Synaptosome and synaptosome fractionation

Rachel Jeffrey – adapted from Carlin RK, Grab DJ, Cohen RS, Siekevitz P (1980) Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. J Cell Biol 86:831-843 and MB Kennedy protocol (see any paper) and Mike Ehlers lab protocol (see Ehlerslab.org)

Preparations for the day before

Solution A (1X)	Final conc.	For 100 mL
Sucrose	0.32 M	10.944 g
$NaHCO_3$ (0.5 M)	1 mM	200 uL
$MgCl_2$ (1M)	1 mM	100 uL
$CaCl_2 \cdot 2H_2O$	0.5 mM	7.351 mg
Na pyrophosphate (FW: 265.9)	10 mM	265.9 mg
Nanopure water		
Protease inhibitors (add right before use)		2 tablets

Solution B (1X)	Final conc.	For 50 mL	For 100 mL
Sucrose	0.32 M	5.472 g	10.944 g
$NaHCO_3$ (0.5 M)	1 mM	100 uL	200 uL
Nanopure water			
Protease inhibitors (add right before use)		1 tablet	

For gradient:

Concentration	1.2 M	1.0 M	0.85 M
Sucrose	8.208 g	6.844 g	5.836 g
1 mM $NaHCO_3$	To 20 mL		

Note: sucrose adds a significant volume to the solution.

For PSD preps, also make 0.5% Triton X with Solution B

Note: Keep everything on ice, pre-cool reagents, add protease and phosphatase inhibitors to everything!

1. Rinse and dissect brains in solution A on ice
2. Homogenize with homogenizer using 12 up and down strokes. Use 20 mL solution A for 5 g brain tissue. One mouse forebrain is about 300 mg.
3. Combine homogenates and dilute to 10% weight/volume in solution A (about double of the original volume of solution A).
4. Centrifuge at low speed 710 g in clinical centrifuge to get soft pellet for 10 minutes (in 15 mL falcon tubes)
5. Resuspend pellent in the solution A supernatant using 3 up and down strokes of Teflon glass homogenizer.
6. Centrifuge at 1400 g for 10 minutes to remove nuclei.
7. Pool supernatants into 30 mL reusable centrifuge tubes and centrifuge 13,800 g for 10 minutes in floor centrifuge.
8. Resuspend pellet (containing synaptosomes and mitochondria) with six strokes of homogenizer in solution B (0.32 M sucrose, 1 mM $NaHCO_3$) using 16 mL solution B per 5 g starting material
9. Layer 8 mL of homogenized pellet on discontinuous sucrose gradient (10 mL in each layer of 1.2 M, 1.0 M, 0.85 M sucrose). Use 25 x 89 mm Beckman centrifuge tubes (reorder number 344058).
10. Spin in ultracentrifuge for 2 hours at 82,500 g (25,000 rpm for the SW28) at 4 ^{o}C. Set acceleration and brake to slow setting.
11. Aspirate top layers carefully with vacuum. Synaptosomes will be in layer between 1.0 M and 1.2 M; should get about ~3-5 mL.

To continue on to make PSDs:

1. Incubate synaptosomes in ice cold 0.5% Triton X in solution B for 15 minutes shaking on ice.
2. Centrifuge 35,000g in Sorvall for 20 min (PSD 1-Triton pellet).
3. Incubate this pellet again in 5 mL Triton X solution for 15 min on ice.
4. Centrifuge 201,800g for 1hr to obtain PSD (two-Triton pellet) in SW41 rotor.
5. For protein extraction, solublize pellet by drying completely, then add SDS up to final concentration of 3%. Sonicate pellet if necessary for 5-10 min. Boil in Sample buffer.