Experiment (3)

Extraction and Determination of Bacterial Proteins

Materials:

Chemical

LB medium, Distilled water, BSA stock solution (62.5 µg/ml), Bradford reagent and Lysis buffer

Preparation of lysis buffer

Containing the following: 140mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4 and 1.8mM KH_2PO_4

Equipment and Glassware

Microfuge centrifuge, electronic balance, water bath, spectrophotometer, microcentrifuge tube, centrifuge tube, Pasteur pipette, micropipette, tips, test tubes, plastic cuvettes.

Protocol:

A) Extraction and isolation of bacterial proteins:

1. Centrifuge the bacterial sample (6 ml of overnight culture) for 5 minutes at 3000 rpm at 4 °C.
2. Discard the supernatant and resuspend the pellet in 1 ml lysis buffer.
3. Sonicate for 30–60 s in ice bucket until the cells are completely disrupted.
4. Transfer the resuspended sample to microcentrifuge tube, then spin 5 min at 13000 rpm at 4°C.
5. Separate soluble proteins (supernatant) from insoluble proteins (pellet). Use supernatant (soluble proteins) for next step. **(Tube F)**
6. Resuspend the pellet in another 1 ml lysis buffer and use supernatant for next step. (Insoluble proteins). **(Tube G)**

B) Determination of total bacterial proteins concentration:

1. Set up 8 test tubes as following:

Tubes	d.H2O (µl)	Volumes from stock BSA (62.5 µg/ml)	Sample [µl]	Bradford reagent (µl)	Protein concentration [µg/ml]
Blank	200	0		800	
A	180	20			
B	150	50			
C	100	100			
D	50	150			

E	-	200			
F **(Unknown soluble proteins)**	-	-	200		?
F'	100	-	100		?
G **(Unknown insoluble proteins)**		-	200		?
G'	100	-	100		?

2. Mix and incubate for 5 min at room temperature, then measure the absorbance at 590 nm.
3. Plot standard curve for absorbance against BSA concentration using results for solutions (A-E).
4. From the standard curve, estimate the concentration of proteins presents in your samples.

Results:

Test tube	Protein concentration [µg/ml]	Absorbance at 590 nm
Blank		
A		
B		
C		
D		
E		
F	_____	
F'	_____	
G	_____	
G'	_____	