

ProteoSep

Standard ProteoSep Solubilization Protocol: Protein Extraction from Cells and Tissues

Cytosolic Protein Solubilization (CPS) Buffer Contents:

7.5M urea
2.5M thiourea
12.5% glycerol
50mM Tris
2.5% n-octylglucoside
1.25mM of protease inhibitor in water solution.

12.5% Glycerol preparation (50 cc)

1. Measure 6.25 cc Glycerol (Sigma, G6279) into a 50 mL volumetric flask.
2. Add distilled water to volume.

CPS buffer preparation (50 cc)

1. Weigh the following into a 100 mL beaker:
22.52 grams urea (Sigma, U0631),
9.52 grams thiourea (Sigma, T7875),
0.304 grams Tris (Fisher, BP154-1, molecular Biology grade),
1.25 grams n-octylglucoside (Sigma, O8001),
1.89 cc protease inhibitor (Sigma, P2714).
2. Add 20 cc of 12.5% glycerol into the beaker. Stir thoroughly until all dissolved.
3. Transfer all solution to a 50 cc 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 cc 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.

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

12.5% Glycerol preparation (50 cc)

1. Measure 6.25 cc Glycerol (Sigma, G6279) into a 50 mL volumetric flask.
2. Add distilled water to volume.

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MPS buffer preparation (50 cc)

1. Weigh the following into a 100 mL beaker:

21.03 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 (Sigma, P2714).
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 .

Membrane Protein Solubilization 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 -20°C 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 cc of a lysis buffer consisting of:

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

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

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