Synaptic Plasma Membrane Fraction (From Dr. Melissa Snyder)

Perfusion Buffer- HEPES Buffered Sucrose (150ml)

4mM HEPES pH 7.4	0.6ml stock solution (1.192g in 5ml ddH_2O, pH7.4)
320 mM Sucrose	16.43g
2mM EGTA	0.1125g
10mM Na pyrophosphate	0.399g
1mM Na Orthovanadate	3ml of 50mM stock solution
10mM NaF	3ml of 500mM stock solution
0.1mM PMSF	0.15ml of 100mM stock (0.0871g in 5ml EtOH)

Bring volume to 150 ml with dH_2O

Homogenization Buffer- HEPES Buffered Sucrose (30ml)

4mM HEPES pH 7.4	0.12ml stock solution (1.192g in 5ml ddH_2O, pH7.4)
320 mM Sucrose	3.286g
2mM EGTA	0.0228g
10mM Na pyrophosphate	0.0798g
1mM Na Orthovanadate	0.6ml of 50mM stock solution
10mM NaF	0.6ml of 500mM stock solution
1ug/ml aprotinin	30ul of 1mg/ml stock
1ug/ml leupeptin	30ul of 1mg/ml stock
0.1mM PMSF	30ul of 100mM stock

Bring volume to 30 ml with dH_2O

dH_2O with inhibitors (10ml)

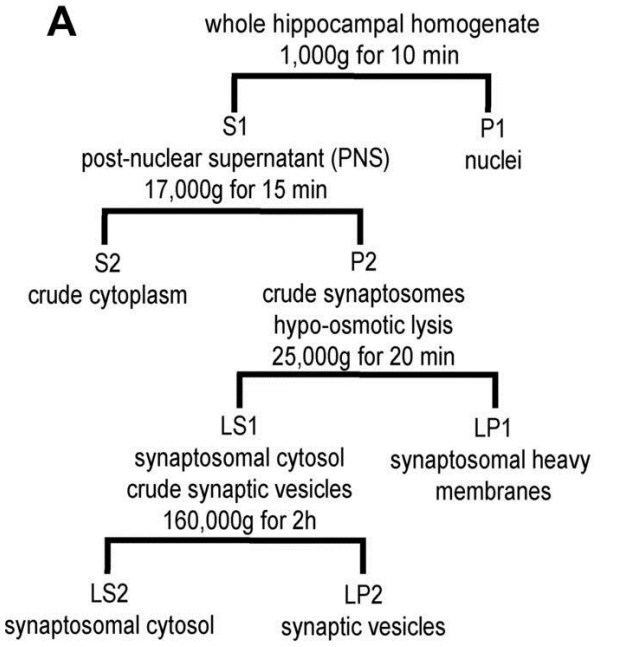
dH_2O	8.57ml
1mM Na Orthovanadate	0.2ml of 50mM stock solution
10mM NaF	0.2ml of 500mM stock solution
10mM Na pyrophosphate	1ml of 100mM stock solution
1ug/ml aprotinin	10ul of 1mg/ml stock solution
1ug/ml leupeptin	10ul of 1mg/ml stock solution
0.1mM PMSF	10ul of 100mM stock solution

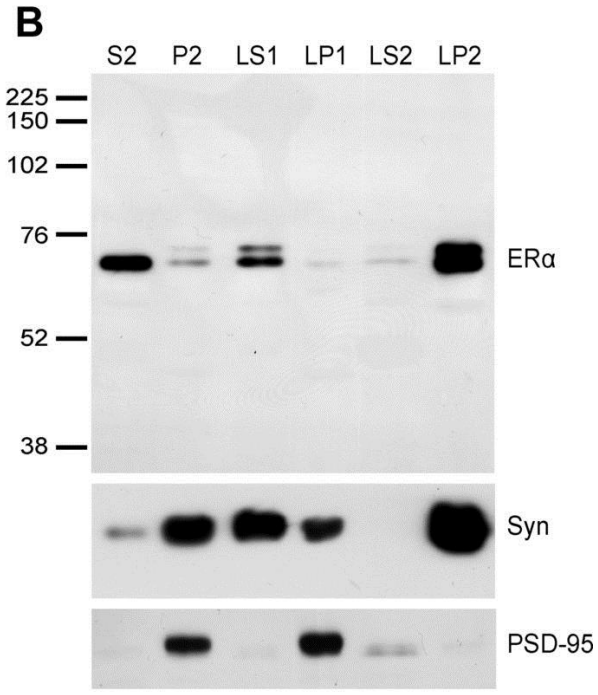
1. Perfuse rat for ~2 minutes with slushy perfusion buffer
2. Remove brain and place in slushy buffer
3. Dissect out brain regions of interest on ice (tissue can be rapidly frozen on dry ice and stored at -80^{o}C)
4. Weigh tissue
5. Homogenize in 9 volumes of **sucrose homogenization buffer** (~1ml) and homogenize (20 strokes) with glass tissue grinder
6. Centrifuge at 1000g for 10 minutes at 4^{o}C
7. Discard P1 which contains nuclei and large tissue fragments
8. Centrifuge supernatant (S1) at 15000g for 15 minutes at 4^{o}C
9. Resuspend pellet (P2) which contains crude synaptosomal fraction in 0.5ml **sucrose homogenization buffer** (save supernatant S2)
10. Spin resuspended pellet P2 at 15000g for 15 min at 4^{o}C (this step removes contaminants; discard supernatant)
11. Lyse pellet via hypoosmotic shock by resuspending washed crude synaptosomal fraction (P2') in 9 volumes of ice cold **ddH_2O containing protease and phosphatase inhibitors** (~**400 µl)**
12. Rapidly adjust to 4mM using 1M HEPES, pH7.4 (**1.6µl** HEPES)
13. Rehomogneize tissue
14. Incubate at 4^{o}C for 30 minutes, mixing continuously
15. Centrifuge lysate at 25,000g for 30 minutes. LP1 contains lysed synaptosomal membrane fraction (save and freeze LS1 which contains crude synaptic vesicle fraction)
16. Resuspend LP1 in 9 volumes **sucrose homogenization buffer** (**150**-**200µl**)
17. Aliquot and store at -20^{o}C

Details of tissue:

For example:

Distribution of ERα in hippocampal synaptosomal fractions





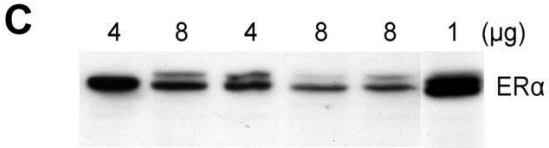


Figure 1. Distribution of ERα in hippocampal synaptosomal fractions. A, Schematic of hippocampal tissue fractionation by differential centrifugation. B, Equal amounts of protein (4 μg per lane) from fractions isolated by differential centrifugation were probed for ERα, the synaptic vesicle marker synaptophysin (Syn), and the postsynaptic density marker PSD-95. ERα was concentrated in crude cytoplasm (S2), synaptosomal cytosol (LS1), and synaptic vesicle (LP2) fractions. ERα did not concentrate with postsynaptic densities and migrated as a double band in synaptosomal fractions. C, To confirm ERα in P2 and subsequent SV-containing fractions, we varied protein loaded per lane. Doubling protein loaded for P2, LP1, and LS2 to 8 μg per lane showed that ERα is present in these fractions. The ERα signal was still strong in LP2, even with only 1 μg loaded. (Tabatadze N et al. Endocrinology 2013;154:819-830)