Subcellular Fractionation

References

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2. Nicholson, A. M., & Ferreira, A. (2009). Increased membrane cholesterol might render mature hippocampal neurons more susceptible to beta-amyloid-induced calpain activation and tau toxicity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(14), 4640–4651. https://doi.org/10.1523/JNEUROSCI.0862-09.2009
3. Kelly, B. L., & Ferreira, A. (2007). Beta-amyloid disrupted synaptic vesicle endocytosis in cultured hippocampal neurons. *Neuroscience*, *147*(1), 60–70. https://doi.org/10.1016/j.neuroscience.2007.03.047

Solution

1. Fractionation buffer: 250 mM Sucrose, 1 mM $MgCl_2$, 2 mM EGTA, 25 mM Hepes, pH 7.4

Abbreviations

1. PBS: Phosphate Buffered Saline
2. EDTA: Ethylenediaminetetraacetic acid
3. TCA: Trichloroacetic Acid

Protocol

1. Scrape cells in 250 µL of PBS with 5 mM EDTA.
   Note: you may need to combine 2 dishes to get enough protein and save some sample to have as whole cell lysate.
2. Spin down for 10 min @ 5,000 rpm (in the cold room) and resuspend in 250 µL of fractionation buffer.
3. Rapidly lyse cells in liquid nitrogen and slowly thaw in a water bath at room temperature.
4. Spin for 30 min @ 100,000 x g.
5. Save the supernatant (“cytosolic fraction”) and resuspend the pellet (“membrane fraction”) in 250 µL of fractionation buffer.
6. Add Triton X-100 to each fraction to solubilize at final concentration of 0.5%.
7. Add 50-100 µL of 2X Laemmli buffer and boil for 5 minutes.
   Note: if you need to concentrate the cytosolic and membrane fractions, you can precipitate them in 10% TCA and resuspend the pellets in 2X Laemmli buffer containing 2% sodium carbonate.