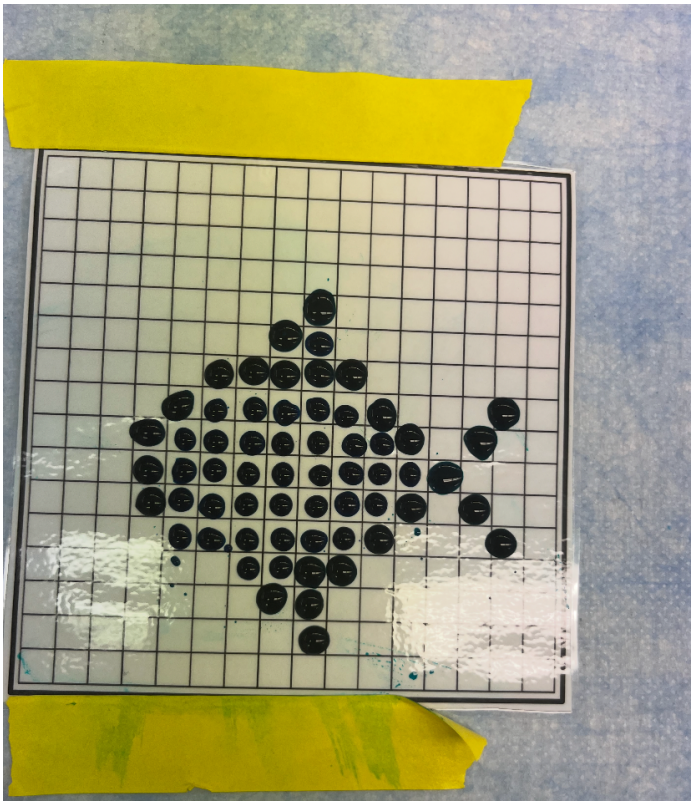
Biomolecules of Life (Laboratory)

MICROPIPETTE AND DYE-BINDING ASSAY WORKSHEET

Your Names: Cindy Qu and Emily Zhang

PART I: DRAW A PICTURE USING A MICROPIPETTE

Q1. Include an image of the masterpiece you completed in the laboratory class in the space below. Ensure that the image you made involves no less than 40 drops and two different colors (blue, red, yellow and green). One color must be consistently 15 µL and the other 30 µL. Your pipetting skills will be graded for this exercise because pipetting is a critical skill that you need to develop in this laboratory class. **(4 pts)**



(Note 1: Random little dots are left over from wiped dots, they are not contributing to the official dots we pipetted)

(Note 2: The two colors we used are green and blue, but they look very similar so that is why everything looks like one color even though we used two colors)

Biomolecules of Life (Laboratory)

PART II: DYE-BINDING ASSAY

Using the spectrophotometer, read the absorbance at 595 nm and record the readings in column 6 for test tubes 1 to 9 after you aliquot 1.0 mL of each tube samples into cuvettes.

Q2. Standard Curve **(3 pts)**

1 Tube No.	2 Standard BSA Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)
1	0	500	-	4.5	0.000	0
2	10	490	-	4.5	0.101	10
3	20	480	-	4.5	0.265	20
4	30	470	-	4.5	0.409	30
5	40	460	-	4.5	0.506	40
6	60	440	-	4.5	0.686	60

Q3. **(2 pts)**

What is the letter of the unknown concentration sample that you are assigned? B

Concentration Determination of An Unknown

1 Tube No.	2 Unknown Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)*
7	15	485	-	4.5	0.309	25.068

* This will be calculated from your standard curve when you plot tubes 1 to 6.

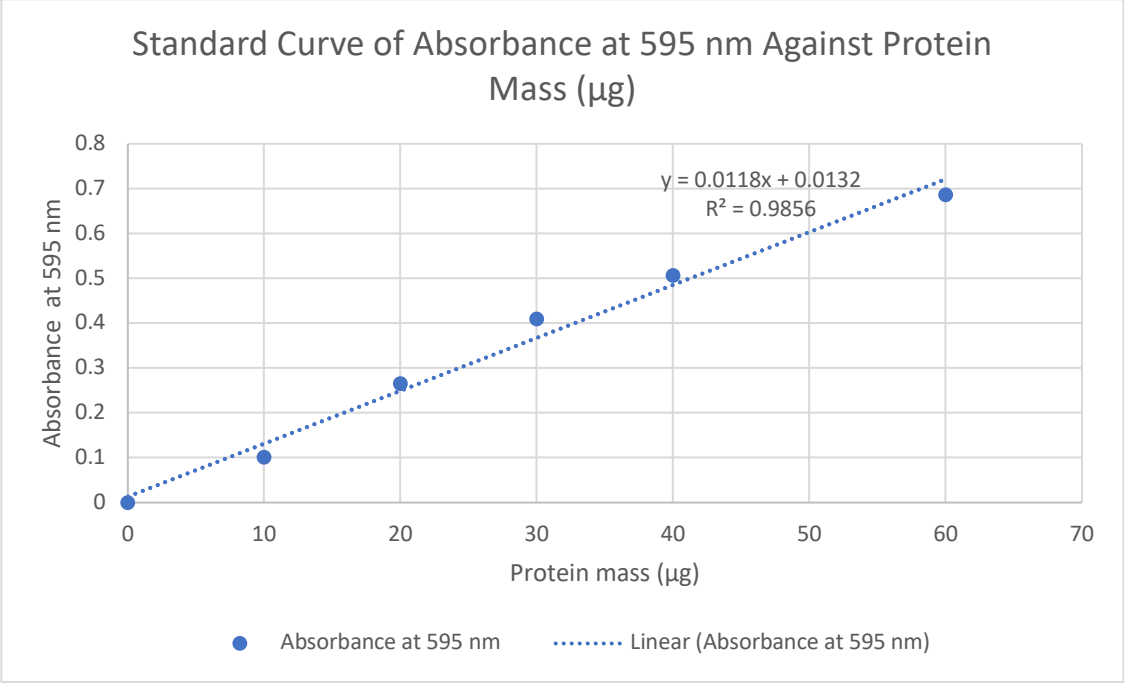
Q4. Determination of the Effect of SDS **(2 pts)**

1 Tube No.	2 Standard BSA Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)	
						Measured*	Expected
8	50	350	100	4.5	0.440	36.169	50
9	-	400	100	4.5	0.433	35.576	-

* This will be calculated from your standard curve when you plot tubes 1 to 6.

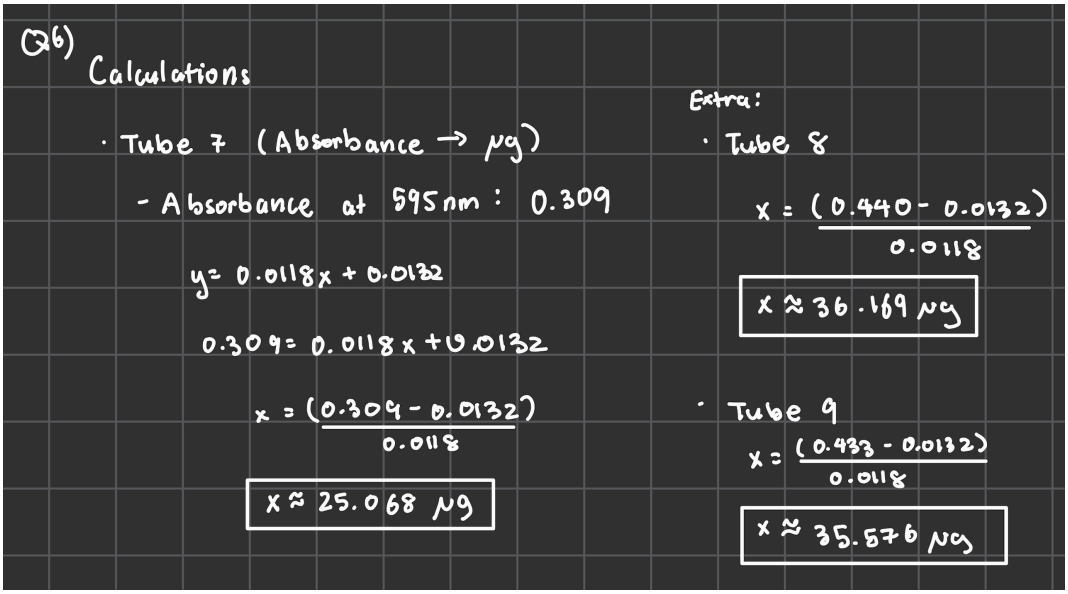
Q5. Standard curve graph **(4 pts)**

Using the data you recorded in the table in Q2, generate standard curves of absorbance at 595 nm against protein mass (µg). Remember to label the axes and include the equation of best fit line in the graph. Insert the graph below.



Q6. Protein mass (column 7) **(2 pts)**

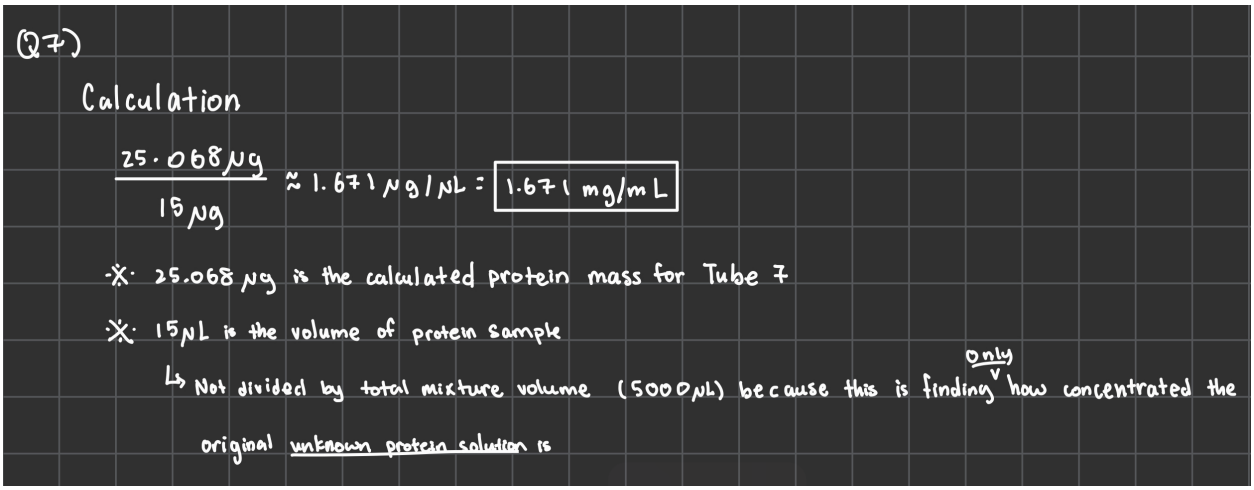
Show a sample calculation for converting absorbance into µg of protein for tube 7. Fill up protein mass (column 7 of tables) for all the tubes in Q3 and Q4.



Q7. Determining unknown concentration **(3 pts)**

What is the determined protein concentration (mg/mL) for your assigned unknown (tube 7) using the dye-binding assay? Show your calculations below.

The determined protein concentration for my assigned unknown (tube 7) is 1.671 mg/mL using the dye-binding assay.



Q8. Effect of interfering substances **(2 pts)**

Based on your data in the table of Q4, does SDS interfere with estimation of protein concentration? Briefly explain why.

Based on my data in the table of Q4, SDS does interfere with the estimation of protein concentration.

Absorbance is proportional to protein concentration, especially when using a consistent dye-binding assay in the linear range (Beer-Lambert Law).

However, even though Tube 8 had a higher protein concentration (50 µL) compared to Tube 9 (no protein), their absorbance at 595 nm were very similar. This showcases that SDS disrupts absorbance and can interfere with the binding of the dye used to the protein.

As a result, SDS led to a decreased absorbance close to the number a tube would be for a tube with no protein.

PART II: DYE-BINDING ASSAY

Using the spectrophotometer, read the absorbance at 595 nm and record the readings in column 6 for test tubes 1 to 9 after you aliquot 1.0 mL of each tube samples into cuvettes.

Q2. Standard Curve **(3 pts)**

1 Tube No.	2 Standard BSA Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)
1	0	500	-	4.5	0.000	0
2	10	490	-	4.5	0.099	10
3	20	480	-	4.5	0.263	20
4	30	470	-	4.5	0.407	30
5	40	460	-	4.5	0.504	40
6	60	440	-	4.5	0.684	60

Q3. **(2 pts)**

What is the letter of the unknown concentration sample that you are assigned? B

Concentration Determination of An Unknown

1 Tube No.	2 Unknown Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)*
7	15	485	-	4.5	0.309	25.21

* This will be calculated from your standard curve when you plot tubes 1 to 6.

Q4. Determination of the Effect of SDS **(2 pts)**

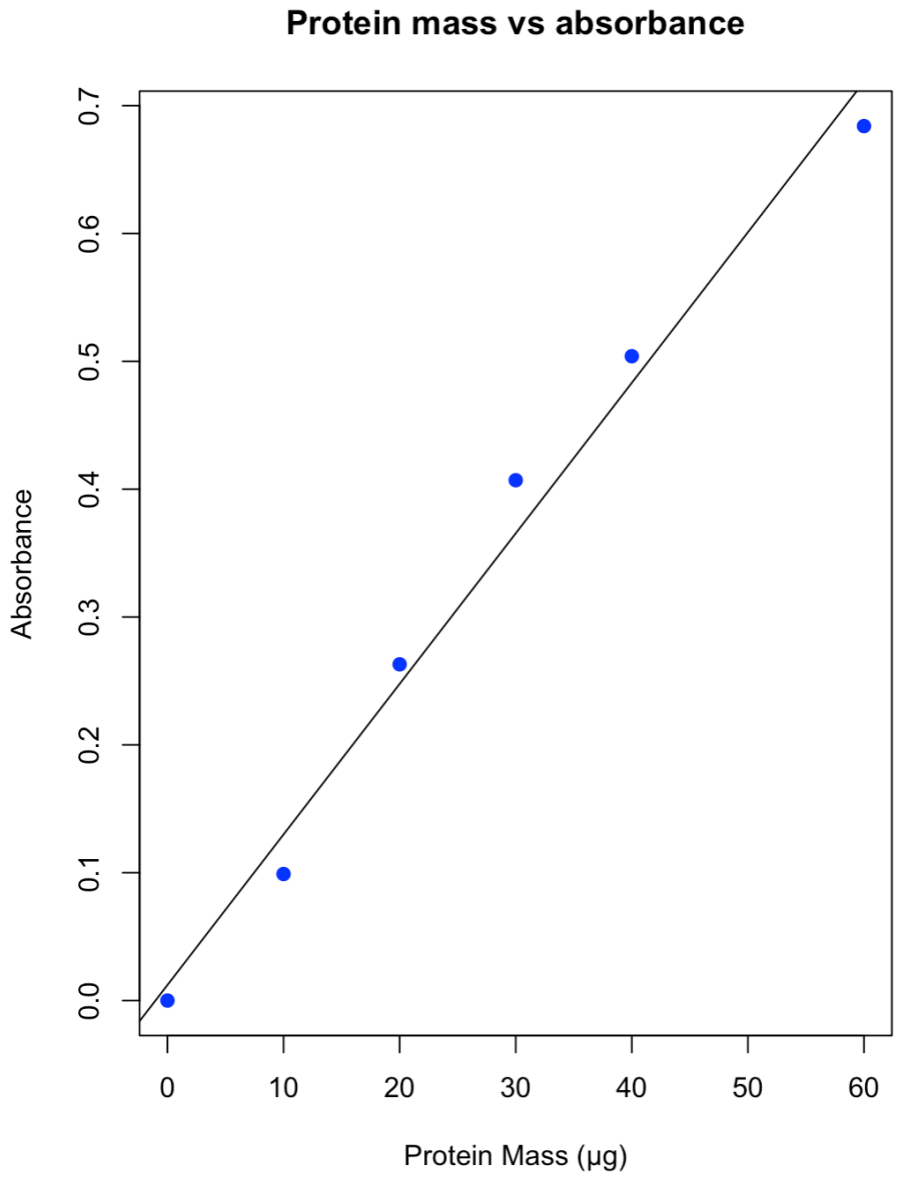
1 Tube No.	2 Standard BSA Protein (µL)	3 Water (µL)	4 SDS (µL)	5 Blue Dye Reagent (mL)	6 Absorbance at 595 nm	7 Protein mass (µg)	
						Measured*	Expected
8	50	350	100	4.5	0.440	36.33	50
9	-	400	100	4.5	0.433	35.74	-

* This will be calculated from your standard curve when you plot tubes 1 to 6.

Biomolecules of Life (Laboratory)

Q5. Standard curve graph **(4 pts)**

Using the data you recorded in the table in Q2, generate standard curves of absorbance at 595 nm against protein mass (µg). Remember to label the axes and include the equation of best fit line in the graph. Insert the graph below.



*absorbance vs protein mass
y = 0.0118*x + 0.0122
R^2 = 0.9821

Q6. Protein mass (column 7) **(2 pts)**

Show a sample calculation for converting absorbance into µg of protein for tube 7. Fill up protein mass (column 7 of tables) for all the tubes in Q3 and Q4.

absorbance = 0.0118*protein + 0.0122
0.309 - 0.0122 = 0.0118*protein
protein = 0.2968/0.0118
protein mass (µg) = 25.15

(the table has a slightly different value because there were a few more sigfigs in the software)

Q7. Determining unknown concentration **(3 pts)**

What is the determined protein concentration (mg/mL) for your assigned unknown (tube 7) using the dye-binding assay? Show your calculations below.

concentration = 25.15 µg/15 µL
= 1.68 µg/µL
= 1.68 mg/mL

Q8. Effect of interfering substances **(2 pts)**

Based on your data in the table of Q4, does SDS interfere with estimation of protein concentration? Briefly explain why.

Yes, SDS interferes with the estimation of protein concentration. As the protein mass increases, the absorbance increases in a positive linear correlation, so given the data points, a protein mass of 50 with the standard curve should produce an absorbance ~0.6022 or between 0.504 and 0.684, and tube 8 had an absorbance that was below this range. Additionally, a protein mass of 0 should produce an absorbance of 0. However, both samples with SDS had roughly the same absorbance that was significantly greater than 0. This suggests that SDS disrupts the absorbance and interaction between the protein and the dye.