

# Data Tutorial

You just finished performing an assay, and now you would like to use your data to produce a semi-logarithmic fit curve using Excel Spreadsheets. This tutorial will give you the tools to process your raw data into a usable format.

## Step One: Excel Skillset

Use the Microsoft Excel Basic Tasks page to clear up confusion when operating in excel.

<https://support.microsoft.com/en-us/office/basic-tasks-in-excel-dc775dd1-fa52-430f-9c3c-d998d1735fca>

## Step Two: Setting Up the Data

- A. List letters A-H down the column A, then list numbers 1-12 above. Draw borders using the border tool.

|    |            |   |   |   |   |   |   |   |   |   |    |    |    |
|----|------------|---|---|---|---|---|---|---|---|---|----|----|----|
| 27 | Plate Map: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 28 | A          |   |   |   |   |   |   |   |   |   |    |    |    |
| 29 | B          |   |   |   |   |   |   |   |   |   |    |    |    |
| 30 | C          |   |   |   |   |   |   |   |   |   |    |    |    |
| 31 | D          |   |   |   |   |   |   |   |   |   |    |    |    |
| 32 | E          |   |   |   |   |   |   |   |   |   |    |    |    |
| 33 | F          |   |   |   |   |   |   |   |   |   |    |    |    |
| 34 | G          |   |   |   |   |   |   |   |   |   |    |    |    |
| 35 | H          |   |   |   |   |   |   |   |   |   |    |    |    |

This grid will be used to map sample concentrations,

- B. Fill out the grid with the concentrations of your standard concentration values, exactly where they are placed on the plate.

|    |            |            |    |   |   |   |   |   |   |   |    |    |    |
|----|------------|------------|----|---|---|---|---|---|---|---|----|----|----|
| 26 |            | ng/mL      |    |   |   |   |   |   |   |   |    |    |    |
| 27 | Plate Map: | 1          | 2  | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 28 | A          | C0         | C1 |   |   |   |   |   |   |   |    |    |    |
| 29 | B          | 2000 --->  |    |   |   |   |   |   |   |   |    |    |    |
| 30 | C          | 1000 --->  |    |   |   |   |   |   |   |   |    |    |    |
| 31 | D          | 500 --->   |    |   |   |   |   |   |   |   |    |    |    |
| 32 | E          | 250 --->   |    |   |   |   |   |   |   |   |    |    |    |
| 33 | F          | 125 --->   |    |   |   |   |   |   |   |   |    |    |    |
| 34 | G          | 62.5 --->  |    |   |   |   |   |   |   |   |    |    |    |
| 35 | H          | 31.25 ---> |    |   |   |   |   |   |   |   |    |    |    |

The standard concentrations have been filled in for the columns 1 and 2. They have been arranged in duplicate rows, with a negative blank, C0, in the cell A1; and a positive control, C1, in the cell A2.

- C. Copy and Paste your plate map, then label it Raw Data.

|    |           |       |       |   |   |   |   |   |   |   |    |    |    |
|----|-----------|-------|-------|---|---|---|---|---|---|---|----|----|----|
| 37 | Raw Data: | 1     | 2     | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 38 | A         | 0.019 | 1.701 |   |   |   |   |   |   |   |    |    |    |
| 39 | B         | 0.268 | 0.29  |   |   |   |   |   |   |   |    |    |    |
| 40 | C         | 0.656 | 0.649 |   |   |   |   |   |   |   |    |    |    |
| 41 | D         | 1.134 | 1.142 |   |   |   |   |   |   |   |    |    |    |
| 42 | E         | 1.43  | 1.423 |   |   |   |   |   |   |   |    |    |    |
| 43 | F         | 1.525 | 1.582 |   |   |   |   |   |   |   |    |    |    |
| 44 | G         | 1.632 | 1.599 |   |   |   |   |   |   |   |    |    |    |
| 45 | H         | 1.663 | 1.684 |   |   |   |   |   |   |   |    |    |    |

The data the user generates can be added to the Raw Data plate map in the corresponding cells of the template for easy analysis and reference.

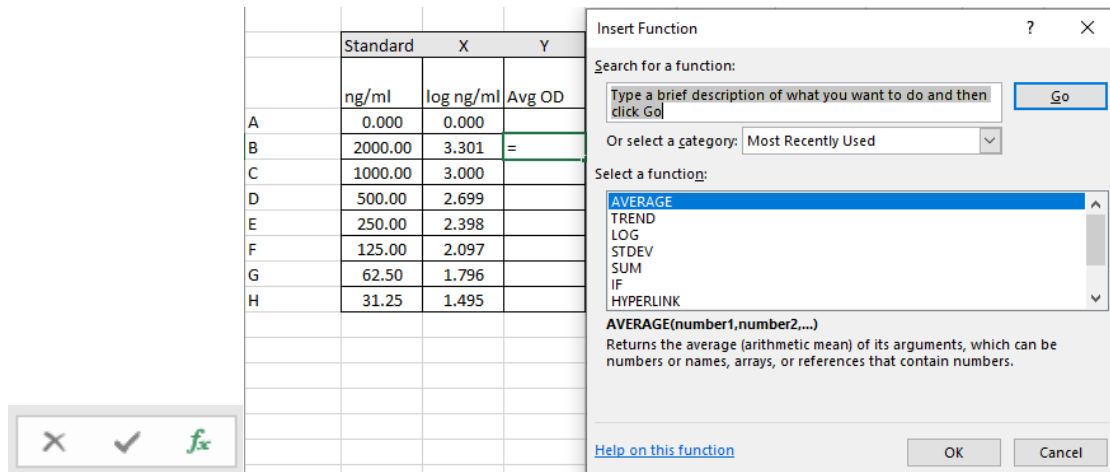
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## Step Two: Standard Calculations

In this step the standard calculations are performed via table, which allows the user to easily define the graph's X and Y values. This table can be referred to for further calculations if necessary.

|   | Standard | X         | Y      |
|---|----------|-----------|--------|
|   | ng/ml    | log ng/ml | Avg OD |
| A | 0.000    | 0.000     |        |
| B | 2000.00  | 3.301     |        |
| C | 1000.00  | 3.000     |        |
| D | 500.00   | 2.699     |        |
| E | 250.00   | 2.398     |        |
| F | 125.00   | 2.097     |        |
| G | 62.50    | 1.796     |        |
| H | 31.25    | 1.495     |        |

- A. Begin by creating a table with A – H running down the left column, followed by the standard concentrations(ng/mL), then the log of the standard concentrations (log ng/mL), and finally the Average Optical Density (Avg OD) going left to right in the row above the A row.



The screenshot shows an Excel spreadsheet with the following data:

|   | Standard | X         | Y      |
|---|----------|-----------|--------|
|   | ng/ml    | log ng/ml | Avg OD |
| A | 0.000    | 0.000     |        |
| B | 2000.00  | 3.301     | =      |
| C | 1000.00  | 3.000     |        |
| D | 500.00   | 2.699     |        |
| E | 250.00   | 2.398     |        |
| F | 125.00   | 2.097     |        |
| G | 62.50    | 1.796     |        |
| H | 31.25    | 1.495     |        |

The 'Insert Function' dialog box is open, showing the 'LOG' function selected. The dialog box contains the following text:

Insert Function

Search for a function:

Type a brief description of what you want to do and then click Go

Or select a category: Most Recently Used

Select a function:

- AVERAGE
- TREND
- LOG
- STDEV
- SUM
- IF
- HYPERLINK

AVERAGE(number1,number2,...)  
Returns the average (arithmetic mean) of its arguments, which can be numbers or names, arrays, or references that contain numbers.

Help on this function

OK Cancel

- B. Take the log of the standard concentration (ng/mL) by pressing the function button, which is located directly under the font tab on the home ribbon. Highlight the desired cell, in this case the cell the user would like to fill is the second row of the log ng/mL column. Once the cell is selected, press the function button. A window will appear with options for each function available, select LOG and press OK. Copy and Paste the previous cell into every empty cell of the desired column.

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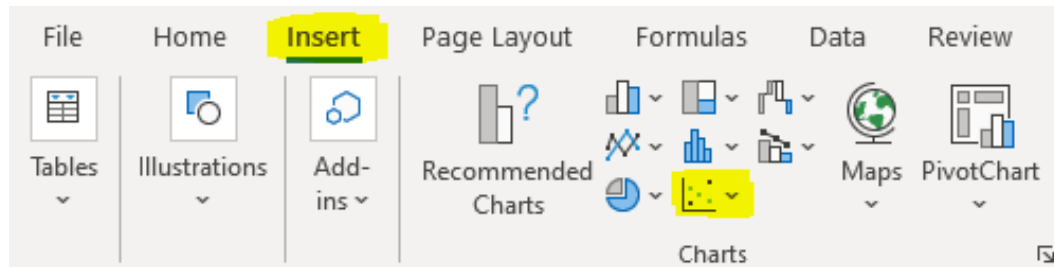
| Raw Data: | 1     | 2     | Function Arguments   |
|-----------|-------|-------|--|
| A         | 0.019 | 1.701 | AVERAGE<br>Number1: B30:C30 = {0.268,0.29}<br>Number2: = number<br>Number3: = number |
| B         | 0.268 | 0.29  |  |
| C         | 0.656 | 0.649 |  |
| D         | 1.134 | 1.142 |  |
| E         | 1.43  | 1.423 |  |
| F         | 1.525 | 1.582 |  |
| G         | 1.632 | 1.599 |  |
| H         | 1.663 | 1.684 |  |

- C. Repeat the process and take the AVERAGE of your standard.

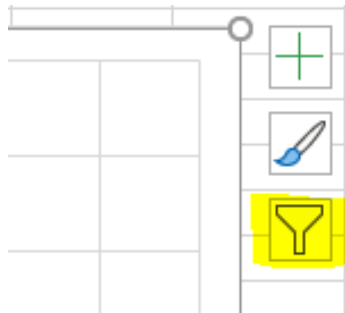
## Step Three: Plotting the Standard Curve

This step of the process allows us to extrapolate information once we know the correct range, also known as the linear portion of the curve.

- A. The first step is to go to the INSERT tab of the TOOL RIBBON select the SCATTER PLOT graph.

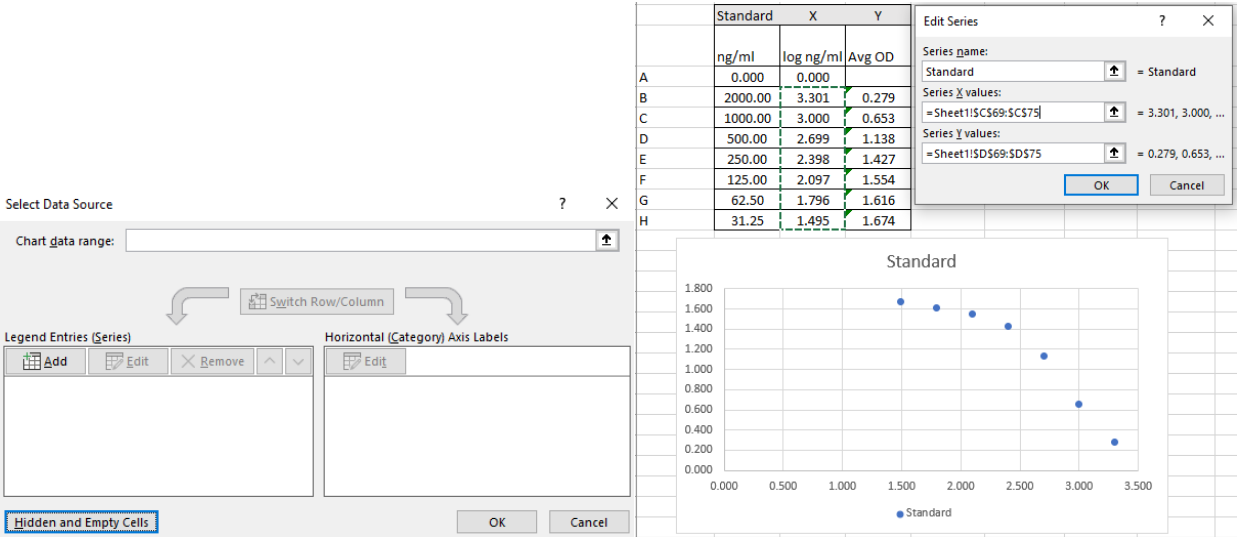


- B. Add DATA to the CHART by clicking the CHART FILTERS icon, located at the top right hand of the CHART. Then click the [Select Data](#) text at the bottom of the pop up.



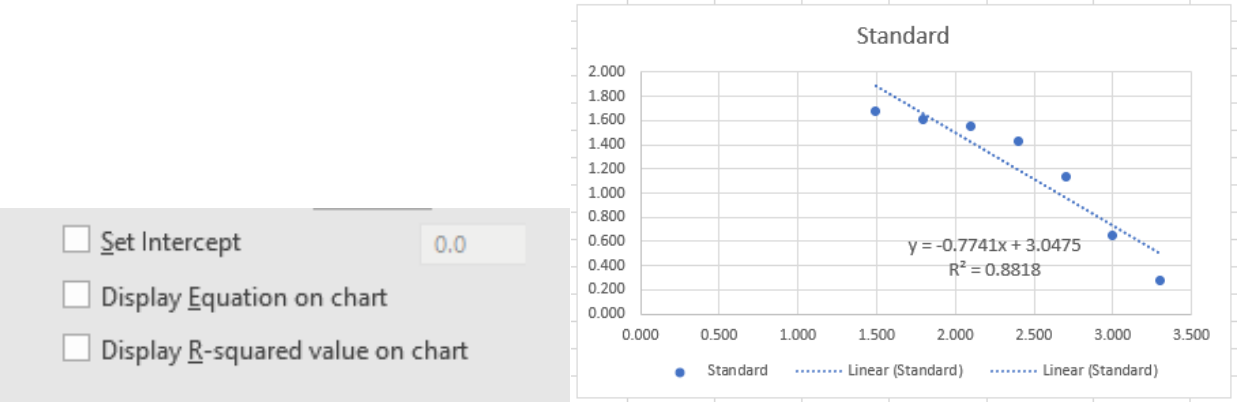
- C. Click on the Add button of the SELECT DATA SOURCE window and name the series, then click and drag to highlight the columns of data. The X VALUES are the LOG NG/ML, and the Y VALUES are the AVG ODS. Click OK.

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D. The data for the Standards has been plotted. Now the user will select the PLUS symbol at the top right hand of the graph. Add Axis Titles, Chart Title, Legend, and Trendline to the graph.

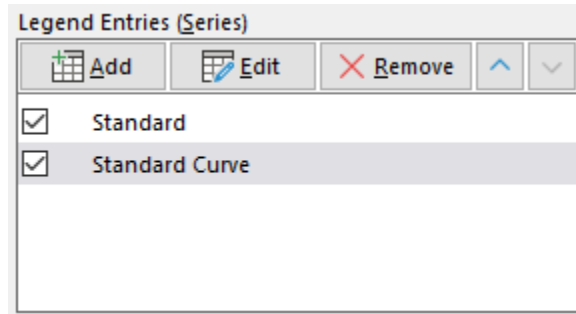
E. Click TRENDLINE, and then click the black arrow next to it for MORE OPTIONS. Check the boxes: Display Equation on chart and Display R-squared value on chart. Check the chart and confirm that the line and data are being displayed.



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## Step Four: Linear Portion of the Curve

The final step is to select a data range which maximizes the R-squared value, while including the most data points on the graph as possible. This step can be subjective and change due to the needs of the user. For this example, the user will select the linear portion which has the highest concentrations of analyte before the curve flattens out at the top left of the graph.

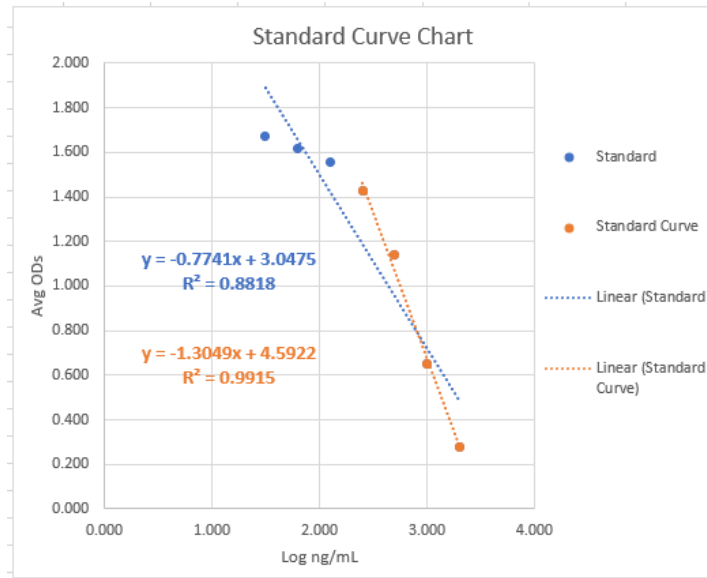


- Repeat the previous portion of Step Three section B and open the SELECT DATA popup from the chart. Add another Series of data and name it, Standard Curve.
- Select the X and Y values for the Series of data once again, but only the data points which the user believes to be on the linear portion of the curve. For this example, the first four points will be selected only.

| Standard | X         | Y      |
|----------|-----------|--------|
| ng/ml    | log ng/ml | Avg OD |
| 0.000    | 0.000     |        |
| 2000.00  | 3.301     | 0.279  |
| 1000.00  | 3.000     | 0.653  |
| 500.00   | 2.699     | 1.138  |
| 250.00   | 2.398     | 1.427  |
| 125.00   | 2.097     | 1.554  |
| 62.50    | 1.796     | 1.616  |
| 31.25    | 1.495     | 1.674  |

- Select the CHART ELEMENTS icon from the previous section, Step E, and click add Trendline, then MORE OPTIONS. Finally, add the Equation to the chart and the R-squared value for the new Standard Curve values.

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The closer the R-squared value is to 1.0, the better the value range is to incorporate in future calculations; for example, the range may be used to calculate the expected concentration value of unknown samples.

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