Oct 16, 2025

Co-Immunoprecipitation

Science Advances

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6zxzkgqe/v1

Tatiana Saccon[1], Mohan Babu[1]

[1]Department of Biochemistry, University of Regina

Szu-Chi Liao
Gladstone Institutes

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6zxzkgqe/v1

External link: https://doi.org/10.1126/sciadv.adu0726

Protocol Citation: Tatiana Saccon, Mohan Babu 2025. Co-Immunoprecipitation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6zxzkgqe/v1

Manuscript citation:
Liao S, Kano K, Phanse S, Nguyen M, Margolis E, Fu Y, Meng JX, Moutaoufik MT, Chatterton Z, Saccon T, Broderick K, Aoki H, Simms J, Suteja FX, Sei Y, Huang EJ, McAvoy K, Manfredi G, Halliday G, Babu M, Nakamura K CHCHD2 mutant mice link mitochondrial deficits to PD pathophysiology. Science Advances 11(46). doi: 10.1126/sciadv.adu0726

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

Created: August 20, 2025

Last Modified: October 16, 2025

Protocol Integer ID: 225024

Keywords: ASAPCRN, mitochondria, protein interaction, protein

Abstract

This is a protocol to analyze protein-protein interactions associated with mitochondria using co-immunoprecipitation.

Materials

- RIPA buffer (Thermo Scientific 89900)
- Halt Protease inhibitor cocktail, PIC (Thermo Scientific 78429)
- Primary antibodies
- µMACS Protein A or G magnetic microbeads (Miltenyi Biotec 130-092-945 or 130-092-947)
- µMACS columns (Miltenyi Biotec 130-042-701)
- Magnetic rack
- 2x Laemmli buffer (Bio-Rad 1610737)

Procedure

1 Resuspend 500 µg of mitochondrial lysate in 1 mL RIPA buffer containing a protease inhibitor cocktail (PIC).

2 Add 3 µL of primary antibody to the lysate and incubate at 4°C with agitation for 1 hour.

3 Add 100 µL of µMACS Protein A (for rabbit antibodies) or Protein G (for mouse antibodies) magnetic microbeads (Miltenyi).

4 Incubate at 4°C with agitation for 4 hours.

5 Pass the microbead-bound protein complexes through µMACS columns.

6 Wash columns twice with 1 mL of 0.1% RIPA buffer containing PIC.

7 Wash columns again with 1 mL of detergent-free RIPA buffer.

8 Elute proteins with 100 µL of 2x Laemmli buffer.

9 Heat at 95°C before collection.

10 Proceed to immunoblotting.