Osmotic Shock

Materials

Samples and Reagents

Induced *E. coli* cultures
Wash Buffer: 10 mM Tris-HCl, pH 8.0
Hypertonic Buffer: 30 mM Tris-HCl, 20% sucrose, pH 8.0
500 mM EDTA
Osmotic Shock Buffer: 5 mM $MgSO_4$, pre-cooled
Spheroplast Lysis Buffer: 100 mM Tris-HCl, pH 8.0
100% (w/v) trichloroacetic acid (TCA)
Acetone, pre-cooled to −20 °C
SDS loading buffer
Liquid nitrogen
Deionized water

Equipment

Spectrophotometer
Refrigerated centrifuge
Microcentrifuge
Ice bath
37 °C water bath
Vortex mixer
Air-drying or vacuum-drying equipment
Centrifuge tubes and microcentrifuge tubes

Procedure

Biomass Normalization and Cell Collection

1. Induce recombinant protein expression under the designated experimental conditions.
2. Measure the OD_{600} of each culture after induction.
3. Normalize the amount of culture collected according to:
   $$\text{Cellunits} = OD_{600} \times \text{culturevolume}$$
   Use the same number of cell units for all experimental groups.
4. Centrifuge the normalized cultures at 5,000 × g for 15 min at 4 °C.
5. Discard the culture supernatant and retain the cell pellet.

Cell Washing

1. Resuspend each cell pellet in 4 mL of ice-cold Wash Buffer.
2. Centrifuge at 5,000 × g at 4 °C and discard the supernatant.
3. Repeat the washing step twice, for a total of three washes.

Hypertonic Treatment

1. Resuspend each washed cell pellet in 7.5 mL of Hypertonic Buffer.
2. Add 15 µL of 500 mM EDTA to obtain a final EDTA concentration of approximately 1 mM.
3. Mix gently and incubate at room temperature for 10 min.
4. Centrifuge at 10,000 × g for 10 min at 4 °C.
5. Carefully discard the supernatant and retain the cell pellet.

Osmotic Shock and Collection of the Periplasmic Fraction

1. Add 7.5 mL of pre-cooled Osmotic Shock Buffer to each pellet and gently resuspend the cells.
2. Incubate the suspension on ice for 10 min with occasional gentle mixing.
3. Centrifuge at 10,000 × g for 10 min at 4 °C.
4. Carefully collect the supernatant as the periplasmic fraction. Retain the pellet for preparation of the cytoplasmic and insoluble fractions.

TCA Precipitation of the Periplasmic Fraction

1. Transfer equal volumes of the periplasmic fractions from each experimental group into clean centrifuge tubes.
2. Add one volume of 100% TCA to four volumes of periplasmic sample to obtain a final TCA concentration of 20%. For example, add 1.875 mL of 100% TCA to 7.5 mL of sample.
3. Mix gently and incubate on ice for 30–60 min.
4. Centrifuge at 15,340 × g for 45 min at 4 °C.
5. Carefully remove the supernatant without disturbing the protein pellet. Briefly centrifuge the tube again and remove the remaining liquid.
6. Add a volume of −20 °C acetone equal to the original sample volume.
7. Centrifuge at 15,340 × g for 5 min at 4 °C and carefully discard the acetone.
8. Air-dry or vacuum-dry the protein pellet.
9. Resuspend the TCA-precipitated pellets from all samples in the same volume of SDS loading buffer, typically 10–20 µL.

Preparation of Cytoplasmic and Insoluble Fractions

1. Resuspend the pellet retained after osmotic shock in 1 mL of Spheroplast Lysis Buffer.
2. Freeze the suspension in liquid nitrogen and thaw it directly in a 37 °C water bath.
3. Repeat the freeze–thaw treatment twice, for a total of three cycles.
4. Centrifuge at maximum microcentrifuge speed for 5 min at 4 °C.
5. Collect the supernatant as the cytoplasmic soluble fraction.
6. Retain the pellet as the insoluble and membrane-associated fraction.
7. Resuspend the pellet in 100 µL of Spheroplast Lysis Buffer by vortexing.

Sample Normalization for SDS-PAGE or Western Blotting

1. Prepare all compared samples from the same number of starting cell units.
2. Use identical extraction volumes, TCA concentration factors, and final resuspension volumes for corresponding samples.
3. Add SDS loading buffer to the cytoplasmic soluble and insoluble fractions using the same sample-to-buffer ratio.
4. Load samples representing the same proportion of the original normalized biomass. When comparing the same type of fraction among different groups, load equal volumes prepared using identical dilution and concentration factors.