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Expression and purification of Syp-VAMP2 complex into native nanodiscs

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We use this protocol and it's working

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Abstract

This is a protocol for the purification of Syp-VAMP2 complex into native nanodiscs.

Mammalian expression constructs

2d

1 Thaw Expi-HEK293 cells and passage 3 times

2 Using a T2A polycistronic vector containing the VAMP2 and Synaptophysin (Syp) genes with FLAG tag, express VAMP2 and Syp in Expi-HEK293 cells using ExpiFectamine transfection reagent for 48:00:00 hours. 2d

3 Spin down transfected cells and rinse in ice-cold PBS.

Solubilization of VAMP2-Syp into native nanodiscs

4h 25m

4 Resuspend cells expressing VAMP2-Syp into lysis buffer ([M] 50 millimolar (mM) Tris HCl pH 7.4 , [M] 150 millimolar (mM) NaCl , [M] 10 % volume glycerol) supplemented with protease inhibitor tablets.

5 Lyse cells using nitrogen cavitation (600 psi, 00:15:00 minutes). 15m

6 Remove debris and nuclei using a 4000 rpm centrifugation spin for 00:10:00 minutes. 10m

7 Ultracentrifuge the clarified lysate at 200,000xg for 01:00:00 hour at 4 °C to collect membranes. 1h

8 Resuspend membranes in 1.5% polymer solution (ChloroSMA80) and incubate at 4 °C for 02:00:00 hours. 2h

9 Post-solubilization centrifuge the sample at 200,000xg for 01:00:00 hour to remove insoluble material. 1h

Native nanodisc enrichment using affinity chromatography

10 Incubate native nanodiscs containing VAMP2-Syp complex with anti-FLAG resin Overnight at 4 °C with rotation.

11 Remove supernatant and wash beads extensively with [M] 50 millimolar (mM) Tris HCl pH 7.4 , [M] 150 millimolar (mM) NaCl , [M] 10 % volume glycerol .

12 Elute nanodiscs using buffer from Step 11 containing 3XFLAG peptide at 10 ug/ml concentration.