chromotek® Anti-rabbit IgG / anti-mouse IgG VHH Agarose for Immunoprecipitation

Product Code: mrIGa

Product Information

Description: The ChromoTek anti-rabbit IgG / anti-mouse IgG VHH Agarose for Immunoprecipitation consists of three VHHs (nanobodies), which are coupled to agarose beads. It can be used for the immunoprecipitation of proteins from cell extracts of various organisms such as humans, mice, dogs, or yeast using primary antibodies raised in rabbits or mice.

Applications: IP, Co-IP

Specificity/Target: The three VHHs bind specifically to rabbit IgG (Fc fragment), mouse IgG1 (Fc fragment), and mouse Ig κ light chain (Fab fragment), thus covering 100% and 98% of available rabbit and mouse primary antibodies, respectively. There is no cross-reactivity to rat, human, bovine, goat, or guinea pig antibodies. Background binding to host cell proteins from a range of organisms, such as human, mouse, and dog cell lines or yeast, is very low.

Binding capacity: >60 µg of rabbit or mouse IgG per 25 µL bead slurry

Bead Size: 90 µm (cross-linked 4% agarose beads)

Elution Buffer: 2x SDS-sample buffer (Lämmli)

Type: Nanobody

Class: Recombinant

Host: Alpaca

Shipment: Shipped at ambient temperature

Storage Buffer: 20% ethanol

Storage Condition: Upon receipt, store at +4°C. Do not freeze!

Stability: Stable for 1 year upon receipt

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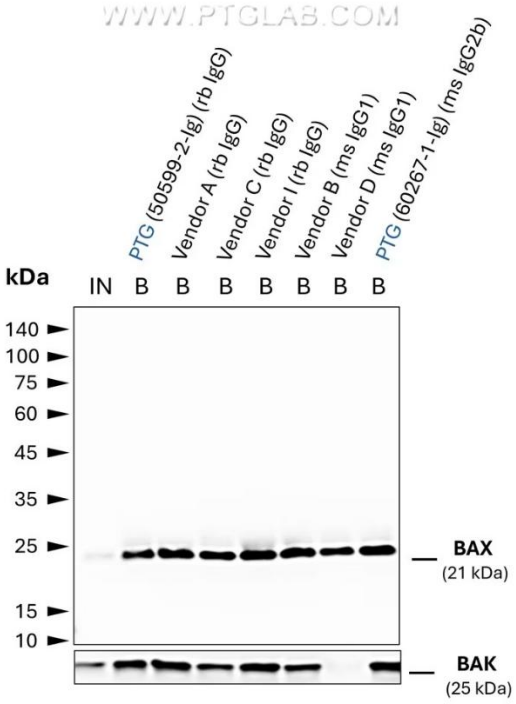
Selected Validation Data



Co-IP of BAX and BAK using anti-BAX IgGs from different vendors using anti-ms / anti-rb IgG VHH Agarose (mrIGa). Tested IgGs include those of rabbit and mouse origin with IgG1 and IgG2b subtypes. 5 µg of respective IgG was spiked into HEK293T cell lysate derived from 0.5x10^7 cells. 1% of input (IN) and 25% of bound (B) fraction was loaded onto an SDS-PAGE gel. For Western Blot analysis, BAX was detected using a polyclonal rb IgG (PTG: 50599-2-Ig) (1:2000) labeled using a conformation-specific HRP-conjugated anti-rabbit secondary. This allows a clean detection of BAX, without interfering signals of IgG heavy and light chains derived from the spiked rabbit antibodies. The presence of BAK co-precipitated with BAX was tested using a polyclonal rb IgG (PTG 29552-1-AP) 1:2000, and a conformation-specific HRP-conjugated anti-rabbit secondary. While all IgGs allowed for efficient IP of BAX, BAK was absent in the case of the primary antibody from vendor D, possibly due to the BAX:BAK interaction interface being masked by this particular IgG.

Suggested Buffer Compositions for IP

Buffer	Composition
Lysis Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% Nonidet™ P40 Substitute (adjust the pH at +4°C)
RIPA Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1% SDS, 1% Triton™ X-100, 1% deoxycholate (adjust the pH at +4°C)
Dilution Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA (adjust the pH at +4°C)
Wash Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.05% Nonidet™ P40 Substitute, 0.5 mM EDTA (adjust the pH at +4°C)

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2x SDS-sample Buffer	120 mM Tris/Cl pH 6.8, 20% glycerol, 4 % SDS, 0.04% bromophenol blue, 10% β- mercaptoethanol
Acidic Elution Buffer	200 mM glycine pH 2.5 (adjust the pH at +4°C)
Neutralization Buffer	1 M Tris pH 10.4 (adjust the pH at +4°C)

Note: Use your equivalent cell lysis buffer for other cell types such as yeast, plants, insects, and bacteria. Consider using a wash buffer without detergent for Co-IP.

Product Sizes

Product	Product Code	Size
anti-rabbit IgG / anti-mouse IgG VHH Agarose for IP	mrIGa-1	1 ml
	mrIGa-2	2 ml
	mrIGa-5	5 ml

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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 cell extract 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

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