

Dose Response Analysis with LabScribe

Introduction

The Dose Response relationship describes the change in the effect on an organism or tissue caused by differing levels of exposure to a chemical stressor after a certain exposure time.

Dose Response parameters are calculated and then a dose-response curve is calculated in *LabScribe's Dose Response Advanced Analysis Module*.

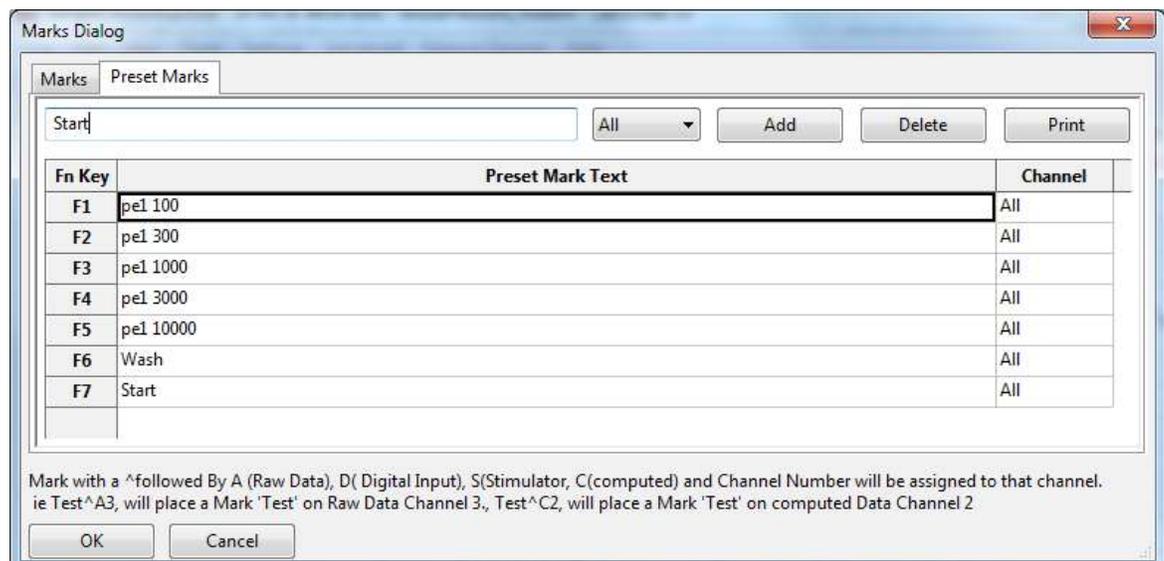
The **Dose Response Advanced Analysis Module** requires a separate license. The first time you select **Dose Response**, you will be asked for a username and a serial number. Contact iWorx Systems for more information.

This document includes a step by step tutorial for using most of the features of the **Dose Response Advanced Analysis Module**. To use the step by step guide, you will need a recording with at least one Dose Response channel. In order to use the online analysis part of the module, you will need to be recording these parameters as you proceed through the tutorial. This file can then be saved and used in the offline analysis tutorial.

Dose Response Measurement

To make it easier later to analyze the data it is beneficial to follow the following procedure when recording the data.

- 1) Make a table of the drug concentrations that you are going to be using. In our example we are going to apply 100, 300, 1000, 3000, and 10000 units of the drug pe1.
- 2) Setup preset marks to make it easier to mark the data during recording. The preset marks are present in the Marks window, which you can access from the Toolbar or the View Menu. It is best to start the mark with the drug name.



Marks dialog with preset marks entered.

3) While recording data, make sure that the marks are placed in the record when the drug is given.

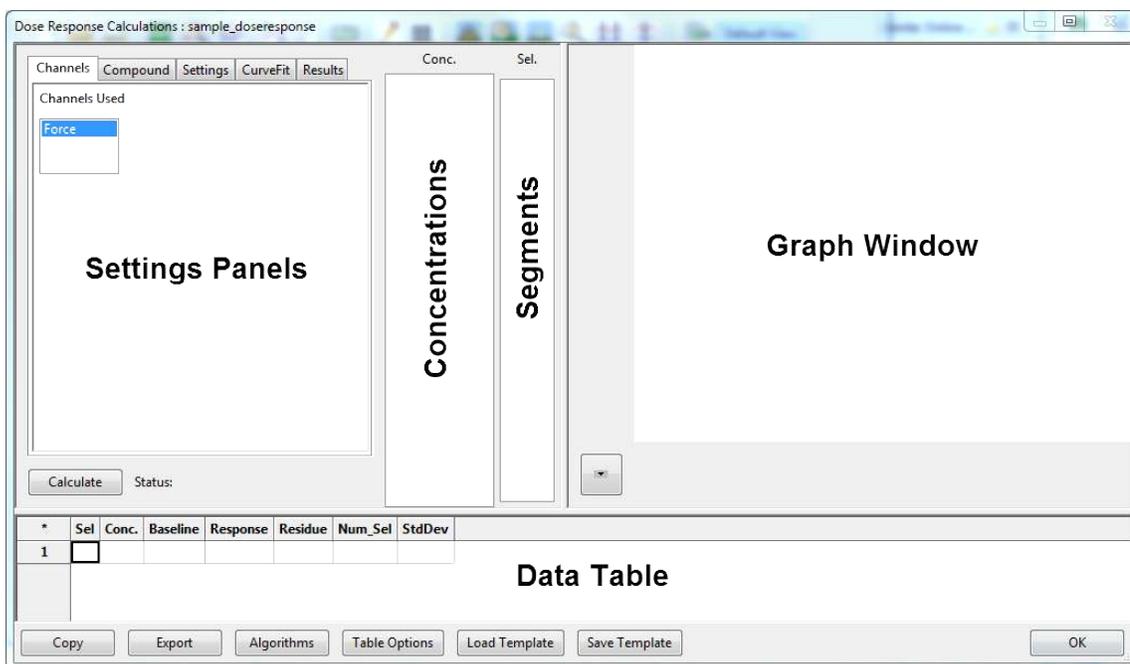
Dose Response Analysis: Step by Step

The **Dose Response Calculations** dialog allows sophisticated offline analysis of previously recorded Dose Response data.

The Offline Dose Response Calculations Dialog

To display the **Dose Response Calculations** dialog and familiarize yourself with its features:

- 1) Open the Dose Response data file.
- 2) Choose **Dose Response** from the **Advanced** menu to open the offline **Dose Response Calculations** dialog. The panels of this dialog can be resized by moving the mouse cursor over the boundaries until a double-headed arrow appears, and dragging the boundaries to resize the panels.

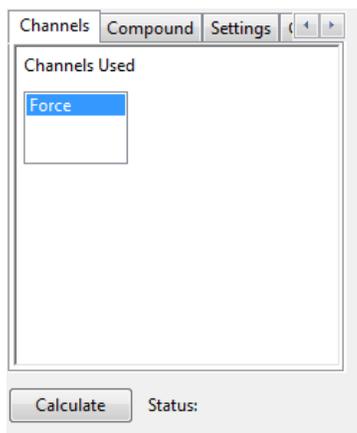


Dose Response Calculations dialog.

- 3) Familiarize yourself with the **Dose Response Calculations** dialog, pictured above.
 - The tabbed **Settings** panels are on the left.
 - The **Concentrations** list, will show the Drug concentrations used.
 - The **Segments** list shows the list of segments for a particular concentration
 - An XY graph window on the right displays the **Dose Response Graph**,
 - Between the configuration dialogs and the XY graph window is the **Cycles Selected** list, an editable list of the cycles that can be displayed and analyzed.
 - The **Data Table** is located on the lower part of the dialog.

To configure the Dose Response Channels:

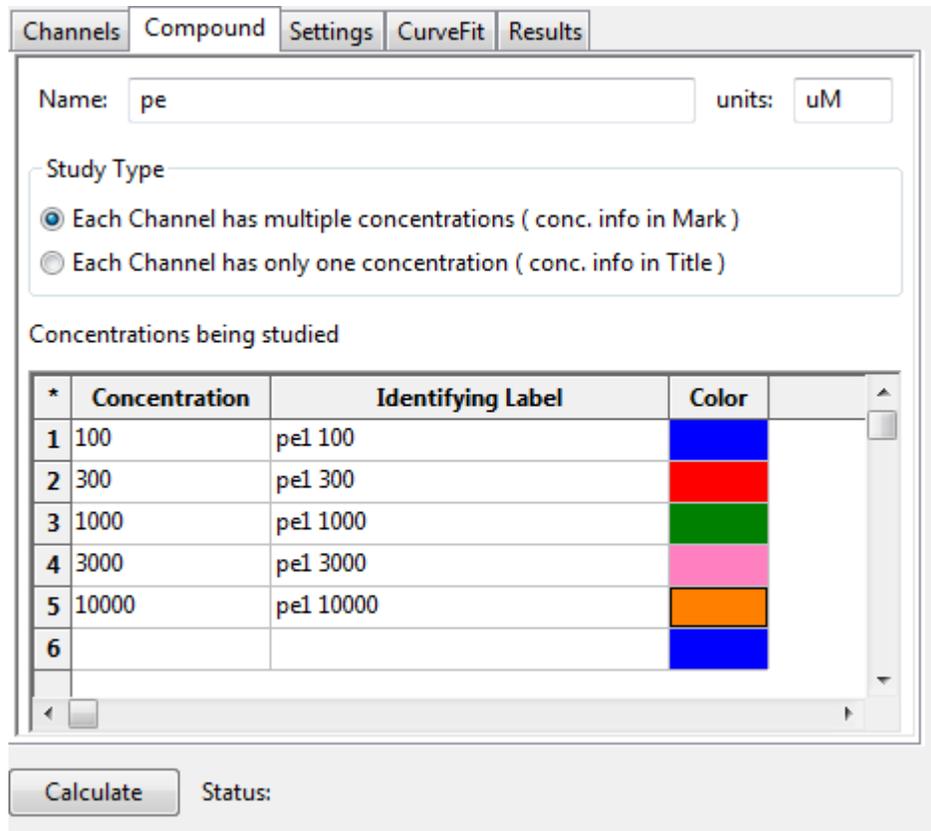
- 1) Click on the **Channels** tab, opening the **Channels** configuration dialog.
- 2) Select the channels that have the Dose Response data.



Dose Response Channels configuration panel.

To configure the Dose Response Compound:

- 1) Click on the **Compound** tab, opening the **Compound** configuration dialog.



Dose Response Compound configuration dialog. Concentration information indicated in marks.

- 2) Enter the name of the drug or compound (as an example, “pe”).
- 3) Enter the name of the units.
- 4) Select the type of Study. Indicate whether each channel has multiple concentrations, which are labeled using marks, or if each channel has only one concentration and the concentration information is in the title. Figures show each option.
- 5) In the table of concentrations being studied, click on the empty Identifying Label cell to see a list of Marks or Titles in the data file. Choose the Mark you want to associate with the concentration and enter the concentration.
- 6) Choose a color for the concentration. The software will create a new row below. Enter the next concentration until all the concentrations are entered.

Channels Compound Settings CurveFit Results

Name: units:

Study Type

Each Channel has multiple concentrations (conc. info in Mark)

Each Channel has only one concentration (conc. info in Title)

Concentrations being studied

*	Concentration	Identifying Label	Color
1	0.1	0.1uM_Amiloride	Light Green
2	0.3	0.3uM_Amiloride	Pink
3	1	1uM_Amiloride	Dark Red
4	3	3uM_Amiloride	Orange
5	30	30uM_Amiloride	Dark Purple
6			Blue

Dose Response Compound configuration dialog. Concentration information comes from channel titles.

To configure the Dose Response Settings:

- 1) Define the selection that will be used to calculate the response. The selection is identified using marks in the data file. In the example illustrated below, LabScribe finds the mark “0.1-30uM_Amiloride”, and indicates the selection should start 120 seconds after the mark. If the marks contain the dose information, it is important that the mark begins with the drug name followed by the concentration, allowing LabScribe to find all the drug delivery marks easily.
- 2) Indicate the desired **Selection Duration** in seconds.
- 3) The Baseline that will be used to calculate the response can be set to zero. It can also be set to the Max, Min, Max-Min, or Mean of a selected region of the data.

- 4) Response is calculated as the Maximum, Minimum, Max-Min, Mean of the selected region of the data. The period of data used to calculate the response is set in the illustrated example as from 0 seconds in the selection to 270 seconds.

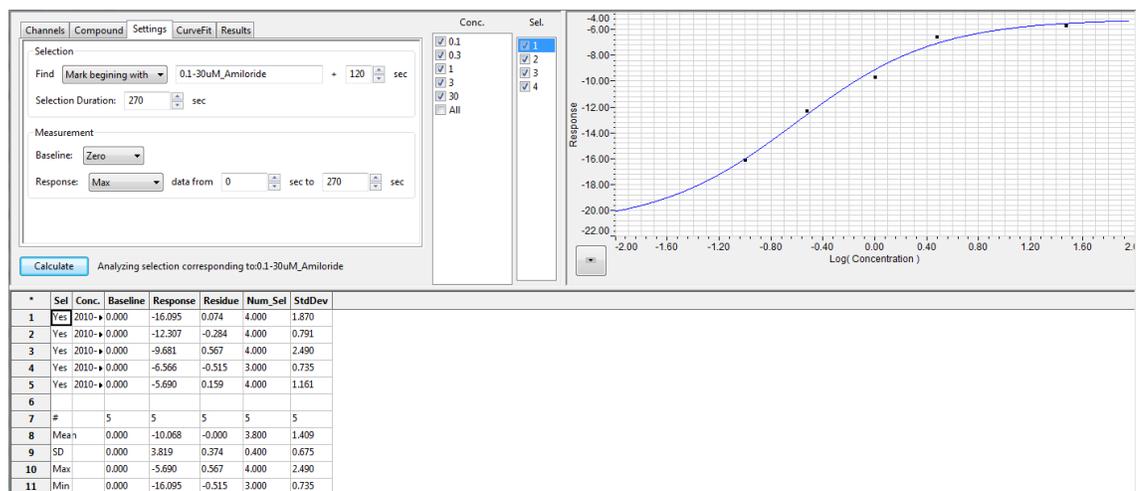
Dose Response Settings configuration dialog.

- 5) Click the **Calculate** button just above the **Data Table** to start the analysis.

Important: After any configuration settings are changed, click **Calculate** again, to trigger the revised analysis.

The software will find all the segments of data that have the dose response measurements. These segments will be sorted, averaged and listed under the **Conc.** list. In the illustrated example below, the concentrations 0.1, 0.3, 1, 3, and 30 are listed. The program will also calculate the **Response** for each concentration and draw the **Schild Plot** in the XY Graph window. The calculated values are displayed in the **Data Table**.

Click on any of the concentration values in the **Conc** list shows the data segments corresponding to that concentration. Clicking on **All** shows all the data segments.

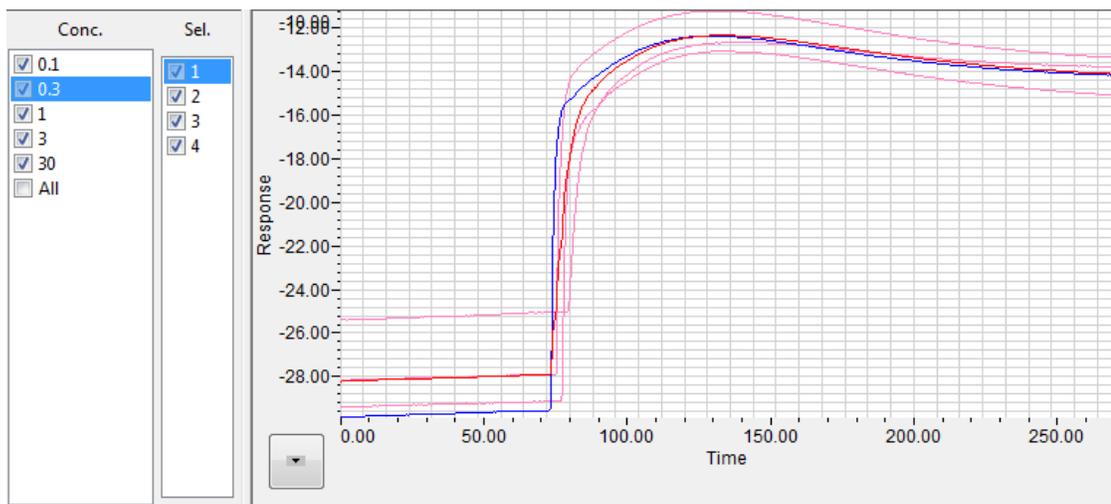


Dose Response Calculations dialog with Schild Plot displayed.

Click the button below the graph to display the XY Graph menu.

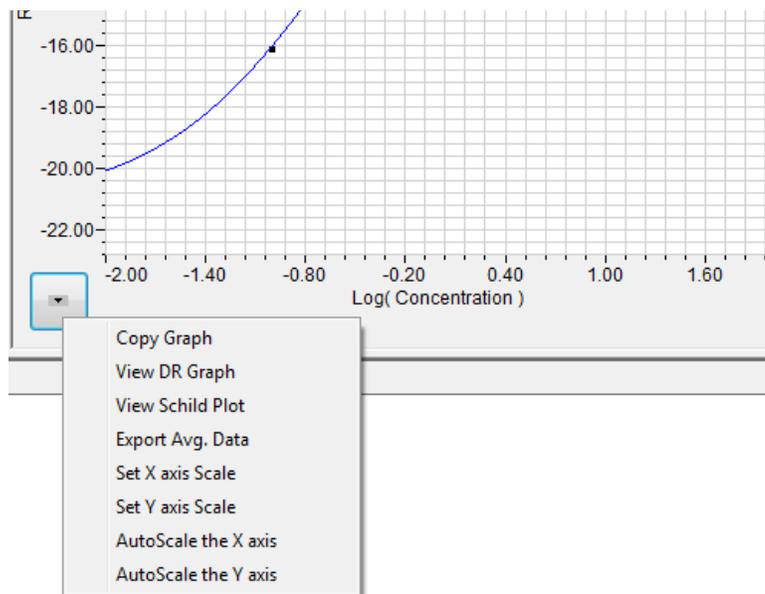
From this menu, one can:

- 1) **Copy Graph:** Copies the current Graph to the clipboard.
- 2) **View the DR Graph:** Displays the segments for one or all concentrations.



DR Graph

- 3) **View the Schild Plot:** Displays the Schild Plot.
- 4) The next 4 options are used to set the X and Y axes. The X and Y axes can also be set by right-clicking on the axis and using the axis submenu.



XY Graph menu.

To display the parameters used to generate the Schild plot, click the CurveFit tab.

	Initial Guess	Fitted Value	Std Dev
Slope	1	0.811417	
Top	0	-5.21048	
Bottom	-13.3192	-21.179	
LogEC50	0	-0.603939	

Dose Response Curve Fit panel.

Data Table

The **Data Table** spans the lower part of the **Dose Response Calculations** dialog and displays the calculated values for each of the segments

The bottom few rows show the sample size, the mean, the standard deviation, minimum and maximum values, and the range of each of the chosen parameters averaged over all the selected cycles.

There are six buttons beneath the **Data Table**: **Copy**, **Export**, **Algorithms**, **Table Options**, **Load Template**, and **Save Template**.

- 1) Click the asterisk at the upper left of the **Data Table** to display two options: **Autosize** and **Copy Selection**. **Autosize** will optimize the size of the **Data Table** boxes, and **Copy Selection** copies any selected **Data Table** cells to the clipboard.
- 2) Click **Copy** to copy all the calculated data in the **Data Table** to the clipboard.
- 3) Click the **Export** button to export the data as a tab (*.txt) or comma (*.csv) separated text file. The currently displayed XY graph can be exported as a Portable Network Graphics (*.png) or JPEG (*.jpg) image.⁷
- 4) Click **Algorithms** to display the mathematical definitions of the parameters included in the **Data Table**.
- 5) Click **Table Options** to open the **Offline Calculations Options Dialog**, which lists the functions from which the **Data Table** parameters can be chosen. All functions are described in the **Dose Response Analysis: Reference** section. Marks indicating temperature and activity can also be added to the file, and those values can be chosen from the **Table Options** list and included in the **Data Table**.
- 6) Click **Load Template** or **Save Template** to display a dialog allowing you to name and save a specific configuration for future use or to load a previously saved template.
- 7) Click **OK** to close the analysis.