

### Synaptosome Preparation

1. Remove cortex from 3 rats and place into 15 mL sucrose; separate into smaller pieces; pour cortex and sucrose into Dounce homogenizer glass tube (ON ICE).
2. Homogenize cortex (10 strokes - nice and slow).
3. Transfer contents into plastic centrifuge tube; add 10 mL sucrose to glass tube and pour it into a conical centrifuge tube.
4. 1<sup>st</sup> spin (2 min) at 4,200 x g.
5. Transfer s/n carefully (do not get any pellet here – BE CONSERVATIVE) and place it into another plastic conical tube (dispose of the pellet).
6. 2<sup>nd</sup> spin (12 min) at 25,200 x g. Set-up can be done during these spins and during the water bath incubation.
7. Dispose of s/n; resuspend pellet into 25 mL sucrose (slowly disperse tissue in 1-2 mL at first, then add remaining).
8. 3<sup>rd</sup> spin (12 min) at 25,200 x g.
9. Dispose of s/n; resuspend in 25 mL HBS (slowly disperse in 1-2 mL as before). Spin for (5 min) at 11,000 x g.
10. Resuspend in the synaptosomes in 2 mL HBS, mixing slowly and thoroughly.
11. Spec and can store at  $-80^{\circ}\text{C}$  or continue.

## **Fractionation and Synaptic Vesicle Purification**

12. Lyse synaptosomes with lysis buffer (hypotonic media: 2mM HEPES and 0.5 mM EGTA in water, pH 7.4). Add in a 1:9 ratiometric solution with synaptosomes (1 mL (1mg) synaptosomes: 9 mL lysis buffer).
13. Lyse on ice for 45 min with gentle shaking.
14. Centrifuge lysed synaptosomes at 1000 x g for 20 min.
15. Pellet is P1 – spec and store in 20 µg aliquots
16. Take the s/n from step 18 and centrifuge at 32,800 x g for 45 minutes. Pellet is P2 – spec and store in 20 µg aliquots
17. Collect s/n from step 19 and centrifuge at 100,000 x g for 1 hr. Pellet is P3 – spec and store in 20 µg aliquots
18. Load 20 µg of each of the following into an SDS-PAGE gel containing the Synaptosome fraction, P1 fraction (enriched in plasma membrane), P2 fraction (enriched in endosomes), and P3 fraction (enriched in vesicles).