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Expression and purification of Syp-VAMP2 complex into native nanodiscs

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Protocol status: Working

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


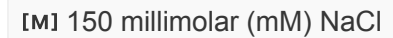








2d

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

2d

Solubilization of VAMP2-Syp into native nanodiscs

4h 25m

- 4 Resuspend cells expressing VAMP2-Syp into lysis buffer ( 50 millimolar (mM) Tris HCl pH 7.4 ,  150 millimolar (mM) NaCl ,  10 % volume glycerol) supplemented with protease inhibitor tablets.
- 5 Lyse cells using nitrogen cavitation (600 psi,  00:15:00 minutes).
- 6 Remove debris and nuclei using a 4000 rpm centrifugation spin for  00:10:00 minutes.
- 7 Ultracentrifuge the clarified lysate at 200,000xg for  01:00:00 hour at  4 °C to collect membranes.
- 8 Resuspend membranes in 1.5% polymer solution (ChloroSMA80) and incubate at  4 °C for  02:00:00 hours.
- 9 Post-solubilization centrifuge the sample at 200,000xg for  01:00:00 hour to remove insoluble material.

15m

10m






1h

2h

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
 50 millimolar (mM) Tris HCl pH 7.4 ,  150 millimolar (mM) NaCl ,
 10 % volume glycerol .
- 12 Elute nanodiscs using buffer from Step 11 containing 3XFLAG peptide at 10 ug/ml concentration.