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Synaptic vesicle preparation from mouse brain

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We use this protocol and it's working

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Abstract

This protocol describes the preparation and purification of synaptic vesicles (SVs) from the brains of mice. Brain tissue is homogenized in sucrose-based SHT buffer, followed by sequential centrifugation steps to obtain the LP2 fraction. Synaptic vesicles are subsequently purified by glycerol gradient centrifugation, and SV-enriched fractions are collected for downstream biochemical analyses.

Materials

- **Sucrose** (≥99.5%) (Sigma-Aldrich)
- **Mg-EGTA**
- **HEPES-Tris**, pH 7.4
- c**Omplete™ Protease Inhibitor Cocktail (11836153001, Roche)**
- **EGTA**
- **$MgCl_2$**
- **Glycerol** (molecular biology grade)

Buffers

- **SHT buffer:** 0.3 M sucrose, 1 mM Mg-EGTA, 10 mM HEPES-Tris, pH 7.4
- **Osmotic lysis buffer:** 8 mM HEPES-Tris, pH 7.4
- **Glycerol gradient buffer (5–25%):** 150 mM NaCl, 10 mM HEPES-Tris (pH 7.4), 1 mM EGTA, 0.1 mM $MgCl_2$, 5–25% (v/v) glycerol (formed as a continuous gradient)

Tissue homogenization

1 Dissect mouse brains and place them in ice-cold SHT buffer supplemented with protease inhibitor.

2 Homogenize the tissue in 10 mL SHT buffer using a glass–Teflon homogenizer (9 strokes at 900 rpm).

Initial fractionation

3 Centrifuge homogenate at **871 × g, 10 min, 4°C**. Collect the supernatant (S1).

4 Centrifuge S1 at **12,000 × g, 15 min, 4°C** to obtain the P2 pellet (crude synaptosomes).

Synaptosome washing and osmotic lysis

5 Resuspend the P2 pellet in SHT buffer and centrifuge at **14,500 × g, 15 min, 4°C**. Discard the supernatant

6 Resuspend the pellet in 8 mM HEPES-Tris (pH 7.4) and incubate on ice for **45 min** to induce osmotic lysis.

7 Homogenize briefly to complete disruption using a glass–Teflon homogenizer (five strokes at 3,000 rpm) .

Preparation of LP2 fraction

8 Centrifuge the lysate at **32,500 × g, 20 min, 4°C** to obtain supernatant LS1.

9 Centrifuge LS1 at **280,000 × g, 2 h, 4°C** to obtain the LP2 pellet.

10 Resuspend the LP2 pellet in SHT buffer diluted to 40 mM sucrose (prepared by diluting standard SHT buffer: 0.3 M sucrose, 1 mM Mg-EGTA, 10 mM HEPES-Tris, pH 7.4).

10.1 Resuspention

a. **Use a 200 µL pipette tip cut at ~1/4 from the distal end** and pipette up and down **100 times**.

b. Switch to a **200 µL tip cut at ~1/8 from the distal end** and pipette **100 times**.

c. Finally, use a **standard (uncut) 200 µL tip** and pipette **100 times** to complete resuspension.

11 Aliquot the resuspended LP2, flash-freeze in liquid nitrogen, and store at **–80°C**.

Glycerol gradient purification

12 Prepare a **5–25% glycerol gradient** using the glycerol gradient buffer using a gradient maker to form a continuous gradient.

13 Layer the LP2 sample onto the gradient.

14 Centrifuge at **270,000 × g for 1 h 4 min at 4°C**.

15 Collect 0.5 mL fractions from the top using a gradient fractionator or connected fraction collector, ensuring smooth and continuous recovery of the gradient.

16 Use fractions 5–12, which usually contain purified synaptic vesicles, for subsequent experiments.