**BCH 333**

**Lab Sheet #3**

**Materials:**

**-Chemicals:**

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**-** **Glassware:**

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**-Instruments:**

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**Method:**

1. **Bradford method:**

A- Set up 9 centrifuge tubes and label them as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bradford reagent** | **Sample with Unknown Concentration**  | **Distilled** **Water** | **Bovine Serum Albumin(BSA)****(150µg/ml)** | **Tube** |
| 5 ml | - | 1 ml | - |  **(blank)** |
| 5 ml | - | 0.93 ml | 0.07 ml | **A** |
|  5 ml | - | 0.87 ml | 0.13 ml | **B** |
|  5 ml | - | 0.74 ml | 0.26 ml | **C** |
|  5 ml | - | 0.6 ml | 0.4 ml | **D** |
| 5 ml | - | 0.34 ml | 0.66 ml | **E** |
| 5 ml | - | - | 1 ml | **F** |
| 5 ml | 1 ml | - | - | **G 1 (sample)** |
| 5 ml | 1 ml | - | - | **G 2 (sample)** |

B- Mix and Incubate at room temperature for 5 min.

C- Measure the absorbance at 595 nm.

**Note: Bovine Serum Albumin(BSA)**

**Results:**

|  |  |  |
| --- | --- | --- |
| **Absorbance at 595 nm** | **Concentration****(µg/ml)** | **Tube** |
|  | 10.5 | **A** |
|  | 19.5 | **B** |
|  | ………… | **C** |
|  | 60 | **D** |
|  | 99 | **E** |
|  | 150 | **F** |
|  | =.................. | **G** |
|  | =.................. | **H** |

-Plot a standard curve of absorbance at 595 nm against BSA protein concentration (μg/ml).

-From the standard curve obtain the concentration of protein with the unknown concentration.

-Average protein concentration in tube [G 1 and G 2] =………………………………….……..………. (μg/ml).

-Which are the Tubes that considered as standard solutions ?…………………………………………………………………….…….

-From the table of additions what is the concentration of your stock standard BSA?

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-Why did you read the absorbance of the tubes at 595 nm and which type of cuvette did you use?

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-what is the concentration of C standard solution?

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1. **Warburg-Christian Method ( A280/ A260 Method):**

Read the absorbance of (protein sample A) sample, at 280nm then, read the same sample at 260nm, then fill the following:

A280= ……………………..……

A260= …………………………..

A280/ A260 ratio = ……………………..

Correction factor from the table= ……………………………

Unknown concentration of protein sample A = ………………………………......……………………………………mg/ml.

-Can you predict the percentage of the nucleic acid, that contaminate the "protein sample A"?

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**ANSWER THE FOLLOWING:**

1- Mention methods which depends on, the absorption properties of proteins molecules only in the solution?

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2-In this lab (Bradford method part), you did use (G 1 and G 2) tube to determine the concentration of the sample with unknown concentration, explain why did you duplicate the same sample?

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True or false (correct if the statement is false)

-Bicinchoninic acid method and biuret test are both quantitative tests and depend on reducing Cu+2. [ ]

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