

Data Reduction of Results Generated by Modulus™ Microplate using GraphPad Prism® 5.0 Software

1. INTRODUCTION

GraphPad Prism® 5.0 is a powerful combination of biostatistics, curve fitting via nonlinear regression, and scientific graphing in one comprehensive program. GraphPad Prism® 5.0 helps users organize, analyze, and graph repeated experiments, pick appropriate statistical tests, and interpret the results. These capabilities make the software ideally suited for data plotting and analysis applications in the life sciences.

For the purpose of demonstrating how GraphPad Prism® 5.0 can be used in conjunction with the Modulus™ Microplate Fluorometer, results previously generated using the Quant-iT™ PicoGreen® DNA assay were used.

2. MATERIALS REQUIRED

- Modulus™ Microplate Multimode Reader (P/N 9300-002)
- Fluorescence Optical Kit - Blue, 490/515 - 580 nm (P/N 9300-046)
- GraphPad Prism® 5.0 for Windows (GraphPad Software, San Diego, California USA: www.graphpad.com)
- Quant-iT™ PicoGreen® DNA Assay Kit (P-7581) (Molecular Probes, Inc.)

3. ANALYSIS OF DATA

3.1 Data Transfer from Modulus™ Microplate

1. After assay completion, transfer data from the Modulus™ Microplate to a PC or Mac computer via a USB flash drive.
2. Insert a USB flash drive into the compatible port located to the left of the Modulus™ Microplate touch screen.

Note: When insertion is detected, an icon depicting a USB flash drive should appear on the **INSTRUMENT STATUS** bar.

3. Touch the **RESULTS** button on the INSTRUMENT CONTROL screen.
4. Touch the **FILES** button and highlight the file to be exported.
5. Touch the **USB** button under the **TOOLS** button.
6. Touch the **OK** button to export the result file.
7. Move the USB flash drive to a local PC or Mac computer. Click on the desired file to open it in Excel.

3.2 Managing Data in Excel

1. Double click on a .csv file to open it.
2. Using Excel, average and subtract buffer values from all known and unknown samples.

3.3 Preparing GraphPad Prism® 5.0 for Data Transfer from Excel

1. Launch the GraphPad Prism® 5.0 software program.
2. Select the option labeled **XY** under the tab **NEW TABLE AND GRAPH**. See **Figure 1** as an example.
3. Select the option **START WITH AN EMPTY DATA TABLE**.
4. Under **CHOOSE A GRAPH**, select the option labeled **POINTS AND CONNECTING LINE**.

- Under **SUBCOLUMNS FOR REPLICATES OR ERROR VALUES**, select the second option using **Figure 1** as an example. Select the number of replicates according to the number used in the specific experiment.

Note: For this experiment an n = 3 was used for both standard and unknown values.

- Click on the button labeled **CREATE** to generate a new data table.

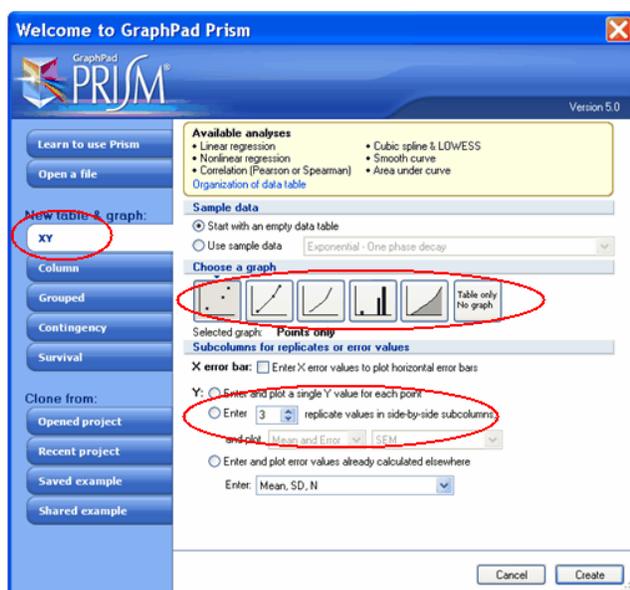


Figure 1.

3.4 Transferring Data from Excel to GraphPad Prism® 5.0

- Select data to be copied from Excel and open the folder labeled **DATA TABLE** in the GraphPad Prism® 5.0 software.
- If raw data is in column format, take the following steps. Highlight cell **A:Y1** and right click to select **PASTE TRANSPOSE**. Select **PASTE DATA** to enter this data into the GraphPad Prism® 5.0 spreadsheet, using **Figure 2** as an example.

- If raw data is in row format, take the following steps. Highlight cell **A:Y1** and right click to select **PASTE DATA**. Enter this data into the GraphPad Prism® 5.0 spreadsheet, using **Figure 2** as an example.

- Label the X-values used for the Standard Curve, located in the X-column of the GraphPad Prism® 5.0 spreadsheet.
- Enter data generated from unknown samples into the GraphPad Prism® 5.0 spreadsheet, using **Figure 2** as an example.
- Label the unknown samples in the X-column as appropriate.
- Click on folder labeled **RESULTS** on left-hand side of the screen. Select **LINEAR REGRESSION** and click on the button labeled **OK** at the bottom of the screen
- If unknown samples are to be determined, select the option box to **INTERPOLATE UNKNOWN FROM STANDARD CURVE**.

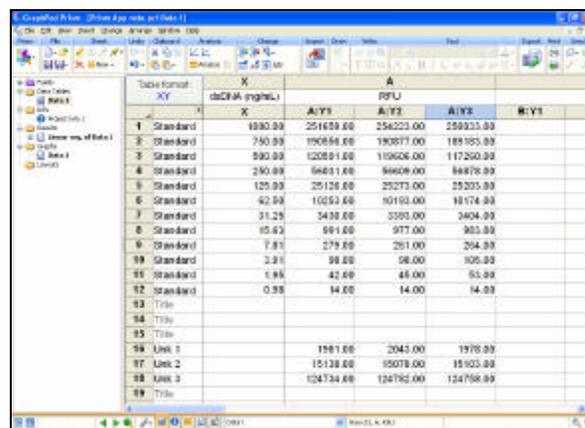


Figure 2.

4. RESULTS

4.1 Viewing and Labeling the Standard Curve Graph

1. The Standard Curve Graph can be viewed by clicking on the tab labeled **DATA 1** under the **GRAPHS** folder.
2. When the graph is open, highlight **DATA 1**, **Y TITLE**, and **X TITLE** and change the labels to specifically appropriate titles.
3. To alter the axes of X and Y, simply double-click on either axis and uncheck the box labeled **AUTOMATICALLY DETERMINE THE RANGE AND INTERVAL**. Manually change the maximum and minimum values for both axes to adjust the range.
4. If the graph needs to change from linear to log scale, click on the **SCALE** arrow and select an appropriate option.
5. After custom formatting the axes, return to the graph by clicking on the button labeled **OK**.

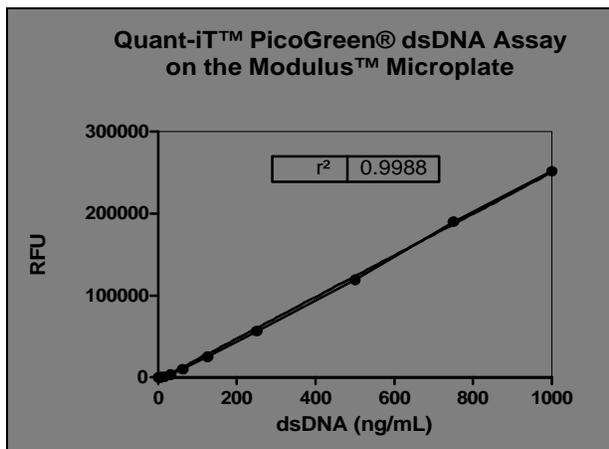
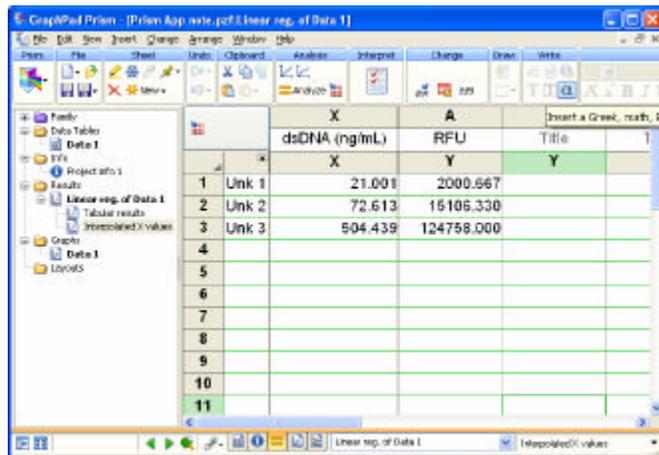


Figure 3. High-range dsDNA standard curve determined using Quant-iT™ PicoGreen® dsDNA Reagent and the Modulus™ Microplate Fluorometer with Blue Fluorescence Optical Kit. Analysis of an XY table with linear regression was performed using GraphPad Prism® 5.0 for Windows from GraphPad Software.

4.2 Determination of Unknown Samples

The results for unknown samples can be viewed by clicking on **INTERPOLATED X VALUES** under the **RESULTS** folder. Results are presented in the format shown below (see **Figure 4**).



	X	Y	Interpolated X values
1	Unk 1	21.001	2000.667
2	Unk 2	72.613	16106.330
3	Unk 3	504.439	124758.000
4			
5			
6			
7			
8			
9			
10			
11			

Figure 4.

5. CONCLUSION

Using GraphPad Prism® 5.0 is an intuitive method for analyzing data generated in today's life science laboratory. With a powerful combination of biostatistics, curve-fitting via nonlinear regression, and scientific graphing, this all-in-one comprehensive program makes data-reduction processing quick and easy.

The Modulus™ Microplate Fluorometer achieves superior performance by use of a dedicated fluorescence detector. The detector is not shared with any other detection modes. The individual Fluorescent Optical Kit of the Modulus™ Microplate Fluorometer uses solid-state optics and a powerful wavelength-matched LED to deliver excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or Absorbance Detection Modules as well as other accessories can be added after initial purchase.

Superior performance, ease of use, and utmost flexibility make the Modulus™ Microplate Reader an ideal choice for today's life science laboratory.

REFERENCE

1. **GraphPad Prism®** (2007): Scientific graphing, curve fitting and statistics. Written web tour.

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