**Bacterial Protein Isolation**

* Centrifugation the bacteria 10min 3000 rpm 4˚C
* Re suspend pellet in 1 ml PBS lysis solution
* place the tubes in ice bucket for 1 min. or more if the cells are not completely disrupted *( lysis is complete when the cloudy cell suspension become translucent)*
* Spin 7 min 1300 rpm 4˚C .
* Separate soluble proteins (supernatant) from insoluble proteins ( pellet).
* Use supernatant for the next step .
* *Keep sample of 40 µl of supernatant for PAGE – SDS and Western blot( soluble proteins)*
* *Re suspend pellet in another 1ml of lysis buffer , Keep sample of 40 µl . for PAGE – SDS and Western blot( unsoluble proteins)*

***Determination of Total Bacterial Proteins Concentration* by Biuret method:**

* Set up 8 test tube A-H, G is soluble sample and H is the insoluble sample.
* **( Concentration of the Stock solution = 140 m(µg / (µl).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test tube** | **Dis. H2O(µl)** | **BSA stock solution(µl)** | **Sample(µL)** | **Protein conc. (mg/ml)** |
| A( blank) | 250 | - | - | 0 |
| B | 200 | 50 | 28 |
| C | 150 | 100 | 56 |
| D | 100 | 150 | 84 |
| E | 50 | 200 | 112 |
| F | - | 250 | 140 |
| G | 200 | - | 50 | ? |
| H | 200 | - | 50 | ? |

* incubate all the tubes in water bath at 37 ˚C for ⑤ min.
* To each tube add 1000 µl of Biuret reaget, mix well and allow standing for10 min in the water bath
* Measure the absorbance of solutions at 540 nm (B-H) using A as blank.
* Plot the standard curve for absorbance against BSA concentration using results for solutions (B-H).
* From your standard curve, estimate the concentration of protein in your sample (G &H)