NOV 12, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4qr62vo5/v1

Protocol Citation: Leonardo A Parra-Rivas 2023. Preparation of an Enriched Synaptic Vesicle Fraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4qr62vo5/v1

Protocol status: Working
We use this protocol and it's working

Created: Nov 12, 2023

Preparation of an Enriched Synaptic Vesicle Fraction

Leonardo A Parra-Rivas[1]

[1]University of California, San Diego

Subhojit Roy Lab

Leonardo A Parra-Rivas
University of California, San Diego

ABSTRACT

Preparation of an Enriched Synaptic Vesicle Fraction

Last Modified: Nov 12, 2023

PROTOCOL integer ID: 90817

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 × *g* for 10 min at 4 °C to remove nuclei and cellular debris (P1). The supernatant (S1) was centrifuged at 15,000 × *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 × *g* for 22 min at 4 °C, and the resulting supernatant LS1(S3) was centrifuged again at 174,900 × *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.