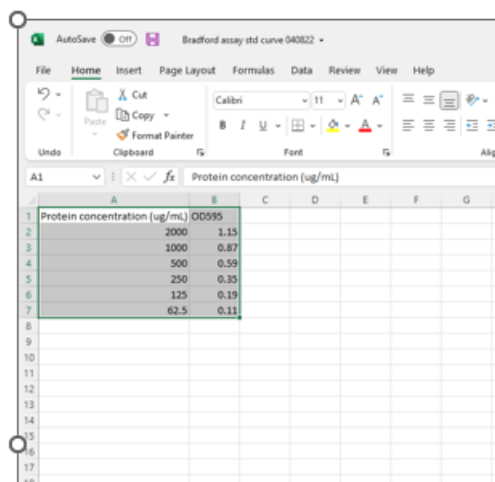


## Graphing a Protein Standard Curve in Excel (PC)

Each team will create a protein concentration standard curve using the Excel program, a commonly used spreadsheet program. Each team will use the equation of the standard curve line and the OD595 reading of their purified protein to calculate the concentration of their purified protein.

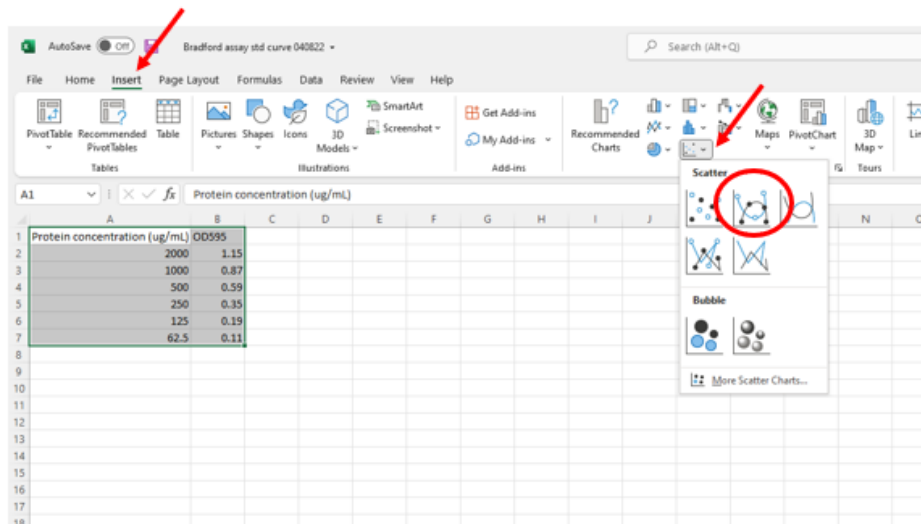
1. Open an Excel spreadsheet.
2. Create a heading in box A1 called 'Protein Concentration (ug/mL)'. Create a heading in Box B1 called 'OD595'. In put your data from highest concentration to the lowest as shown in the example below.



The screenshot shows an Excel spreadsheet with the following data:

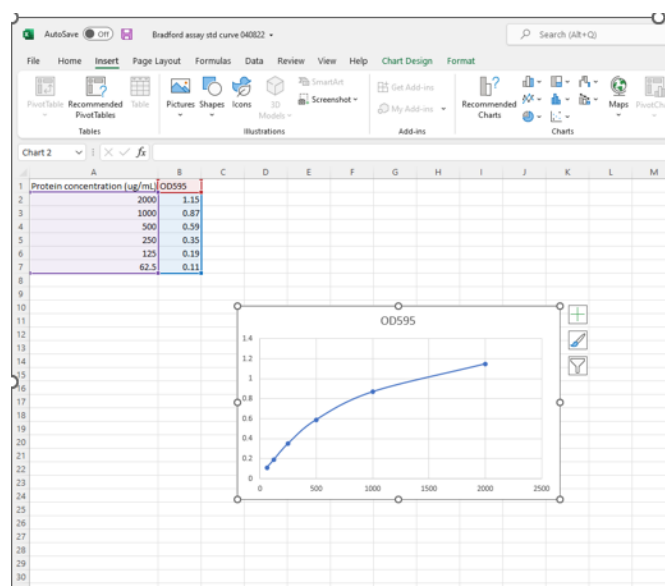
Protein concentration (ug/mL)	OD595
2000	1.15
1000	0.87
500	0.59
250	0.35
125	0.19
62.5	0.11

3. Select the entries in both column A and B. Select the 'Insert' tab at the top of the page (indicated by the slanted red arrow). Then select the line graph icon (indicated by the horizontal red arrow). When that window opens, select the type of graph shown in the figure below (red circle).

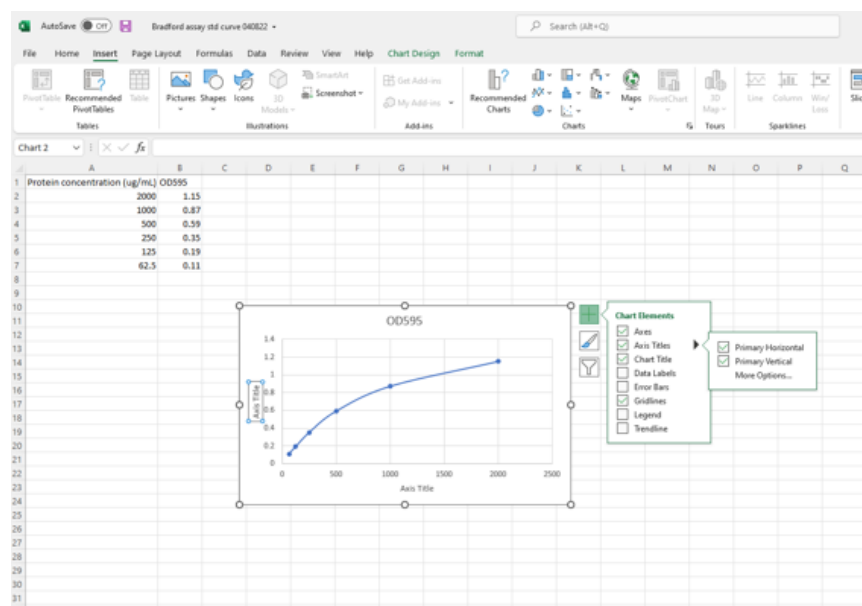


## Module 3, Lesson 5: Graphing a Standard Curve Protocol

- This will plot your data with the OD595 readings on the y-axis and the protein concentration on the x-axis. The axes are not properly labeled yet and the graph title is incorrect.

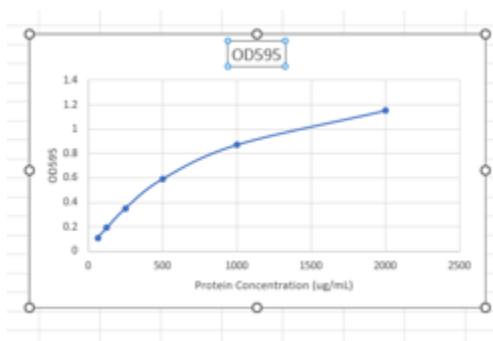


- Click inside of the graph box to make the 3 icons appear to the right of the graph box. Click on the '+' icon. Make sure the 'Axis Titles' box is checked. Click on the arrow next to 'Axis Titles' and 'Primary Horizontal' and 'Primary Vertical' choices will appear. Make sure both of those boxes are checked. Now the axis labels will be activated and you can click on them and rename the axes.

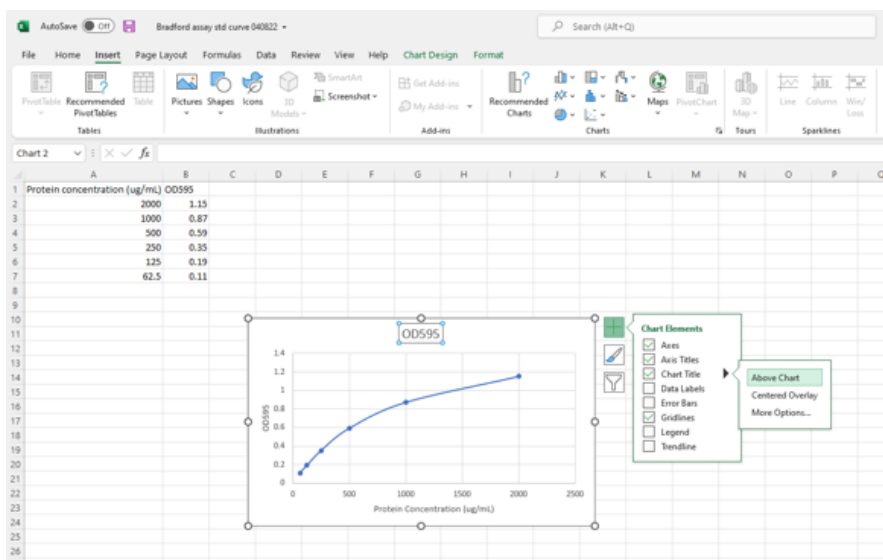


## Module 3, Lesson 5: Graphing a Standard Curve Protocol

6. Change the x-axis label to: Protein Concentration (ug/mL). Change the y-axis label to: OD595. Now the graph should look like this.

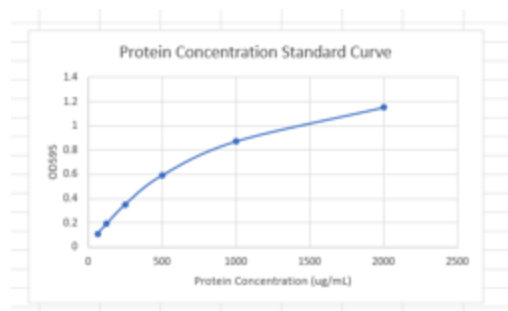


7. The chart title will still be incorrect. Click on the '+' icon again. Make sure the 'Chart Title' box is checked. Click on the arrow to the right of the 'Chart Title' box. This will open a window with choices for where the title appears. It also activates the chart title text. Click on the chart title text and change it to: Protein Concentration Standard Curve.

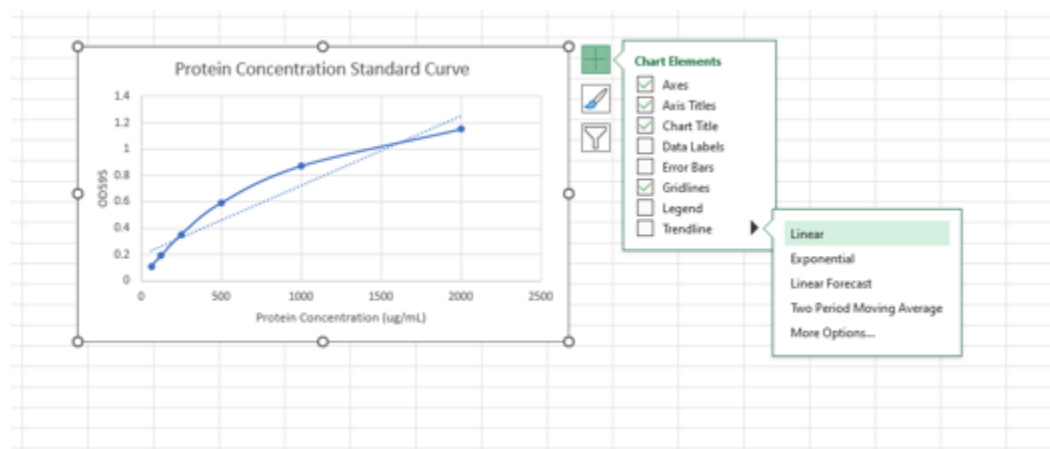


8. Now your graph should look like this.

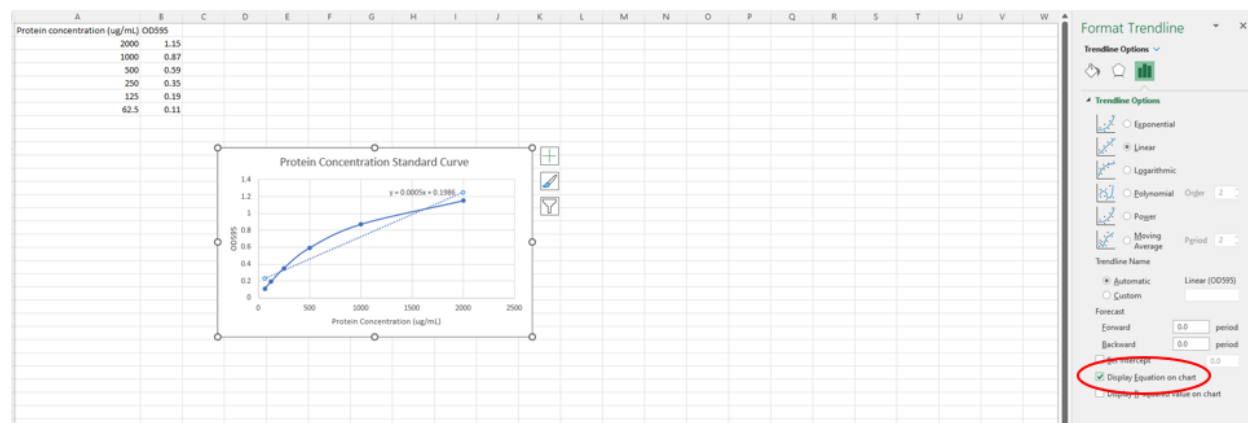
## Module 3, Lesson 5: Graphing a Standard Curve Protocol



9. Now you need to plot a linear 'best fit' line. Click inside the graph box to get the '+' box again. Click the 'Trendline' box and choose 'Linear'. Now a linear best fit line will display.



10. To find the equation of the line, click on the '+' button again. Go to 'Trendline'. This time choose 'More Options'. A menu will appear on the right side of the screen. Click on the 'Display equation on chart' box. Now the equation of the 'best fit' line will display in the graph box.



11. You can use this equation to calculate the concentration of your unknown – your purified protein. The equation is written in the form of:  $y = mx + b$ .  
m is the slope of the line.

b is the y-intercept of the line.

y is the OD595 reading.

x is the concentration of the sample.

To solve for x use:  $x = \frac{y-b}{m}$

12. So, in the case of the example line, the equation is:

$$y = 0.0005x + 0.1986$$

13. If a sample of unknown concentration (like the purified protein) gave an OD595 reading of 0.65, we can calculate its concentration. In this case:

$$y = 0.65$$

$$m = 0.0005$$

$$b = 0.1986$$

SO :

$$x = \frac{0.65 - 0.1986}{0.0005} = 902.8 \text{ ug/mL}$$

14. Once you know the protein concentration of your purified protein, you can calculate your protein yield by multiplying the concentration by the volume of the sample you collected. Make sure your volume units are the same when you do the calculation.

15. Calculate the protein concentration and yield of the protein that you purified. Enter this information in the Downstream Process Batch Record.