

### **Immunoisolation of synaptic vesicles from nerve terminals**

1. Purify synaptic vesicles as described by the fractionation of synaptic vesicles in “Synaptosome preparation” protocol.
2. Adjust desired pellet fraction to 500  $\mu$ l with 2 M sucrose.
3. Load the pellet-sucrose fraction to the bottom of a 0.3, 0.8, and 1.2 M sucrose gradient. Centrifuge the gradient for 3 hours at 350,000 x g. The light membrane fractions float to the top.

### ***Preparation of antibody-protein A conjugated to dynabeads complex***

4. Make a homogenous suspension of protein A conjugated to dynabeads (pA-DB) (Dyna) by vortexing 1-2 minutes.
5. Transfer 200  $\mu$ l of pA-DB to a centrifuge tube.
6. Place the tube on Magnetic Isolation Device (MID) for 1 min and pipette off the fluid.
7. Add 200  $\mu$ l of PBS and resuspend.
8. Repeat step 3.
9. After final wash, remove washing buffer and resuspend in 200  $\mu$ l coating buffer (0.1 M borate buffer, pH 9.5).
10. Add chosen linker IgG to DB and mix thoroughly for 1-2 min. The ratio of pA-DB to IgG should be 200  $\mu$ l pA-DB: 60  $\mu$ g IgG.
11. Incubate at 37°C for 16-24 hr (or longer at lower temperatures) with bidirectional mixing.

12. After incubation collect pA-DB with MID. Wash pA-DB twice for 5 minutes at 4°C with PBS/0.1% BSA, pH 7.4.
13. Resuspend pA-DB in 200 µl incubation buffer containing: 2mM EDTA, 5% FBS, PBS, pH 7.4 and add affinity-purified antibody against the target protein at the ratio of 200 µl pA-DB: 30 µg IgG.
14. Incubate the mixture for 30 minutes at 4°C with bidirectional mixing.
15. Collect pA-DB with MID and wash 4x 5 min at 4°C with PBS/0.1% BSA.
16. Resuspend pA-DB in 500 µl incubation buffer.

*Isolation of synaptic vesicle populations*

17. Add 500 µl of purified synaptic vesicle (can use P3 fraction of fractionation experiment) to the pA-DB complex. Incubate for 1-12 hours at 4°C with continuous mixing.
18. Collect pA-DB with MID, save the supernatant (nonbound fraction), and wash 4 times for 15 minutes each in 1 mL incubation buffer.
19. Add SDS sample buffer and run on SDS-PAGE gel to analyze vesicle contents (bound fraction).