Obtaining Membrane-rich Protein Fractions

Reference

1. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. Neuroscience, 275, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017

Solutions

1. Homogenizing Tris-EDTA Buffer: 10 mM Tris-HCL, 5 mM EDTA pH 7.4, 320 mM Sucrose, 0.1 mM Na_3VO_4, 1 mM NaF
2. Pellet Resuspension Buffer: 10 mM Tris-HCL pH 7.4, 0.1 mM Na_3VO_4, 1 mM NaF

Protocol

1. Homogenize hippocampus in ice-cold Homogenizing Tris-EDTA Buffer.
2. Centrifuge at 700 x g for 10 minutes at 4°C.
3. Remove supernatant and centrifuge supernatant at 37,000 x g for 40 minutes at 4°C .
4. Resuspend pellet in 10 mM Tris-HCL pH 7.4 containing enzyme inhibitor mixture.
5. Obtain protein concentration and store aliquots in -80°C until needed.

For Western Blot

1. Add 0.1 volume of 10% sodium deoxycholate in 500 mM Tris – HCl pH 9.0
2. Incubate for 30 minutes at 36°C .
3. Add 0.1 volume of 500 mM Tris – HCl pH 9.0 with 1% Triton X.
4. Centrifuge at 37,000 x g for 10 minutes at 4°C .
5. Add equal volume of 2X Laemmli Buffer to supernatant.
6. Boil for 10 minutes.
7. Store in -20°C until needed.