



Rat Brain Fractionation

- 1) Weigh two adult rat brains, should be \approx 4-5 grams total.
- 2) Place in 40 ml of ice-cold homogenization buffer (0.32 M sucrose, 5 mM Na Phosphate buffer pH 7.4, 0.1 M Na fluoride, with fresh addition of 2 μ g/ml aprotinin, 1 μ g/ml leupeptin, 2 μ g/ml antipain, 10 μ g/ml benzamidine and 0.5 mM PMSF) in a 50 ml Potter-Elvehjem Tissue Grinders
- 3) Homogenize on ice using 10 strokes of a Teflon-glass homogenizer 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, 4°C X 10 min.
- 5) Collect the supernatant and save (this has brain membranes = crude synaptosomes)
- 6) Recover additional membranes from pellet by scraping off top, lightly colored layer (do not disturb deeper layer of red blood cells). Save pellet as "low speed pellet" or LSP.
- 7) Rehomogenize pellet using 40 ml of buffer, this time use only 8 strokes.
- 8) Centrifuge this homogenate in Sorvall SS-34 rotor or equivalent at 750 X g, 4°C X 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, 4°C for 90 min (or ultracentrifuge at 100,000 X g for one hour if available).
- 11) Save pellets, these are the crude membrane fraction. Resuspend in 2.5 ml for every gram of brain used.
- 12) Rehomogenize using hand held Dounce glass-glass homogenizer. Should yield \approx 10-15 mg protein/ml.

Can modify protocol to brains from any mammalian species by proportionally adjusting volumes.