

**Presynaptic (synaptic vesicle) enrichment fractionation protocol for mouse brain
[based on Huttner et al, J Cell Biol. 96(5): 1374-88 (1983)]**

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MWS 9/26/06

Note: Remember to save a portion (eg. 50-100 μ L) of each fraction! Also, prepare solutions the day before you perform the fractionation and store them at 4°C.

- 1. Homogenize 2 cortexes together in 1.6 mL of sucrose buffer (see bottom)**
 - Use 4 cortexes total per prep; 3.2 mL buffer equals approx. 5.3-6 vol. per brain volume
 - Homogenize 12 strokes w/ a Dounce Teflon-tip homogenizer (loose fit)
- 2. Centrifuge in two 2-mL eppendorf tubes in microcentrifuge 10 min at 800 x g [3100 rpm in Epp. 5415C] at 4°C.**
- 3. Resuspend each large loose pellet from step 2 in another 300 μ L (600 μ L total) and spin pellet again; combine supernatants.**
- 4. Discard pellet P1 and spin supernatant S1 at 9200 x g [10, 600 rpm in Epp. 5415C] for 15 min at 4°C.**
- 5. Set aside supernatant S2 and resuspend each pellet P2 in 1 mL sucrose buffer (2 mL total/ 4 cortexes).**
- 6. Spin resuspended P2 at 10,200 x g [11,200 rpm in Epp. 5415C] 15 min at 4°C.**
- 7. Remove supernatant S2' and resuspend pellet P2' in 200 μ L sucrose buffer (per 2 cortexes; 400 μ L total); combine & transfer to a Dounce homogenizer.**
 - Note: Final total volume will be approx. 725 μ L.
- 8. Add 9 volumes (6.5 mL) ice-cold dH₂O and immediately homogenize with 3 vigorous strokes.**
- 9. Pour lysate into 15-mL Falcon tube with 54 μ L (to 7.5 mM final concentration) 1 M HEPES and store on ice for 30 min.**
- 10. Transfer lysate into 2 4-mL ultracentrifuge tubes and centrifuge lysate 20 min at 25,000 x g [25,000 rpm in TLA 100.3 microultracentrifuge rotor].**
 - I suggest adding protease inhibitors at this step to be safe.
- 11. Transfer LS1 (supernatant) to new 4-mL tube and centrifuge 2 hr at 165,000 x g [63,000 rpm in TLA 100.3 rotor]. Resuspend LP1 (pellet) in 500 μ L (total volume) 1% NP-40 IP buffer (see bottom) + protease inhibitors.**
 - Combine the S2 and S2' supernatants and spin these along with the LS1 fraction at 165,000 x g for 2 hr. This will give S3 (supernatant) and P3 (pellet).

- To resuspend LP1, pipet up and down followed by 25 strokes w/ Dounce homogenizer (if necessary).
- To resuspend P3, use 400 μL total volume 1% NP-40 IP buffer.

12. Remove supernatant; this is LS2 fraction. Resuspend pellet in 1% NP-40 IP buffer.

- LP2 pellets will be clear & glassy, and difficult to resuspend. I resuspend in 150 μL total volume. You may want to Dounce homogenize these pellets also.

Prepare these solutions and store at 4°C the night before you prep the fractions:

1. Sucrose buffer:

0.32 M sucrose in 4 mM HEPES pH 7.3 + protease/phosphatase inhibitors

Per 20 mL sucrose buffer:

- 2.19 g sucrose
- 80 μL 1 M HEPES pH 7.3
- Bring to final vol. of 20 mL with dH₂O.

2. 1% NP-40 IP buffer:

20 mM HEPES, 150 mM NaCl, 2 mM EDTA pH 8.0, 1% NP-40

Per 25 mL IP buffer:

- 0.5 mL 1 M HEPES
- 0.75 mL 5 M NaCl
- 100 μL 0.5 M EDTA
- 250 μL NP-40
- 23.4 mL dH₂O

3. ice-cold dH₂O

When you are finished with your fractionation, you should have the following fractions: S1, S2, P2, S3, P3, LS1, LP1, LS2, LP2.

The approximate yield of each fraction is as follows:

- S1: 8-9 $\mu\text{g}/\mu\text{L}$**
- S2: 5 $\mu\text{g}/\mu\text{L}$**
- P2: 5 $\mu\text{g}/\mu\text{L}$**
- S3: 2.5 $\mu\text{g}/\mu\text{L}$**
- P3: 13-15 $\mu\text{g}/\mu\text{L}$**
- LS1: 1.5 $\mu\text{g}/\mu\text{L}$**
- LP1: 10 $\mu\text{g}/\mu\text{L}$ [total amt: 5 mg]**
- LS2: 1.5 $\mu\text{g}/\mu\text{L}$**
- LP2: 2 $\mu\text{g}/\mu\text{L}$ [total amount: 300 μg]**

Fractions*:

S1-cortex homogenate

P1-nuclei, lg. debris

S2-cytosol, light membranes

P2-crude synaptosomes

S3-cytosol

P3-light membranes (Golgi & ER)

LS1-cytosol of synaptosomes

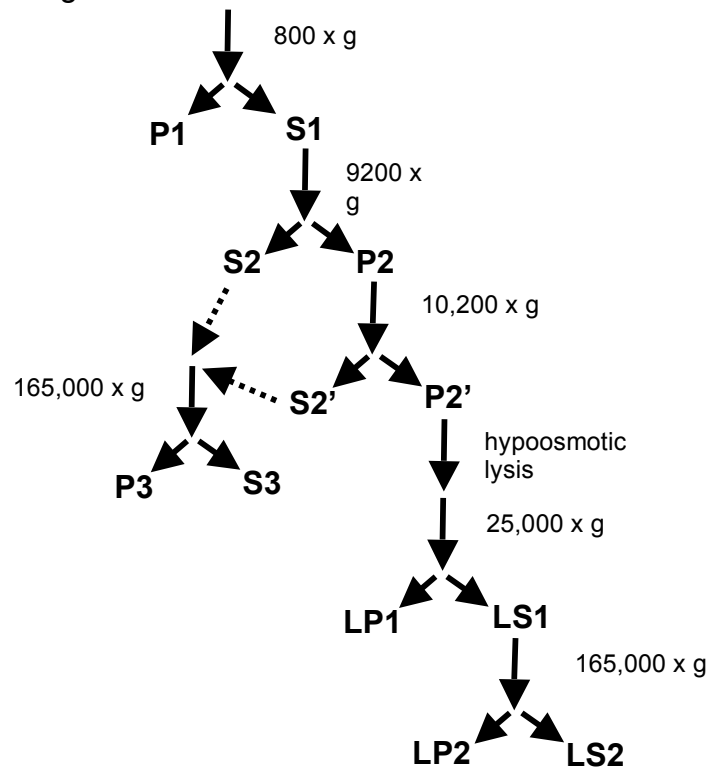
LP1-synaptosomal membranes

LS2-LS1 fraction minus LP2

fraction

LP2-synaptic vesicle(SV)-enriched

homogenize cortex in sucrose buffer



*definitions of synaptic vesicle (presynaptic) enriched fractions as written in Niethammer et al, Neuron 28: 697-711(2000) [Li-Huei Tsai lab]

Original reference for synaptic vesicle enrichment protocol:

Huttner et al, J Cell Biol 96(5): 1374-1388 (1983)

A second reference for presynaptic enrichment fractionation protocol:

Dunah and Standaert, J. Neurochem 85(4): 935-943 (2003).