Membrane/Organelle Fractioning

References

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3. Nicholson, A. M., Wold, L. A., Walsh, D. M., & Ferreira, A. (2012). β-Amyloid carrying the Dutch mutation has diverse effects on calpain-mediated toxicity in hippocampal neurons. *Molecular medicine (Cambridge, Mass.)*, *18*(1), 178–185. https://doi.org/10.2119/molmed.2011.00366

Solutions

1. Microtubule Stabilizing Buffer (MTSB): 130 mM HEPES, 4 mM $MgCl_2$,10 mM EGTA,pH 6.9
2. Membrane/Organelle Fractioning Buffer: Microtubule Stabilizing Buffer + 0.02% Saponin

Abbreviations

1. MTSB: Microtubule Stabilizing Buffer
2. PBS: Phosphate Buffered Saline

Protocol

1. Before beginning, warm 10 ml of MTSB in 37°C in water bath.
2. Discard N2 media and wash once in PBS, remove and discard.
3. Add ~1 ml of detergent-free MTSB buffer for 30 seconds to stabilize, remove and discard.
4. Add ~1 ml of MTSB containing Saponin for 1 min to extract.
5. Rinse carefully in ~1 ml of detergent-free MTSB for 30 seconds, remove and discard.
6. Harvest fraction in 2X Laemmli buffer for Western blotting.
7. Boil samples for 10 minutes and store at -20°C.