chromotek® Anti-IgG VHH Agarose for Immunoprecipitation

Product Code: mrIGa, mIGa, rIGa, mIG1a, mIG2aa, mIG2ba, mIG3a

Immunoprecipitation Protocol

Cell Material

The following protocol describes the preparation of a mammalian cell lysate.

For other types of cells, we recommend using 500 µg of cellextract and start the protocol with step bead equilibration.

Mammalian Cell Lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend adding protease inhibitors to the lysis buffer to prevent degradation of your target protein and its binding partners.

For one immunoprecipitation reaction, we recommend using ~10^6-10^7 cells.

1. Choice of lysis buffer:
   a. For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold lysis buffer by pipetting up and down. Supplement lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
   b. For nuclear / chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNase I (f.c. 75-150 Kunitz U/mL), $MgCl_2$ (f.c. 2.5 mM), protease inhibitor cocktail, and PMSF (f.c. 1 mM) (not included).
2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.
3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre-cooled tube and add 300 µL dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 µL of diluted lysate for further analysis (input fraction).

Bead Equilibration

1. Resuspend the beads by gently pipetting them up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

Note: Alternatively, ChromoTek Spin columns (sct-10; -20; -50) can be used to equilibrate the beads.

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Option 1: Incubation of lysate with primary antibody followed by precipitation using beads:

Binding of protein of interest

1. Add 1 to 5 µg of primary rabbit or mouse antibody specific for protein of interest to the cleared, diluted lysate.
2. Rotate end-over-end for 1 hour at +4°C.

Bead Equilibration

1. In the meantime, resuspend the beads by gently pipetting them up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

Immunoprecipitation

1. Add diluted lysate, including the primary antibody, to the equilibrated beads.
2. Rotate end-over-end for 1 hour at +4°C.

Option 2: Immobilization of primary antibody on beads followed by incubation with lysate:

Bead preparation:

1. Resuspend the beads by gently pipetting them up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.
5. Add 500 µL ice-cold dilution buffer.
6. Add 1 to 5 µg of primary rabbit or mouse antibody specific for protein of interest.
7. Rotate end-over-end for 30 min at 4 °C.
8. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

Immunoprecipitation

1. Add diluted lysate to the equilibrated beads comprising the primary antibody.
2. Rotate end-over-end for 1 hour at +4°C.

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Continue for all options:

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL wash buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the remaining.
6. Repeat this step at least twice.
7. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the wash buffer, test various salt concentrations, e.g., 150 - 500 mM, and/or add a non-ionic detergent, e.g., Triton™ X-100 (see wash buffer compatibility table for maximal concentrations).

Note: Alternatively, ChromoTek Spin columns (sct-10; -20; -50) can be used to wash the beads.

Elution with 2x SDS-sample buffer (Laemmli)

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

Note: For Western blot detection, we recommend using a Western blot-validated antibody that is derived from a different species than the immunoprecipitation antibody or at least a different clone (if possible). Using different antibodies for immunoprecipitation and Western blot detection will (1) reassure you of the identity of the detected protein and (2) avoid the staining of the heavy and light chains of the immunoprecipitation antibody, which may mask the signal for your protein(s) of interest. For secondary antibodies for Western blot, we recommend Multi-rAb HRP-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (Proteintech RGAR001) or Multi-rAb™ HRP-Goat Anti-Mouse Recombinant Secondary Antibody (H+L) (Proteintech RGAM001). If a Western blot detection antibody of a different species is unavailable, we recommend labeling the detection antibody using the FlexAble HRP Labeling kit (e.g., for rabbit IgG: Proteintech KFA005; for mouse IgG1: Proteintech KFA025), which will avoid staining of the heavy and light chains of the immunoprecipitation antibody.

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Related Products

Product	Code
anti-rabbit IgG / anti-mouse IgG VHH Magnetic Agarose for Immunoprecipitation	mrIGma, mIGma, rIGma, mIG1ma, mIG2ama, mIG2bma, mIG3ma

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