Cytoskeletal Fractioning of Tissue

Reference

1. Afreen, S., & Ferreira, A. (2019). Altered Cytoskeletal Composition and Delayed Neurite Elongation in tau$_{45-230}$-Expressing Hippocampal Neurons. Neuroscience, 412, 1–15. https://doi.org/10.1016/j.neuroscience.2019.05.046

Solutions

1. RIPA Homogenization Buffer: 500 mM Tris–HCl pH 7.4, 1.5 M NaCl, 2.5% Deoxycholic Acid, 10 mM EDTA, 1 mM Phenylmethylsulfonyl fluoride (PMSF), 1mM Aminobenzamide
2. RIPA Lysis Buffer: 500 mM Tris–HCl pH 7.4, 1.5 M NaCl, 2.5% Deoxycholic Acid, 10 mM EDTA, 1 mM Phenylmethylsulfonyl fluoride (PMSF), 1 mM Aminobenzamide, 1% Triton X-100
3. Tris-Urea Buffer: 1 M Tris–HCL, pH 8.5, 7 M Urea, 2 M Thiourea

Protocol

Tissue Homogenization

1. Obtain previously collected frozen brain tissue.
2. Homogenize in chilled RIPA homogenization Buffer.
3. Centrifuge homogenate at 20,000 x g for 20 minutes at 4°C to remove cellular debris.
4. Collect supernatant.
5. Obtain protein concentration readings.

Cytoskeletal Fractioning

1. Combine lysate from homogenization with 1:1 RIPA Lysis Buffer.
2. Centrifuge lysate at 100,000 x g for 1 hour at 4°C.
3. Collect supernatant (RIPA buffer soluble proteins).
4. Sonicate pellets (Insoluble proteins - Cytoskeleton).
5. Centrifuge at 100,000 x g for 30 minutes at 4°C.
6. Dissolve pellets in Urea Buffer.
7. Sonicate.
8. Dilute 1:1 in 2X Laemmli Buffer and boil for 10 minutes prior to use.
9. Analyze by Western Blot.