

Sarkosyl Fractionation Assay

Human Tissue

References

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Solutions

1. Tris-Buffered Saline (TBS) with Protease Inhibitors (Store at -20°C): 25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 5 mM Sodium pyrophosphate, 1% Protease Inhibitor Cocktail
2. Salt/Sucrose Buffer (Store at -20°C): 10 mM Tris-HCl, pH 7.4, 800 mM NaCl, 1 mM EGTA, 1% Protease Inhibitor Cocktail, 10% (w/v) Sucrose

Protocol

1. Weigh ~100 mg of brain tissue.
2. Homogenize in 300 µL of ice-cold TBS with protease inhibitors.
3. Save some of this (~20 µL) as total protein – brain homogenate.
4. Centrifuge for 15 minutes in Airfuge at 30 PSI (160,000 x g).
5. Supernatant contains soluble proteins.
6. Re-homogenize pellet in 300 µL Salt/Sucrose Buffer.
7. Centrifuge again for 15 minutes at 160,000 x g.
8. Incubate supernatant for 1.5 hours with rocking at room temperature in 1% sarkosyl.
9. Centrifuge for 30 minutes at 150,000 x g and re-suspend pellet in 30 µL TBS.
10. Supernatant is sarkosyl soluble fraction – final volume should be adjusted up to 300 µL.
11. Dilute 1:1 in 2X Laemmli Buffer, Boil for 10 minutes, and run SDS-Page.