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Western blotting of Syp-VAMP2 complex

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

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Abstract

The intricate molecular environment of the native membrane profoundly influences every aspect of membrane protein (MP) biology. Despite this, the most prevalent method of studying MPs uses detergent-like molecules that disrupt and remove this vital local membrane context impeding our ability to quantitatively decipher the local molecular context. Using a library of membrane-active polymers we have developed a platform for the high-throughput analysis of the membrane proteome enabling near-complete spatially-resolved extraction of target MPs directly from their endogenous membranes into native nanodiscs that maintain the local membrane context. We accompany this advancement with an open-access database that quantifies the polymer-specific extraction variability for 2065 unique mammalian MPs and provides the most optimized condition for each of them. Our method enables rapid and near-complete extraction and purification of target MPs directly from any endogenous organellar membrane at physiological expression levels. To further validate this platform, we took several independent MPs and demonstrated how our resource can enable rapid extraction and purification of target MPs from different organellar membranes with high efficiency and purity. Further, taking two synaptic vesicle MPs, we show how the database can be extended to capture multiprotein complexes between overexpressed MPs. We expect these publicly available resources to empower researchers across disciplines to efficiently capture membrane ‘nano-scoops’ containing a target MP and interface with structural, functional, and other bioanalytical approaches.

Reagents needed for protocol

1 Acquire the following antibodies for the Western blotting protocol:

1.1 Primary Antibody: rabbit Na/K ATPase (Abcam Cat# ab167390, 1:5000)

1.2 Primary Antibody: Synaptophysin Rabbit mAb (ABClonal Cat#A19122, 1:5000)

1.3 Primary Antibody: VAMP2 Rabbit mAb (ABClonal Cat#A4235, 1:5000)

1.4 Secondary Antibody: HRP Goat Anti-Rabbit IgG (H+L) (ABClonal Cat#AS014, 1:10000)

Running the gel and transfer

2 Measure protein concentration with Bradford Kit, dilute into 4x Laemmli buffer to bring final concentration to 1x, and load to a 4-20%Mini-PROTEAN TGX precast gel.

3 Run the gel until the loading dye front runs off the ends of the gel.

4 Using a TurboBlot system, transfer gel onto a PVDF membrane.

Blotting

1h

5 Block membrane in 5% milk for 01:00:00 and wash with TBST

1h

6 Incubate with the primary antibody at the appropriate concentration listed above Overnight at 4 °C

7 Wash with TBST

8 Incubate with the secondary antibody at the concentration listed above for 01:00:00 and wash with TBST 1h

9 Add HRP and detect chemiluminescence using an ImageQuant system.