NeuroMab

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Rat Brain Fractionation

1) Weigh two adult rat brains, which should total approximately 4 to 5 grams.

2) Place in 40 mL of ice-cold homogenization buffer (recipe below) in a 50 mL Potter-Elvehjem Tissue Grinder.

3) Homogenize on ice using 10 strokes of the Potter-Elvehjem Tissue Grinder attached to a 3/8" variable speed drill at 2,500 rpm.

4) Centrifuge homogenate in Sorvall SS-34 rotor or equivalent at 750 X g at 4°C for 10 min.

5) Collect the supernatant and save, this has brain membranes as crude synaptosomes.

6) Recover additional membranes from pellet by scraping off the top lightly colored layer and avoiding disturbance of the deeper layer of red blood cells. Save pellet as "low speed pellet".

7) Rehomogenize pellet as above using 40 mL of buffer, but using only 8 homogenization strokes.

8) Centrifuge this homogenate in Sorvall SS-34 rotor or equivalent at 750 X g at 4°C for 10 min.

9) Collect supernatant and combine with supernatant from first homogenization.

10) Centrifuge pooled supernatants in Sorvall SS-34 rotor or equivalent at 40,000 X g at 4°C for 90 min (or in ultracentrifuge at 100,000 X g for one hour).

11) Save pellets, which contain crude membrane fractions, and resuspend in 2.5 mL for each original gram of brain used.

12) Rehomogenize using hand-held Dounce glass-glass homogenizer; overall yield is approximately 100 mgs total protein, at a concentration of 10 to 15 mg/mL, as determined by protein assay.

Protocol can be modified to accommodate brains from any mammalian species by proportionally adjusting the weight/volume ratio.

Homogenization Buffer:
0.32 M sucrose
5 mM Na Phosphate buffer pH 7.4
100 mM Na Fluoride
Add fresh just before homogenization: 2 µg/mL aprotinin, 1 µg/mL leupeptin, 2 µg/mL antipain, 10 µg/mL benzamidine and 0.5 mM PMSF

Reference: Trimmer 1991 PNAS (http://www.ncbi.nlm.nih.gov/pubmed/1961744)

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