BCA Assay: Absorbance Analysis

In this section, you will use Google Sheets to take the absorbance data from last year's BCA Assay Experiment and determine the lysate protein concentration in the PC9 and PC9 AXL KO Cells.

1. **Examine the well layout** recorded in your lab notebook (left) and compare that to the absorbance data (right). This ensures that you know which absorbance value corresponds to each standard triplicate and sample duplicate.
2. **Rearrange and label the standard data** so that it is easier to work with. Copy-paste the relevant absorbance data into the concentration-absorbance data table.

STEP 2	[Standard] (ug/mL)	Absorbance 1	Absorbance 2	Absorbance 3
	2000			
	1000			
	500		PASTE	
	250		VALUES	
	50		HERE	
	5			
	0			

3. **Subtract the blank absorbance (0.096) from the standard absorbance readings**. A blank well plate will register a small level of light absorbance. This blank absorbance needs to be subtracted from the outputted absorbance values before making the standard curve. To accomplish this, type "= corresponding cell number - 0.096" For instance, to correct the absorbance for 2000 ug/mL Standard 1 (in cell C26), type:

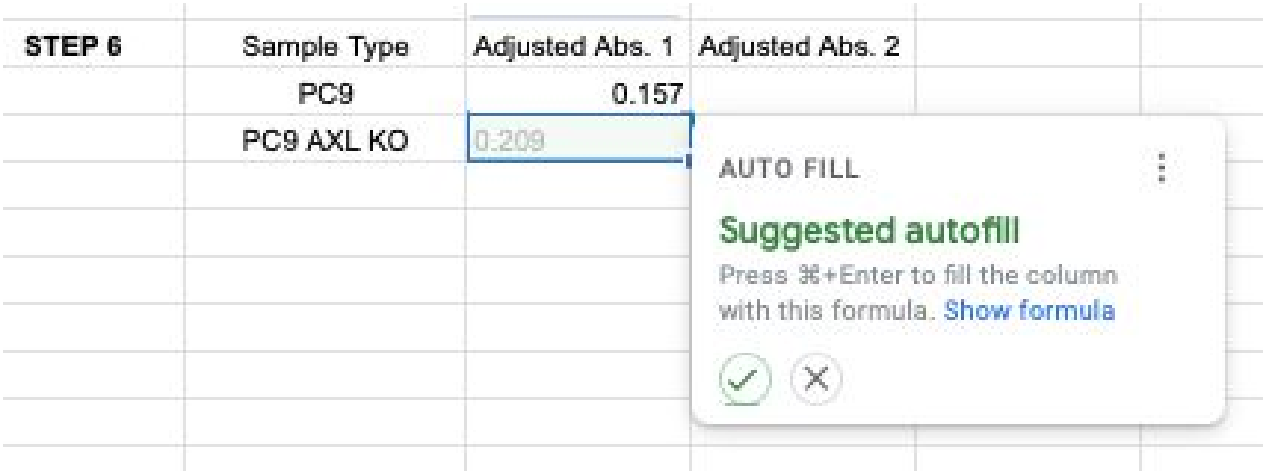
`=C26-0.096` into cell C36. Then, drag down to autofill the other Standard 1 values.

STEP 3	[Standard] (ug/mL)	Adjusted Abs. 1	Adjusted Abs. 2	Adjusted Abs. 3
	2000	2.703		
	1000			
	500			
	250			
	50			
	5			
	0			

4. **Find the absorbance average and error for the standard wells**. To obtain the average, type “=AVERAGE(cell 1, cell 2, cell 3)”. This will yield the mean of the three standard wells. To obtain the error, type “STDEV.S(cell 1, cell 2, cell 3)/SQRT(3).” This will yield the standard error of the mean for the three standard wells. For example, to obtain the mean and standard error of the 2000 ug/mL wells, type `=AVERAGE(C36,D36,E36)` into cell C45 and `=STDEV.S(C36,D36,E36)/SQRT(3)` into cell D45, respectively. Drag down to autofill the other concentration values
5. **Rearrange and label the sample data** so that it is easier to work with. Similar to Step 2, copy-paste the relevant absorbance data into the concentration-absorbance data table.

STEP 5	Sample Type	Absorbance 1	Absorbance 2
	PC9	PASTE	VALUES
	PC9 AXL KO	IN	HERE

6. **Subtract the blank absorbance (0.096) from the sample absorbance readings**. Similar to Step 3,type “= corresponding cell number - 0.096” For instance, to correct the absorbance of the first PC9 sample, type: `=C54-0.096` into cell C58. Drag down or use the autofill feature to blank the PC9 AXL KO sample.



7. **Find the absorbance average and error for the sample wells**. To obtain the average, type “=AVERAGE(cell 1, cell 2)”. This will yield the mean of the two sample wells. To obtain the error, type “STDEV.S(cell 1, cell 2)/SQRT(2).” This will yield the standard error of the mean for the two sample wells. For example, to obtain the mean and standard error of the PC9 wells, type into `=AVERAGE(C58,D58)` cell C62 and `=STDEV.S(C58,D58)/SQRT(2)` into cell D62, respectively. Drag down to autofill the other concentration values.

STEP 6	Sample Type	Adjusted Abs. 1	Adjusted Abs. 2
	PC9	0.157	0.15
	PC9 AXL KO	0.209	0.164

STEP 7	Sample Type	Average Abs.	Error Abs.
	PC9	=AVERAGE(C58,D58	
	PC9 AXL KO		

AVERAGE(value1, [value2, …])

EXAMPLE
AVERAGE(A2:A100, B2:B100)

ABOUT
Returns the numerical average value in a dataset, ignoring text.

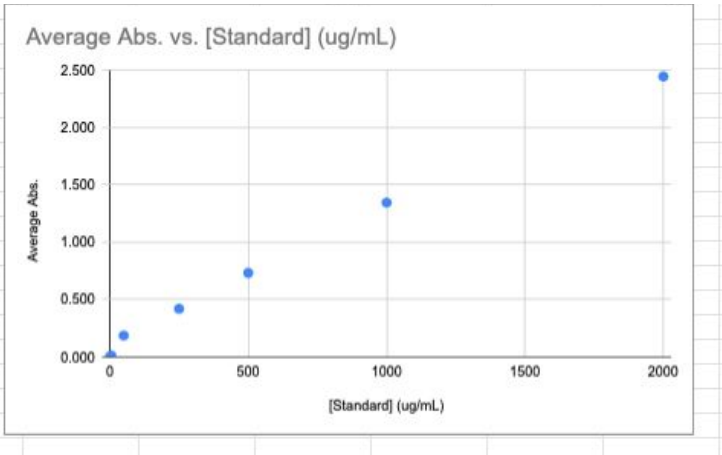
value1
The first value or range to consider when calculating the average value.

value2… – [optional] repeatable
Additional values or ranges to consider when calculating the average value.

8. **Make your standard curve** by plotting Standard Concentration (ug/mL) on the x-axis against Standard Absorbance on the y-axis. Select the concentration and average absorbance data for your samples (In Step 4, cells C45-C51, D45-D51).

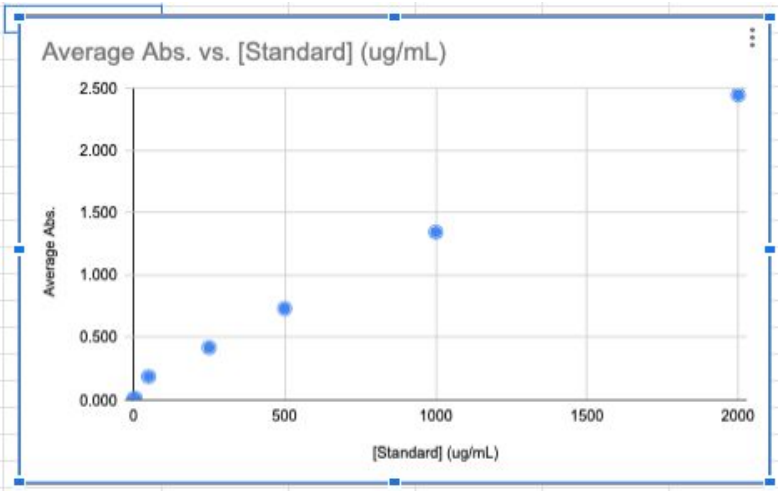
STEP 4	[Standard] (ug/mL)	Average Abs.	Error Abs.
	2000	2.445	0.1349374341
	1000	1.346	0.1024049695
	500	0.732	0.03910669167
	250	0.420	0.03357743952
	50	0.186	0.07817999176
	5	0.013	0.005364492313
	0	0.000	0.000333333333

Then, click Insert -> Chart. You should get this very ugly standard curve.

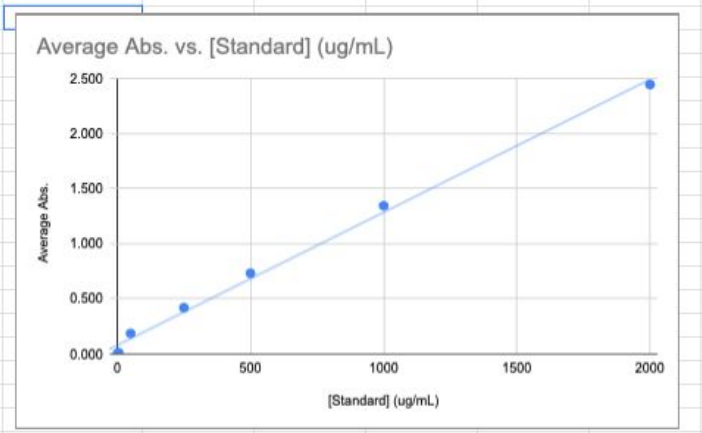


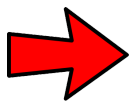
9. Now, we need to add two essential features to the standard curve: a line of best fit and an **equation of the line of best fit**. This will allow us to convert our sample absorbance data into concentrations. Double click on a data point to reveal the Chart Editor. Check the trendline box to add a line of best fit. Scroll down and change the Label from “None” to “Use Equation” to see the equation of the line of best fit.

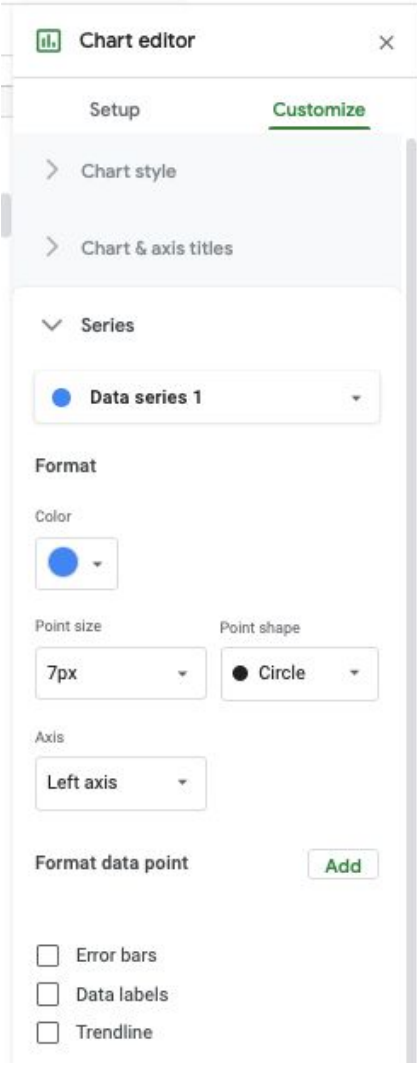
STEP 9



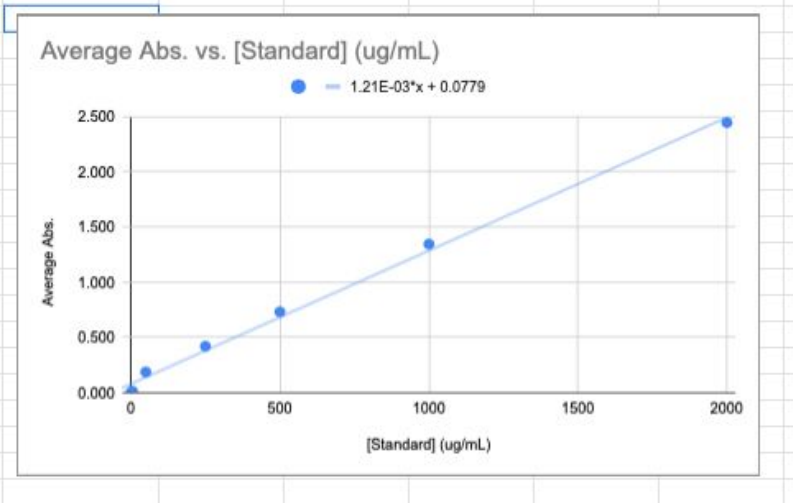
STEP 9

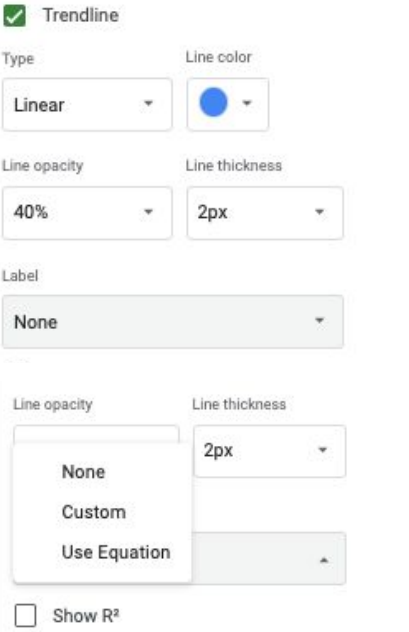






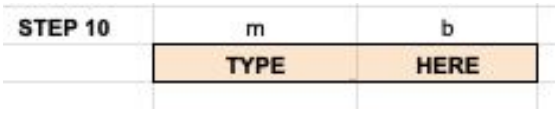
STEP 9



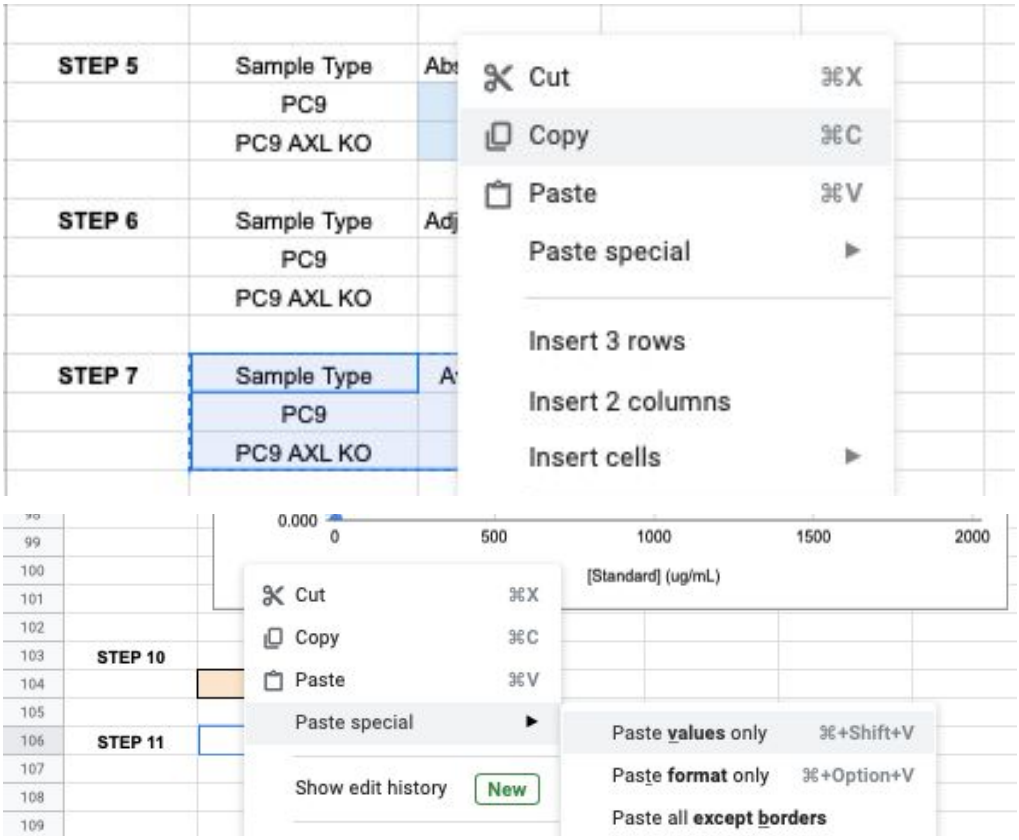


Now, we have the equation $y = (1.21 \cdot 10^{-3})\,x + 0.0779$

10. **Rearrange the equation** of the line of best fix to solve for concentration (y = mx + b x = $(\frac{1}{m})(y - b)$. Paste the m and b values into cells C104 and D104.



11. Now we can find the concentrations of the sample wells! Copy-paste the values from Step 7 into Step 11. Use “Paste-Special -> Values only” to ensure the final numbers copy over rather than the formulas.



Now, solve for sample protein concentration using the rearranged equation and saved values from Step 10. For example, for PC9 protein concentration, type:

`=(1/B104)*(C107-C104)` into cell D107.

Your protein concentration for PC9 should be in cell D107 and your protein concentration for PC9 AXL KO should be in cell D108. Which one has a higher protein concentration? Why do you think it has a higher protein concentration?

Additional Practice: Manufacturing Associate Interview Assignment

1. From a stock solution of 2 M KCl, you need to prepare 50 mL of 600 mM KCl. How much water and KCl do you need to add? Please include all the steps of the calculation in your response.

2. Below is the information for the KCl, water and 50 mL conical tube. Please write a protocol on how to prepare the 50 mL of 600 mM KCl solution in a 50 mL conical tube. The protocol should be clear and detailed so a person can complete this task with minimal supervision. Include all the steps and all the tools and consumables needed.

   2M KCl:
   https://www.thermofisher.com/order/catalog/product/AM9640G?us&en#/AM9640G?us&en

   Water:
   https://www.thermofisher.com/order/catalog/product/10977015#/10977015

   50 mL conical tube:
   https://www.thermofisher.com/order/catalog/product/339650?SID=srch-srp-339650#/339650?SID=srch-srp-339650

3. The 600 mM KCl solution is being used as part of the production process. You are in charge of making batches of this solution for other team members to use. One day after making one batch of the 600 mM KCl solution, you realized you added NaCl instead of KCl for that batch.
   A. Outline your next steps to deal with this mistake.
   B. Part of the response includes sending out an email to the team members. Please draft a one-paragraph email.