# chromotek® HA-Trap Magnetic Agarose

Product Code: atma

## Product Information

**Description:** The ChromoTek HA-Trap Magnetic Agarose consists of an anti-HA Nanobody/VHH, which is coupled to magnetic agarose beads. It can be used for the immunoprecipitation of HA-fusion proteins from cell extracts of various organisms such as humans, mice, dogs, yeast and plants.

**Applications:** IP, Co-IP

**Specificity/Target:** Binds specifically to the HA-tag (sequence YPYDVPDYA) fused to a protein of interest at N-, C- or internal position. Please note that the affinity is highest for a C-terminal fusion. There is no cross-reactivity to other common peptide tags such as the His6-tag, FLAG-tag, Spot-Tag, V5-tag, Strep-tag or C-tag (other tags not tested). Background binding to host cell proteins from a range of organisms such as human, mouse and dog cell lines or yeast and plants is low.

**Binding capacity:** 20 µg of recombinant HA-tagged protein (~30 kDa) per 25 µL bead slurry

**Bead Size:** 40 µm (cross-linked 6 % magnetic agarose beads)

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

**Wash Buffer Compatibility:** 2 M NaCl, 5 mM DTT, 5 mM β-mercaptoethanol, 5 mM TCEP, 2% NP40, 2% Triton X-100, 0.1% SDS, 3-4 M Urea

**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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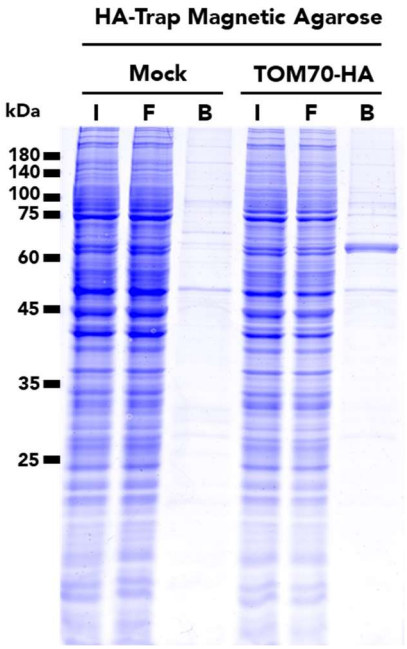
## Selected Validation Data




Immunoprecipitation of TOM70-HA fusion protein from HEK293T cells using HA-Trap Magnetic Agarose. IP was done using both un-transfected (Mock) and transfected (TOM70-HA) cells. I: Input, F: Flow-through, B: Bound.

## 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) |
| 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) |
| Peptide elution buffer | 500 µM (0.64 mg/ml) HA-peptide (ap-1) reconstituted in PBS |

*Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria. Consider using a Wash buffer without detergent for Co-IP.*
*Note: Use Peptide elution buffer for elution under native conditions.*

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## Product Sizes

| Product | Product Code | Size |
|---|---|---|
| HA-Trap Magnetic Agarose | atma-10 | 10 reactions |
| | atma-20 | 20 reactions |
| | atma-100 | 100 reactions |

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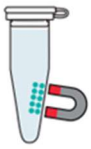
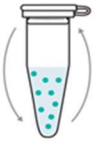
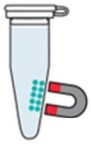
## Protocol at a glance

| | | |
|---|---|---|
| **General** | | • Perform all steps at 4°C<br>• Use your preferred cell lysis buffer and cell lysis conditions |
| **Cell Lysis** |  | • Use $10^6$-$10^7$ cells and 200 µL Lysis buffer.<br>• Perform cell lysis and clear lysate<br>• Mix 200 µl cleared lysate with 300 µL dilution buffer. |
| **Bead Equilibration** |  | • Transfer 25 µL bead slurry into a 1.5 mL tube<br>• Equilibrate beads 3x with 500 µL dilution buffer |
| **Protein binding** |  | • Add 500 µL diluted lysate to beads<br>• Rotate end-over-end for 1 hour at 4°C. |
| **Washing** |  | • Wash beads 3x with 500 µL wash buffer<br>• Transfer beads to a new tube during the last washing step |
| **Elution with SDS-sample buffer** |  | • Resuspend beads in 80 µL 2x SDS-sample buffer<br>• Boil beads for 5 min at 95°C<br>• Analyze the supernatant in SDS-PAGE/ Western Blot |

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## Immunoprecipitation Protocol

### Cell Material

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

For other type 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. Separate the beads with a magnet until the supernatant is clear. Discard the supernatant.

### Protein Binding

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

### Washing

1. Separate the beads with a magnet until the supernatant is clear.
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. Separate the beads with a magnet until the supernatant is clear. Discard the remaining supernatant.
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 information for maximal concentrations).

**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. Separate the beads with a magnet.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

**Note:** For Western blot detection we recommend HA Tag Recombinant antibody (Proteintech 81290-1-RR) and Multi-rAb HRP-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (Proteintech RGAR001).

**Elution with Acidic Elution Buffer**

1. Remove the remaining supernatant.
2. Add 50-100 µL Acidic elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Separate the beads with a magnet until the supernatant is clear.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with 5-10 µL Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

**Note:** Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.

**Elution with HA-peptide**

1. Reconstitute 1 mg HA-peptide (ap-1) in 1550 µl PBS, which results in a final concentration of 500 µM (0.64 mg/ml). Vortex for 1 min to dissolve the powder.
2. Remove the remaining supernatant.
3. Add 80 µL HA-peptide (500 µm) and mix using a pipette.
4. Incubate at 25-37 °C for 5-10 min under regular pipetting to ensure thorough mixing.
5. Separate the beads with a magnet until the supernatant is clear.
6. Transfer the supernatant to a new tube.
7. Repeat this step at least once to increase elution efficiency.

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**Note:** Elution will be most efficient for N-terminal and internal HA-tag fusions. For C-terminal HA-tag fusions, elute at 37 °C for up to 15 min.

## Related Products

| Product | Code |
|---|---|
| HA-peptide | ap-1 |
| HA-Trap Agarose | ata |
| HA-Trap Magnetic Particles M-270 | atd |
| HA-Trap Agarose Kit | atak |
| HA-Trap Magnetic Agarose Kit | atmak |
| HA-Trap Magnetic Particles M-270 | atdk |

## Contact


**Proteintech North America (HQ)**

Proteintech Group, Inc.
5500 Pearl Street, Suite 400
Rosemont, IL 60018 USA

1-888-472-4522
proteintech@ptglab.com

**Proteintech Europe**

Transmission (6th Fl)
6 Atherton Street
M3 3GS, Manchester, UK

+44 161 839 3007
europe@ptglab.com

**ChromoTek & Proteintech Germany**

Fraunhoferstr. 1
82152, Planegg-Martinsreid
Germany

+49 89 124 148 850
germany@ptglab.com

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## Disclaimer

For research use only (RUO), not for diagnostic or therapeutic use.