**Lab# 3 BCH 462**

**Extraction and Determination of Bacterial Proteins.**

**Experiment protocol**

**A] Extraction and Isolation of Bacterial Proteins:**

**You are provided with bacterial culture sample extract the total protein following the steps shown below:**

1.Centrifuge the sample 5 minutes at 3000 rpm at 4°C.

2. Resuspend the pellet in 1 ml lysis buffer.

3. Sonicate in ice bucket for 10 seconds or more if the cells are not completely disrupted.

4. Transfer the Resuspended sample to eppendorf tube, then spin 5 min at 13000 rpm 4°C.

5. Separate soluble proteins (supernatant) from insoluble proteins (pellet). Use supernatant (soluble proteins) for next step.

6. Resuspend the pellet in another 1 ml lysis buffer and use supernatant for next step. (Insoluble proteins).

**B] Determination of Total Bacterial Proteins Concentration:**

1. Set up 8 test tubes as following: blank, A-E [standard BSA solution] and F-G [soluble and insoluble protein samples]. Concentration of stock solution = 140 µg/µl.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test tube** | **Distilled water [µl]** | **Stock BSA solution 140 µg/µl .**  **[µl]** | **Sample [µl]** | **Protein concentration [µg/µl]** |
| **Blank** | 250 | - | - |  |
| **A** | 200 | 50 | - |  |
| **B** | 150 | 100 | - |  |
| **C** | 100 | 150 | - |  |
| **D** | 50 | 200 | - |  |
| **E** | - | 250 | - |  |
| **F** | - | - | 250 | ? |
| **G** | - | - | 250 | ? |

2. Incubate all the tubes in water bath at 37°C for five minutes.

3. To each tube, add 1000 µl of Biuret reagent. Mix well and allow standing for 20 minutes in the 37°C water bath.

4.Measure the absorbance of solutions at 540 nm.

5. Plot standard curve for absorbance against BSA concentration using results for solutions (A-E).

6. From the standard curve, estimate the concentration of proteins presents in your samples.

**Results:**

|  |  |  |
| --- | --- | --- |
| **Test tube** | **Protein concentration [µg/µl]** | **Absorbance at 540 nm** |
| **Blank** |  |  |
| **A** |  |  |
| **B** |  |  |
| **C** |  |  |
| **D** |  |  |
| **E** |  |  |
| **F** | ……………...? |  |
| **G** | ………..…….? |  |