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Preparation of an Enriched Synaptic Vesicle Fraction V.1

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We use this protocol and it's working

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Abstract

Preparation of an Enriched Synaptic Vesicle Fraction

- 1 Mouse brain tissue was homogenized in a buffer containing 0.32 M sucrose in PBS, supplemented with protease/phosphatase inhibitors.

- 2 The homogenate was centrifuged at $1,000 \times g$ for 10 min at 4 °C to remove nuclei and cellular debris (P1). The supernatant (S1) was centrifuged at $15,000 \times g$ for 15min at 4 °C to yield a crude synaptosomal pellet (P2).

- 3 This pellet was hypo-osmotically lysed in water containing protease inhibitors for 5 min at 4 °C and passed through both a 22- and 27½-gauge needle (10 times each).

- 4 This suspension was centrifuged at $23,000 \times g$ for 22 min at 4 °C, and the resulting supernatant LS1(S3) was centrifuged again at $174,900 \times g$ for 2 hours at 4 °C in a STi32 Beckman rotor. The final pellet (LP2 or P4) containing an enriched fraction of synaptic vesicles was used for subsequent experiments.