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α-Synuclein binding assay to isolated synaptic vesicles

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We use this protocol and it's working

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Abstract

The binding of α-synuclein (α-syn) to synaptic vesicles was assessed by pull-down using C-terminally His-tagged α-syn and Co Dynabeads (10103D, Invitrogen). Synaptic vesicles (SVs) were isolated from mouse brain (LP2 fraction), incubated with recombinant α-syn, and the bound fractions were analyzed by immunoblotting.

Materials

- Co Dynabeads (10103D, Invitrogen)
- Synaptic vesicles (SVs; LP2 fraction) purified from mouse brain
- Reaction buffer: 150 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES, pH 7.4
- BSA (A8806, Sigma)
- SHT buffer: 0.3 M sucrose, 1 mM Mg-EGTA, 10 mM HEPES-Tris, pH 7.4
- Recombinant His-tagged α-synuclein
- Wash buffer: 150 mM KCl, 1 mM $MgCl_2$, 5 mM imidazole, 20 mM HEPES, pH 7.4
- Elution buffer: 150 mM KCl, 1 mM $MgCl_2$, 200 mM imidazole, 20 mM HEPES, pH 7.4
- Antibody: synaptophysin (mouse monoclonal, 1:1,000; S5768, Sigma-Aldrich)

Beads Preparation

1 12.5 µL per reaction of Co Dynabeads (10103D, Invitrogen) are transferred to a 1.5 mL low-adhesion microcentrifuge tube.

2 Wash the beads three times with 200 µL reaction buffer (150 mM KCl, 1 mM MgCl2, 20 mM HEPES, pH 7.4).

3 Pre-block Co Dynabeads with 1% BSA in reaction buffer for 30 min at room temperature with gentle rotation.
- This step reduces nonspecific binding of SVs to the beads.

4 Wash the beads three times with reaction buffer.

Binding reaction

5 Resuspend the LP2 fraction in SHT buffer (1mg/mL)

6 Incubate purified recombinant His-tagged α-syn (0–25 µg) with 25 µg of LP2 in reaction buffer (total volume 100 µL) for 3 h at room temperature with gentle agitation. Collect a mixture as input for Western blotting analysis.

7 Add the mixture to the pre-blocked beads and incubate for 15 min at room temperature with gentle rotation.

Washing and elution

8 Place tubes on a magnetic stand and collect the beads. Wash three times with 1 mL wash buffer.

9 Elute bound proteins by incubating beads in 25 µL elution buffer for 15 min at room temperature with gentle rotation.

10 Spin briefly at 1,000 × g for 30 s.

11 Place on a magnetic stand and collect the eluted fraction.

Analysis

12 Analyze pull-down efficiency by immunoblotting.

13 Quantify band intensities using ImageJ.

14 Normalize synaptophysin levels in the elution fraction to the input.