**Lab sheet #3**

**-Spectrophotometric Methods for Determination of Proteins Concentration-**

**-Objectives:**

* To determine the concentration of extracted protein by Bradford method.
* Using Warburg-Christian method to estimate the concentration for a protein sample.

**A. Bradford method:**

**-Method:**

1. Set up 9 tubes and label them as following:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bradford reagent (ml)** | **Sample with Unknown Concentration (ml)** | **Distilled**  **Water**  **(ml)** | **Bovine Serum Albumin (BSA)**  **(150µg/ml) (ml)** | **Tube** |
| 5 | - | 1 | - | **Blank** |
| - | 0.93 | 0.07 | **A** |
| - | 0.87 | 0.13 | **B** |
| - | 0.74 | 0.26 | **C** |
| - | 0.6 | 0.4 | **D** |
| - | 0.34 | 0.66 | **E** |
| - | - | 1 | **F** |
| 1 | - | - | **Dialyzed sample** |
|  | 0.5 | 0.5 | - |

1. Mix and incubate at room temperature for 5 min then record the absorbance at 595nm.

**-Results:**

|  |  |  |
| --- | --- | --- |
| **Absorbance at 595 nm** | **Concentration**  **(µg/ml)** | **Tube** |
|  |  | **A** |
|  |  | **B** |
|  |  | **C** |
|  |  | **D** |
|  |  | **E** |
|  |  | **F** |
|  |  | **Dialyzed sample** |

* Record the absorbance in the table.
* Calculate the concentration of the standard solutions (A-F) using:

C1 x V1 = C2 x V2 formula.

* Plot a standard curve of absorbance at 595nm against BSA protein concentration (μg/ml).
* From the standard curve obtain the protein concentration of dialyzed sample (using TREND formula).

**Related questions:**

1. Which are the tubes that considered as standard solutions?

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1. What is BSA?

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

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1. Did you dilute your sample? If yes, what is your dilution factor? Write down the calculation.

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

**-Method and results:**

1. Read the absorbance of (protein sample A) at 280nm, then read the same sample at 260nm against distal water blank.
2. Record your results:

* A280= \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* A260= \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* A280 /A260= \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Correction factor from the table= \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* **Unknown concentration of protein sample A:**

A280 x correction factor =\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ mg/ml protein.

OR

**Grove's formula:** Protein concentration= [1.55 X A280]-[0.76 X A260] = \_\_\_\_\_\_\_\_\_\_\_\_\_ mg/ml

Unknown concentration of protein sample A = \_\_\_\_\_\_\_\_\_\_\_\_\_ mg/ml

**Related questions:**

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

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1. Why this method considered as "direct" assay?

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