Protocol for Preparation of Brain Lysate, P2, LP1 and LP2 Fractions

All steps should be carried out at 4°C

Materials and reagents

- **Homogenization buffer**: 320 mM sucrose, 4 mM HEPES, pH 7.3
- **PMSF solution**: 200 mM PMSF in EtOH
- **HEPES buffer:** 1 M HEPES-KOH, pH 7.4

Procedure

The protocol is recommended for one brain, but can be scaled up for more brains if necessary.

1. Wash rat brain in 12 ml ice-cold **homogenization buffer**.
2. Homogenize brain in 12 ml fresh ice-cold **homogenization buffer** (9 strokes at 900 rpm).
3. Add 1:1000 **PMSF solution** and centrifuge for 10 min at 2700 rpm (870x g) in SS34 or comparable rotor. Discard the pellet. The supernatant can be used as total brain lysate.
4. Centrifuge supernatant (S1) for 15 min at 10,000 rpm (11,952x g) in SS34 or comparable rotor.
5. Resuspend the pellet (P1) in 12 ml ice-cold **homogenization buffer** and centrifuge for 15 min at 11,000 rpm (14,462x g) in SS34 rotor. **The resulting pellet is the P2 fraction** and contains enriched synaptosomes.
6. For the LP2 fraction continue and resuspend pellet in 1.2 ml **homogenization buffer**.
7. Split the suspension into two equal portions and transfer into homogenizator. Lyse by adding 9 ml ice-cold H_2O to each portion and homogenize 3 strokes at 2000 rpm.
8. Add 50 µl **HEPES buffer** and 1:1000 **PMSF solution** and centrifuge for 20 min at 16,500 rpm (32,539x g) in SS34 rotor. **The resulting pellet is the LP1 fraction** and contains plasma membranes.
9. Centrifuge the supernatant (LS1) for 2 h at 50,000 rpm (22,5634x g) in 50 Ti rotor. **The resulting pellet is the LP2 fraction** and contains enriched synaptic vesicles.

Remarks

To prepare sufficient amounts of P2 to proceed to the preparation of LP2 one brain in general is not enough and the protocol should be scaled up.