and adjust the settings to match the following figure:

![Comparison settings](image)

This comparison determines whether the difference between the two logEC$_{50}$ values is statistically significant.

Now you can click **OK**.

Step 6: Review the graph
Prism fits the curves and automatically places them on the graph. Before looking at the numerical results of the fit, inspect the graph to make sure the data and fit make sense.

![Graph of dose vs. response](image)

**Normalize of Transform of dose vs. response**

- No inhibitor
- Inhibitor

[Agonist], M
Step 7: Review the results

Go to the results sheet titled **Nonlin fit of ...** Prism summarizes the result of the F test (curve comparison) at the top of the results sheet (highlighted by the green box below). The P value is small, so the null hypothesis that both curves have the same EC$_{50}$ is rejected. No surprise. It is obvious by looking at the graph that the two curves are distinct and have substantially different EC$_{50}$s.

Prism then presents the best-fit parameter values from both models lower on the table. Only the results of the “preferred model” will be shown on the graph.

The dotted red arrows indicate that, by scrolling down, you will find the best-fit parameter values from fitting with the non-preferred model, where one curve is fit to both data sets.

Prism fits the logEC$_{50}$, but also shows the EC$_{50}$, which is its antilog (ten to that power). Don’t just look at the best-fit values, but also look at their 95% confidence intervals, which tell you how precisely you have determined the values.
Step 8: Review the analysis check list

All statistical analyses are based on assumptions. When you are viewing the results sheet, click the Analysis Check List button in the toolbar to review the assumptions of nonlinear regression.

Clicking on the Analysis Check List the analysis in this example will load the Prism help web page that discusses comparing nonlinear fits. Notice the list of help topics of the left side of the web page. The help section is not limited to information for navigating Prism; it is also provides a useful reference for questions on statistical analysis. The links to Statistics Guide, Curve Fitting Guide, and Prism Guide at the top of the page (highlighted by the red arrows) will take you to the different sections of the Prism help.