**BCH 333**

***Spectrophotometric Methods for Determination Of Proteins***

**Method:**

1. **Bradford method:**

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration**  **(µg/ml)** | **Sample with unknown concentration** | **Distilled**  **Water** | **Bovine Serum Albumin(BSA)**  **(150µg/ml)** | **Tube** |
| ………… | - | 1 ml | - | **(blank)** |
| ………… | - | 0.93 ml | 0.07 ml | **A** |
| ………… | - | 0.87 ml | 0.13 ml | **B** |
| ………… | - | 0.74 ml | 0.26 ml | **C** |
| ………… | - | 0.6 ml | 0.4 ml | **D** |
| ………… | - | 0.34 ml | 0.66 ml | **E** |
| ………… | - | - | 1 ml | **F** |
| ………… | 1 ml | - | - | **G** |
| ………… | 1 ml | - | - | **H** |

B- Add 5ml of Bradford reagent to each tube [blank – H].

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

D- Measure the absorbance at 595 nm

**Results:**

|  |  |  |
| --- | --- | --- |
| **Absorbance at 595 nm** | **Concentration**  **(µg/ml)** | **Tube** |
|  |  | **A** |
|  |  | **B** |
|  |  | **C** |
|  |  | **D** |
|  |  | **E** |
|  |  | **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 and H] =………………………………….……..………. (μg/ml).

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

Read the absorbance of protein solution A, 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 sample A ?

……………………………………………………………………………………………………………………………………………………………………………………………………

