Jan 13, 2025

Mild Immunoprecipitation with Low Background

DOI
dx.doi.org/10.17504/protocols.io.eq2lywe9pvx9/v1

Cole Sitron[1], F Ulrich Hartl[1]

[1]Max Planck Institute of Biochemistry

C Cole Sitron
Max Planck Institute of Biochemistry

DOI: https://dx.doi.org/10.17504/protocols.io.eq2lywe9pvx9/v1

Protocol Citation: Cole Sitron, F Ulrich Hartl 2025. Mild Immunoprecipitation with Low Background. **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2lywe9pvx9/v1**

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

Created: July 02, 2024

Last Modified: January 13, 2025

Protocol Integer ID: 103395

Keywords: ASAPCRN, immunoprecipitation of protein, immunoprecipitation protocol, mild immunoprecipitation with low background, mild immunoprecipitation, immunoprecipitation, bait protein, bait protein of interest, antibody complex to immuno, antibody, antibody complex, immuno, protein aggregate, protein complex, protein, using gentle lysis condition, bind tube, gentle lysis condition

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282
Aligning Science Across Parkinson's
Grant ID: ASAP- 024268

Abstract

There is often a trade off between buffer stringency and background when performing an immunoprecipitation. One can reduce background with a stringent buffer, but this stringency could also cause a loss of interactors. This immunoprecipitation protocol circumvents this dilemma by using gentle lysis conditions to preserve protein complexes, while lowering the background with BSA blocking, Tween, and low-bind tubes. We have found that this protocol works well for immunoprecipitation of proteins from lysates that contain "sticky" protein aggregates. This protocol uses a protein G-antibody complex to immuno-precipitate the bait protein of interest.

Materials

1. 4 80% confluent 6-wells of cells expressing your protein of interest.
2. Dynabeads™ Protein G Immunoprecipitation Kit (Invitrogen Cat. no. 10007D)

Dynabeads™ Protein G Immunoprecipitation Kit **Thermo Fisher Catalog #**10007D

3. An appropriate antibody (Ab) against your protein of interest
4. Low bind 1.5 ml centrifuge tubes (Eppendorf cat. no. 0030108116)

Protein LoBind Tubes, 1.5 mL **Eppendorf Catalog #**0030108116

5. Microcentrifuge
6. Magnetic tube rack
7. Tube rotator
8. Pierce Rapid Gold BCA Protein Assay Kit (Thermo Fisher Scientific A53225)

Pierce™ Rapid Gold BCA Protein Assay Kit **Thermo Fisher Catalog #**A53225

9. PBS pH 7.2 (Thermo Fisher Scientific cat. no. 20012068)

PBS, pH 7.2 **Thermo Fisher Catalog #**20012068

10. Lysis buffer: PBS pH 7.2, 0.1% Triton X-100 (Thermo Fisher Scientific cat. no. 85111)

Triton™ X-100 Surfact-Amps™ Detergent Solution **Thermo Fisher Catalog #**85111 , 0.02% Tween-20 (Sigma-Aldrich cat. no. P9416-100ML)

Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9416-100ML , 1X cOmplete, Mini EDTA-free Protease Inhibitor Cocktail (Roche cat. no. 11873580001)

cOmplete™ EDTA-free Protease Inhibitor Cocktail **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**11873580001

+ 1X PhosStop (Sigma-Aldrich cat. no. 04906837001)

Roche PhosSTOP™ **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**4906837001 + 0.75 U/ml Benzonase (Max Planck Institute of Biochemistry Core Facility)

11. 2X Blocking Buffer: PBS pH 7.2, 0.1 % Triton X-100, 0.02% Tween-20, 6% BSA (Cell Signaling Technologies cat. no. 9998S) BSA **Cell Signaling Technology Catalog #**9998S
12. Wash Buffer: PBS pH 7.2, 0.1 % Triton X-100, 0.02% Tween-20, additional 113 mM NaCl (final concentration 250 mM)
13. 5 M NaCl
14. 1 M DTT
15. 4X NuPAGE LDS Sample Buffer (Invitrogen cat. no. NP0007)

NUPAGE LDS sample buffer (4x) **Thermo Fisher Scientific Catalog #**NP0007 + 5% β-mercaptoethanol (Sigma Aldrich cat. no. M6250-100ml)

2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250

16. TrypLE Express (Gibco cat. no. 12605036)

TrypLE™ Express Enzyme (1X), phenol red **Thermo Fisher Catalog #**12605036

17. 10% FBS (Thermo Fisher Scientific cat. no. 10270106) in PBS

Fetal Bovine Serum **Gibco - Thermo Fisher Scientific Catalog #**10270106

Troubleshooting

Bead preparation:

30m

1 For each IP, prepare two low-bind tubes with 30 µL Protein G beads and 300 µL Ab Binding Buffer (buffer comes from the Dynabeads Protein G IP Kit; one tube will be the no antibody control to assess background).

> Note
>
> Remember to always pipette the beads with a cut (or wide-bore) pipette tip to avoid damaging the beads.

2 Add the antibody to one tube and an equivalent amount of PBS to the other and rotate all tubes at 10 rpm, Room temperature, 00:30:00 .

30m

2.1 The amount of antibody should be appropriate for 200 µg lysate. The exact amount must be determined empirically or gleaned from the manufacturer's suggestions.

2.2 Check the Protein G Dynabeads manufacturer datasheets to ensure that the antibody you are using can be bound by Protein G.

3 Wash 1X with Ab binding buffer on the magnetic tube rack.

4 Block 01:00:00 with 1X Blocking Buffer (diluted in Lysis Buffer) on the tube rotator.

1h

5 Wash 1x with Lysis Buffer.

Lysate preparation:

3m

6 While blocking beads, remove the medium from the wells and trypsinize with 500 µL TrypLE Express.

7 Quench the TrypLE Express with 500 µL 10% FBS and transfer cells into a centrifuge tube.

8 Pellet cells at 1500 x g, 4°C, 00:03:00 . 3m

9 Wash cells with 1 mL PBS and pellet again.

10 Lyse pellets in 150 µL Lysis Buffer by gentle pipetting.

11 Incubate On ice for 00:20:00 . 20m

11.1 To reduce nonspecific binding of lysate proteins to the beads, the lysate can be centrifuged for 1000 x g, 4°C, 00:05:00 . The supernatant can then be further processed and prepared for immunoprecipitation. While this will reduce background, it can come at the expense of losing some signal if the protein of interest pellets after centrifugation. 5m

12 Quantify total protein concentration after diluting a small sample 1:10 in PBS (BCA Gold).

13 Set up samples 480 µg in 300 µL . To each sample, add: 287 µL Lysis Buffer + 13.56 µL [M] 5 Molarity (M) NaCl.

IP: 1h

14 Add 250 µL lysate (200 µg) to each tube of beads.

> Note
>
> The remaining amount of sample left can be reserved to run as an input fraction.

15 Rotate at 4 °C for 01:00:00 . 1h

16 Take off flow through and set aside to run as a flow-through fraction.

17 Wash beads 3x with Wash Buffer.

18 Allow the tubes to rotate briefly on the first wash and transfer to a new tube for the last wash.

19 Elute proteins by adding 30 µL 4X NuPAGE LDS Sample Buffer + 5% β-mercaptoethanol and boiling the sample at 95 °C for 00:05:00 . 5m

20 Optional: dilute the eluate 1:2 with Wash Buffer + [M] 10 millimolar (mM) DTT and boil again.

20.1 This will ensure total reduction of the antibody to reduce its detection by secondary antibodies upon immunoblotting. We use Veriblot (Abcam cat. no. ab131366) as a secondary antibody to more specifically detect the primary antibody used in the immunoblot rather than the denatured antibody used in the immunoprecipitation.