Recombinant Protein Solubility Analysis

Materials and Equipment

Microtube rack
15 ml conical tubes
Micropipette
Micropipette tips
Microtubes (0.6 ml)
Laminar flow hood
Shaker
Centrifuge
Polytron

Reagents

50 ml liquid LB medium with proper antibiotic (KAN 15 mg/ml, CAM 35 mg/ml, AMP 100 mg/ml)
IPTG 1 M
300 mM KCl, 20 mM Imid 20 mM KH_2PO_4 and 10 mM KCl (pH 8) solution
Laemmli buffer

Methodology

1. Centrifuge 40 mL of medium induced with IPTG, 14000 rpm, 10 min. The medium is divided in 4 tubes of 10 mL.
2. Resuspend the pellet in 4 mL of a cold 20 mM KH_2PO_4 and 10 mM KCl (pH 8) solution (1 mL to each tube).
3. Lyse cells with Polytron. Three cycles of 30 seconds at 12,000 rpm, with intervals of 1 min. Always keep on ice.
4. Centrifuge cell lysate at 14,000 rpm for 10 minutes. If the supernatant is not crystalline, the sample has to be centrifuged again.
5. Take 20 µL of the supernatant and mix with 20 µL of Laemmli buffer.
6. The insoluble phase obtained from previous centrifugation has to be washed twice with a 1% SDS solution and 20 mM KH_2PO_4 and 10 mM KCl (pH 8) solution. Then, mix insoluble phase with 20 µL Laemmli buffer.
7. SDS PAGE analysis of both samples (soluble and insoluble phases).

*Do not cease to contemplate that all samples should be perfectly mixed with the indicated reagents (Laemmli Buffer) before they are boiled for 5 minutes. In case of inclusion bodies, the boiling should be carried out for 10 minutes.