eGFP Purification

Buffer: 1xPBS (137mM NaCl, 2.7mM KCl, 8mM Na2HPO4•7H2O, 1.5mM KH2PO4, pH 7.45)

1. eGFP Expression

1. Select monoclonal colonies from eGFP-28a BL21 plate, inoculate into 1L LB K+C+ medium and cultivate in 37°C, 220rpm overnight.

2. Bacterial centrifugation

Centrifugate bacteria solution at 4°C 4000rpm for 15 min. Discard the supernatant and resuspend the cells with 20ml 1x PBS.

3. Bacteria lysis with high pressure, 4°C.

4. Protein centrifugation

Centrifugate the bacteria lysate at 4°C 15,000rpm for 30 min. The eGFP protein exist in the supernatant of the bacteria lysate. Collect the supernatant as primary sample.

5. Ni Column affinity chromatography

Equilibrate the Ni column with 1x PBS buffer. Place the sample through Ni column to make sure the target protein be attached to the column. Edulcorate impurity protein with 10mM imidazole(PBS) until the OD280 of washout solution lower than 0.02. Elute target protein with 200mM imidazole(PBS) until the OD280 of washout solution lower than 0.02 to collect eGFP.

6. Protein concentration

Concentrate eGFP with ultrafiltration concentration centrifuge tube (10 kD).

7. Molecular sieve chromatography

Equilibration: 1x PBS buffer

Flow rate: 1ml/min

Remain time: 120 min

Pressure: 0.5

Collect all tubes of elution protein at the purification peak.

8. Protein verification

Purified protein SDS-PAGE at 280v, 36min. Verify the purification of eGFP with a single 29kDa band on the gel.