

Quantitative Detection of Protein (activity)

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Experimental Background

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

Dilute BSA Standards

1. Label 9 tubes 1-9
2. Combine the components of the table below to generate appropriate concentration of solutions

Solution (ml)	BSA Standard Dilution						Unknown		Blank
	1	2	3	4	5	6	7	8	9
	0.1mg/ml	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1.0mg/ml			0.0mg/ml

BSA	0.1	0.2	0.4	0.6	0.8	1	-	-	-
H₂O or Buffer	0.9	0.8	0.6	0.4	0.2	-	-	-	1
Sample	-	-	-	-	-	-	1	1	-
Biuret	4	4	4	4	4	4	4	4	4

3. Place tube 9 (**blank**) into a cuvette and measure absorbance (**A**) in the spectrophotometer at 540 nm

4. Calibrate the spectrophotometer to read 0 at **A_{540nm}**

5. Sequentially read each sample at **A_{540nm}** and record values in table below

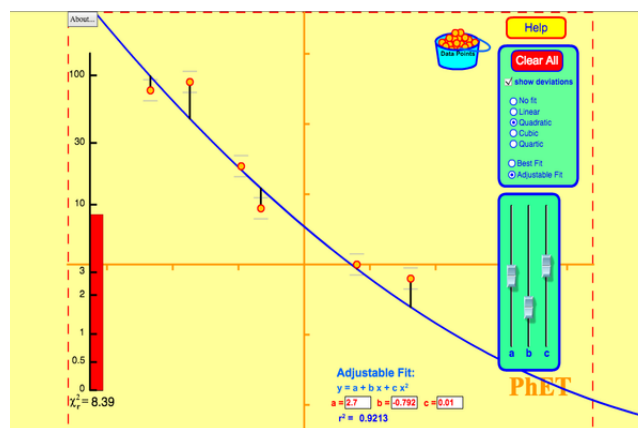
Test Tube	BSA (mg/ml)	Absorbance 540 nm
1	0.1	
2	0.2	
3	0.4	
4	0.6	
5	0.8	
6	1.0	
Use the Plot to determine the conc		

<p>entrations for the values below. Do not add the points below to your plot.</p>		
<p>7</p>	—	
<p>8</p>	—	

6. Plot each BSA dilution in a spreadsheet program like Excel as a scatterplot
7. Generate best-fit line for these standards with the equation of the line
8. Use the equation of the line to estimate the concentration of the unknown sample.

Curve Fitting

Run the simulation below to understand how you can use the standard dilution series to estimate your sample concentrations.



Click on image above to begin simulation on curve fitting

Scatterplot Tutorial

Use the tutorial below and watch at 1.25X to plot your own data.

Tags: [quantitative reasoning](#)