Synaptosomal Preparations

Reference

1. Hallett, P.J., Collins, T.L., Standaert, D.G. and Dunah, A.W. (2008), Biochemical Fractionation of Brain Tissue for Studies of Receptor Distribution and Trafficking. Current Protocols in Neuroscience, 42: 1.16.1-1.16.16. https://doi.org/10.1002/0471142301.ns0116s42

Solutions

1. Homogenizing TEVP Buffer: 10 mM Trizma Base, 5 mM NaF , 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, 320 mM Sucrose, 1% Protease Inhibitor Cocktail, pH 7.4
2. P1 Resuspension TEVP Buffer: 10 mM Trizma Base, 5 mM NaF , 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, pH 7.4
3. P2 Resuspension TEVP Buffer 10 mM Trizma Base, 5 mM NaF , 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, 35.6 mM Sucrose, pH 7.4

Abbreviations

1. WH: Whole Homogenate
2. S1: Supernatant 1
3. P1: Pellet 1
4. S2: Supernatant 2
5. P2: Pellet 2
6. LS1: Lysis Supernatant 1
7. LP1: Lysis Pellet 1

Protocol

1. Homogenize tissue (~200 mg frozen weight) in 5 mL TEVP Buffer + 320 mM sucrose + 1 % Protease Inhibitor Cocktail.
2. Aliquot 300 µL for step 3 and save remainder as whole homogenate (WH).
3. Centrifuge the 300 µL aliquot at 800 x g for 10 minutes at 4°C.
4. Remove supernatant (S1).
5. Resuspend Pellet (P1) in 300 µL TEVP buffer and save.
6. Save 10 µL of S1 and centrifuge remaining S1 at 9,200 x g for 15 minutes at 4°C.
7. Remove supernatant (S2) and save.
8. Resuspend Pellet (P2) in 300 µL TEVP buffer + 35.6 mM sucrose + protease inhibitor cocktail, sonicate.
9. Centrifuge P2 at 25,000 x g for 20 minutes at 4°C.
10. Remove supernatant (LS1) and save.
11. Resuspend Pellet (LP1) in 300 µL TEVP buffer.
12. Add 300 µL 2X Laemmli Buffer to LP1 and boil 10 minutes.
13. Aliquot all fractions 1:1 in 2X Laemmli and boil for 10 minutes, analyze by Western blot.