Protein purification

E.coloi cells grown Over night - liquid culture. (Western and SDS)

Lysis

1. Transfer 2 mL from each culture sample into a 2 mL centrifuge tube and spin for 5 min at 13.000 rpm and 4°C.
2. Remove supernatant.
3. Re-suspend the pellets in 500 uL Lysis Buffer.
4. Sonicate each sample for ~10-15 sec, until solution is clear.
5. Spin down: 1500 rpm 4°C 5 minutes
6. Transfer to new 15 ml Falcon tubes
7. Ni-NTA beads are prepared:
    a. Spin them down
    b. Remove the ethanol
8. Resuspend beads in lysis buffer (just enough to cover them)
9. Take 70-100 µl and add to the falcon tubes
10. Incubate 1 hour in cold room turning table (continuous, slow invertions)
11. Spin down: 1000 rpm 4°C 5 minutes
12. Start purification protocol

Purification protocol

1. Remove supernatant. This is the “Flow through”. If for some reason your protein did not bind to the His-beads, you need to use this to re-purify or run it on gel to see if there is any protein there.

2. Add 1-2 ml of wash buffer. Resuspend by inversion (do not pipette)

3. Spin 1500 rpm 4°C 2 min

4. Remove supernatant with pipette. Keep this “wash” in case the wash buffer removes the protein (imidazole concentration).

5. Repeat wash step one more time. Remove and keep supernatant.

6. Add 200 µL of Elution buffer. Resuspend beads by mixing with the tip. Do not resuspend by pipetting.

7. Spin 1500 rpm 4°C 2 min

8. Take 100 µL of the supernatant and place it in an eppendorf. This is your Elution 1.

9. Add 100 µL of Elution buffer and mix with the tip.

10. Spin 1500 rpm 4°C 2 min.

11. Take 100 µL of the supernatant. This is your Elution 2.

12. Repeat for a 3rd/4th elution if necessary (depends on the amount of protein)

13. On your last elution take all 200 µL.

14. Run 8 µL of each elution + 2 µL loading dye on a TGX Stain-free gel (12%) from Biorad.

15. Visualise the bands using the Gel doc from Biorad.

Buffers

Lysis buffer (20 mL)

- 25 µL Imidazole (4M) at 25 mM
- 2,0 mL NaCl (5M) at 0,5 M
- 800 µL Tris-HCl (0,5M) at 20 mM pH 7,9

- Fill with MQ H_2O

Wash buffer (20 mL)

- 125 µL Imidazole (4M) at 25 mM
- 2,0 mL NaCl (5M) at 0,5 M
- 800 µL Tris-HCl (0,5M) at 20 mM pH 7,9
- Fill with MQ H_2O

Elution buffers (250 mM Imidazole) 5ml

- 312 µL Imidazole (4M) at 250 mM
- 0,5 ml NaCl (5M) at 0,5 M
- 0,2 mL Tris-HCl (0,5M) at 20 mM pH 7,9
- Fill with MQ H_2O

Elution buffer (500 mM Imidazole) 5mL

- 625 µL Imidazole (4M) at 500 mM
- 0,5 ml NaCl (5M) at 0,5 M
- 0,2 mL Tris-HCl (0,5M) at 20 mM pH 7,9
- Fill with MQ H_2O

Elution buffer (1000 mM Imidazole) 5mL

- 1,25 mL Imidazole (4M) at 1000 mM
- 0,5 ml NaCl (5M) at 0,5 M
- 0,2 mL Tris-HCl (0,5M) at 20 mM pH 7,9
- Fill with MQ H_2O

Elution buffer (2000 mM Imidazole) 5mL

- 2,5 mLImidazole (4M) at 2000 mM
- 0,5 ml NaCl (5M) at 0,5 M
- 0,2 mL Tris-HCl (0,5M) at 20 mM pH 7,9
- Fill with MQ H_2O