



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 benzamidin and 0.5 mM PMSF

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

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