ProteoSep

Standard ProteoSep Solubilization Protocols: Protein Extraction from Cells and Tissues

Standard Cytosolic Protein Solubilization (CPS) Buffer Contents:

7.5M urea
2.5M thiourea
12.5% glycerol
50mM Tris
2.5% n-octylglucoside
1.25mM protease inhibitor (optional)

12.5% Glycerol preparation (50 mL)

1. **Measure 6.25 mL Glycerol (Sigma, G6279) into a 50 mL volumetric flask.**

2. **Add distilled water to volume.**

CPS buffer preparation (50 mL)

1. **Weigh the following into a 100 mL beaker:**

   i) **22.52 grams urea (Sigma, U0631),**
   ii) **9.52 grams thiourea (Sigma, T7875),**
   iii) **0.304 grams Tris (Fisher, BP154-1, molecular Biology grade), grams n-octlyglucoside (Sigma, O8001),**
   iv) **1.89 mL protease inhibitor solution [Sigma P2714 vial contents dissolved in 10 mL DI water then diluted to 100 mL total volume with DI water.] (optional)**

2. **Add 20 mL of 12.5% glycerol into the beaker. Stir thoroughly until all dissolved.**

3. **Transfer all solution to a 50 mL flask.**

4. **Add 12.5% glycerol to volume.**

5. **Thoroughly mix and dispense into separate 2.0 mL vials. Store at –20°C.**

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Cell Lysis Procedure

1. **Mix ~0.5 mL of a thoroughly washed cell pellet (or ~ 10^8 cells) in 2.0 mL of CPS buffer and vortex vigorously (see Note below).**

   The CPS buffer will also extract many of the more soluble Membrane proteins as well. If you wish to co-extract all Cytosolic and Membrane proteins, use the Membrane Protein Solubilization buffer in Step 1 instead of CPS Buffer.

2. **Centrifuge the lysate at >20,000g (>15,000 rpm) for 60 minutes.**

3. **Remove the supernatant from the cell debris (save cell debris). Store at –20°C before use or proceed to the desalting step with a PD-10 column for a PF2D run with a ProteoSep Kit.**

4. **Proceed to Membrane Protein Solubilization in the cell debris.**

Standard Membrane Protein Solubilization (MPS) Buffer Contents:

5M urea
2M thiourea
10% glycerol
2.5% SB3-10 (N-decyl-N,N-dimethyl-3-ammonio-1-propane sulfonate)
50mM Tris
2.0% n-octylglucoside
1.0mM protease inhibitor (optional)

12.5% Glycerol preparation (50 mL)

1. **Measure 6.25 mL Glycerol (Sigma, G6279) into a 50 mL volumetric flask.**

2. **Add distilled water to volume.**

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MPS buffer preparation (50 mL)

1. **Weigh the following into a 100 mL beaker:**

   15.01 grams urea (Sigma, U0631),
   7.61 grams thiourea (Sigma, T7875),
   0.24 grams Tris (Fisher, BP154-1, molecular Biology grade),
   1.0 grams SB3-10,
   1.0 grams n-octylglucoside
   3.0 mL protease inhibitor solution (optional)

2. **Add 20 mL of 12.5% glycerol into the beaker. Stir thoroughly until all dissolved.**

3. **Transfer all solution to a 50 mL flask.**

4. **Add 12.5% glycerol to volume.**

5. **Thoroughly mix and dispense into separate 2.0 mL vials. Store at –20°C.**

MPS Procedure

1. **Suspend cell debris in 2.5ml of above MPS buffer. Vortex rigorously**

2. **Centrifuge the solution at >20,000g for 30 minutes.**

3. **Remove the supernatant and store at –20ºC before use or proceed to the desalting step with a PD-10 column for a PF2D run with a ProteoSep Kit.**

Note: For difficult to lyse samples like embryo's, certain tissue samples or spores you may need a more stringent higher salt content lysis protocol.

In this case homogenize the sample briefly with a pipette, and sonicate using a Cell Disruptor B15 and 3 mm tip on ice with continuous pulses for 3-4x10 seconds in 2.5 mL of a lysis buffer consisting of:

20mM HEPES, pH 7.85,
30 mM NaCl,
10% glycerol,
2 mM mM EDTA,
1.0 mM protease inhibitor (Optional)

Add 2.5 mL of either CPS or MPS and proceed to do 2 x PD-10 exchange (2.5 mL each) and combine the effluents with no further dilution. Use 5.0 mL (from the 7.0 mL total) for the ProteoSep run.

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