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Method and validation of synaptosomal preparation for isolation of synaptic membrane proteins from rat brain

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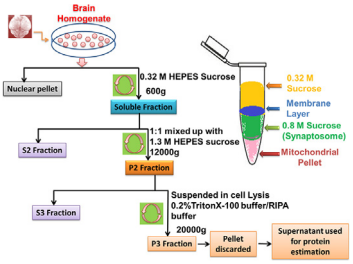
GRAPHICAL ABSTRACT

The method depicts the modified method of synapse protein preparation. The flow diagram depicts the step-by-step synapse protein preparation by differential centrifugation method. Tissues isolated from rat brain were used for synapse preparation. However, details of procedure used for synapse preparation have been described in methodology section.



ABSTRACT

The ability to isolate and observe molecular changes in protein composition and function at synapses is important in understanding the disease mechanisms. Because signal transmission is highly regulated by transient phosphorylation of neuronal proteins at the synapse, preservation of this protein modification during synaptosome preparation is essential. Therefore, enriched preparations of synaptic particles called synaptosome are necessary to study synapse function. Because of insufficiency of ample sample for quantitative and qualitative analysis via old method, we applied some modifications that were resultant in high synapse yield. Interestingly, we found that modified methods produced more protein as well as more clear protein band on electrophoresis. Therefore, the modified procedure was better than the older method in effort to isolate more pure synapse protein for improved result outcome.

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To advance the method for our study, the following modifications were made to the regularly used protocols:

- The pellet consisting of synaptosomes was cleaned two to three times in HEPES buffer containing proteases inhibitor and centrifuged at 12,000 × *g* for 15 min each. This step is highly essential to remove any contamination of sucrose-HEPES buffer and other organelle's which interfere with protein purification analysis.
- Following this step, the synaptosome pellets were suspended in RIPA buffer (mixed with protease inhibitor and PMSF) along with 0.2% TritonX-100 and further centrifuged at 20,000 × *g*.
- Further, the resulting pellet was discarded and suspended in RIPA buffer (mixed with protease inhibitor and PMSF) only. The sample was immediately used for protein estimation and protein electrophoresis.

ARTICLE INFO
Method name: Enriched synapse protein preparation
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Method details

Material required

1. Sterile surgical instrument to harvest brain.
2. Syringe pump for cardiac perfusion to make brain free from blood contamination.
3. Chilled normal saline.
4. Labeled sterile petri-dishes kept on ice cube bucket.
5. HEPES buffer.
6. Ultrapure water or autoclaved water.
7. Autoclaved or sterile Eppendorf tube.
8. Centrifuge machine.
9. Protease inhibitor cocktail, RIPA buffer and PMSF.
10. Sodium phosphate buffer for washing of brain sample.
11. Ether anesthesia used to make animal anesthetized.

Reagent preparation

Normal saline

Normal saline was prepared by dissolving 0.9% of sodium chloride (NaCl), i.e. 9 g of NaCl in 100 ml of distilled water.

Sodium phosphate buffer

To prepare the 0.1 M sodium phosphate buffer, 0.2 M of NaOH was added to 250 ml of 0.02 M $NaH_2PO_4{\cdot}2H_2O$ to adjust pH to 8.0 and volume made up to 500 ml with distilled water (DW). To prepare the sodium phosphate buffer of 0.03 M, pH 7.0. 150 ml of 0.1 M sodium phosphate buffer (pH 8.0) was taken and pH was adjusted to 7.0 and volume made up to 500 ml with DW.

HEPES buffers

HEPES-buffer solution: 145 mM NaCl (8.41 g), 5 mM KCl (37.2 mg), 2 mM $CaCl_2$ (22.1 mg), 1 mM $MgCl_2$ (9.51 mg), 5 mM glucose (9.0 mg), 5 mM HEPES (1.19 g), pH 7.4 dissolved in DW, volume made up to 1000 ml.