PROTOCOL: Proccesing of samples for SDS-PAGE

Processing of samples with B-PER reagent and urea

Material and chemicals:
B-PER reagent
Lysozyme (50 mg/ml)
DNase I (2500 U/ml)
8 M urea
Cell cultures

Preparation of solutions:
B-PER reagent mix
Mix 1 µL of lysozyme and 1 µL of DNase I per 1 ml of B-PER Reagent.

Workflow:

1. Collect samples at desired time of cultivation and dilute them to obtain OD_{600} = 1 in 2 ml. Centrifuge them for 10 minutes, 4000 g, at 4°C. Discard the liquid and work with the pellet.

2. Add 100 µl of B-PER reagent mix per pellet

3. Pipette the suspension up and down until it is homogeneous with plastic Pasteur pipette.

4. Gently shake and incubate 15 minutes at room temperature.

5. Centrifuge lysate at 14000 g for 30 minutes at 4°C to separate soluble proteins (cell-free extract) from the insoluble proteins. After centrifugation, manipulate with samples only ON ICE.

6. Collect the supernatant (CFE), save the pellet.

7. Add 80 µL of 8 M urea to the pellet.

8. Centrifuge for 4 minutes, 4°C, 13000 g.

9. Collect the supernatant, discard the pellet

Proccesing of samples with PMSF lysis buffer

Material and chemicals:
PMSF Lysis buffer

Preparation of solutions:
Lysis buffer**:**
Create a solution of dH_2O consisting of 1 M Tris, 2 M NaCl and 100 µM PMSF.

Workflow:

1. Collect samples at desired time of cultivation. Centrifuge them for 3 minutes, 6000 g. Discard the liquid and work with the pellet.
2. Add 100 µl of PMSF Lysis buffer and resuspend.
3. Leave for 10 minutes at room temperature.
4. Centrifuge for 10 minutes and 14000 g.
5. Separate supernatant and pellet.

Processing of samples with water

Workflow:

1. Collect samples at desired time of cultivation. Centrifuge them for 5 minutes, 13000 g. Discard the liquid and work with the pellet.
2. Wash the pellet twice with dH_2O.