Protocol for Intracellular protein extraction from E.coli BL21 strain.
Protocol Code: ExEc

Materials

- Lysis Buffer:
  40 mM Tris pH 8
  100 mM NaCl
- PMSF
- DNase
- MgCl2
- Lysis buffer + 7M Urea
- Tube rotator
- Centrifuge

Procedure

For every pellet of 50 mL:

1. Resuspend every pellet in 5 mL of lysis buffer. The lysis buffer needs to be close to 0°C.
2. Join resuspended pellets to a final volume of 20 mL.
3. Add 200 uL of PMSF, 1 uL of DNase, 400 uL of MgCl2 and 1,4 uL of β-mercaptoethanol. Incubate for 30 min in a tube rotator.
4. Centrifuge at 12 000 rpm for 20 min.
5. Name the supernatant as “soluble intracellular proteins”.
6. Resuspend the pellet with lysis buffer + 7M Urea and name it as “insoluble intracellular proteins”.