Jun 18, 2025

# Sucrose Density Gradient Co-flotation of PPM1H and Rab10

The Journal of Biological Chemistry

DOI
https://dx.doi.org/10.17504/protocols.io.q26g79o2kvwz/v1

Ayan Adhikari[1], Suzanne R. Pfeffer[1]

[1]Stanford University School of Medicine, Stanford, California USA 94305-5307

Suzanne R Pfeffer
Stanford University School of Medicine



OPEN ACCESS

**DOI:** https://dx.doi.org/10.17504/protocols.io.q26g79o2kvwz/v1

**External link:** https://doi.org/10.1016/j.jbc.2025.110679

**Protocol Citation:** Ayan Adhikari, Suzanne R. Pfeffer 2025. Sucrose Density Gradient Co-flotation of PPM1H and Rab10. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g79o2kvwz/v1

**Manuscript citation:**
Adhikari A, Tripathi A, Chiang CY, Sherpa P, Pfeffer SR (2025) Allosteric regulation of the Golgi-localized PPM1H phosphatase by Rab GTPases modulates LRRK2 substrate dephosphorylation in Parkinson's disease. The Journal of Biological Chemistry 301(10). doi: 10.1016/j.jbc.2025.110679

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

**Created:** June 18, 2025

**Last Modified:** June 18, 2025

**Protocol Integer ID:** 220555

**Keywords:** ASAPCRN, ppm1h phosphatase to unphosphorylated rab10, unphosphorylated rab10, liposome, ppm1h phosphatase, diameter liposome, rab10 sucrose, potential binding of mneon, bound protein, protein, flotation assay, rab10, recombinant mneon

**Funders Acknowledgements:**
**Aligning Science Across Parkinson's**
Grant ID: ASAP-000463

## Abstract

Sucrose gradient co-flotation assays are employed to assess the potential binding of mNeon-PPM1H phosphatase to unphosphorylated Rab10. Recombinant mNeon-PPM1H and Rab10 are incubated with 50 nm-diameter liposomes for 30 min at 30°C. The mixture is layered beneath a discontinuous sucrose gradient and then subjected to ultracentrifugation to resolve liposome-bound proteins. Following centrifugation, fractions are analyzed by immunoblotting to detect the distributions of both mNeon-PPM1H and Rab10.

## Materials

1. Eppendorf LoBind tubes, 0.5mL Catalog No. 0030108434
2. Eppendorf LoRetention tips, 0.1-10uL Catalog No. 022493018
2. Bacterially expressed, full length untagged mNeon PPM1H and full length untagged Rab10 Q68L.
3. Molecular grade sucrose (Millipore #573113)
4. Ultra-clear centrifuge tubes (Beckman Coulter #344090)
5. Freshly prepared 50nm liposomes (produced by using a mini hand extruder (Avanti # 610000 1EA).

## Safety warnings

Freshly prepared liposomes work best

## Purification of mNeon PPM1H and Rab10

1 mNeon PPM1H and Rab10 are expressed in Escherichia coli BL21 (DE3) pLysS Rosetta cells. Protein purification is performed using Cobalt-affinity chromatography followed by size-exclusion chromatography. Details are available at:

**https://www.protocols.io/view/ppm1h-purificationfrom-e-coli-d67v9hn6.**

For Rab10 purification, 20 µM GTP is included in all buffers to stabilize the GTP-bound conformation. The Rab10 Q68L mutant is used to enrich for the GTP-bound state.

## Preparation of Small Unilamellar Vesicles (SUVs)

2 Small (50nm) unilamellar vesicles (SUVs) are formed by extrusion using (18:0-20:4)PC, (18:0-20:4)PI, (18:0-18:2)PS, (18:1) plus PI(4)P, and cholesterol at a molar ratio of 78:7:5:1:9 (Avanti Polar Lipids; ThermoFisher) to reproduce the composition of the trans Golgi network. Details can be found at:

3 https://www.protocols.io/view/preparation-of-unilamellar-liposomes-5qpvo3ke7v4o/v1

## Sucrose Density Gradient Co-flotation

4 50 nm liposomes (1.5 mM) are incubated with mNeon PPM1H (1µM final) and Rab10 (3µM final) for 30 minutes at 30°C in a total volume of 33 µl of HKM buffer (20 mM HEPES pH-7.5, 150 mM potassium acetate and 1 mM magnesium chloride) containing 20µM GTP (HKM/GTP).

5 167 µl of 60% w/v sucrose (in HKM/GTP) is then added and mixed well with the above-mentioned suspension to adjust it to 50% sucrose final, 1X HKM/GTP.

6 This high sucrose suspension is carefully overlaid with 200 µl 25% w/v sucrose in HKM/GTP followed by 100 µl of HKM/GTP buffer.

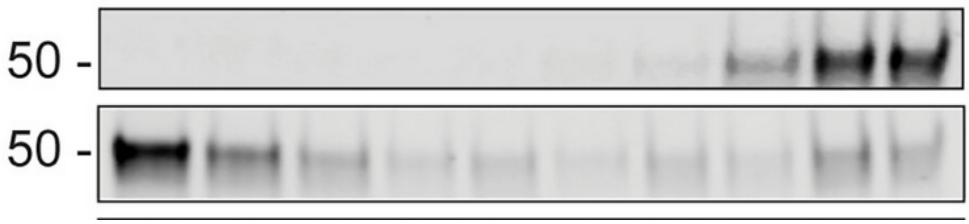
7 The sample is centrifuged at 45000 RPM for 3 hours using an SW55Ti swinging bucket rotor.

8 Ten 50 µl fractions are manually collected from the top of the sucrose gradient by using a micropipette held against the side of the tube.

9 The fractions are then analyzed by Western blotting to detect mNeon PPM1H and Rab10 using anti-rabbit PPM1H antibody and anti-mouse Rab10 antibody.

## Untitled section

10




This immunoblot shows the sucrose gradient flotation profile of PPM1H in the absence (top) or presence (bottom) of small 50nm liposomes. Top of the gradient is shown at left; mass of molecular weight marker in kDa is also shown at left. Reprinted from https://doi.org/10.1073/pnas.2315171120.