

# chromotek® HA-Trap Magnetic Agarose Kit

Product Code: atmak

## Product Information

**Description:** The ChromoTek HA-Trap Magnetic Agarose Kit consists of an anti-HA Nanobody/VHH, which is coupled to magnetic agarose beads. It also contains lysis, wash, and elution buffers that 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 organism 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

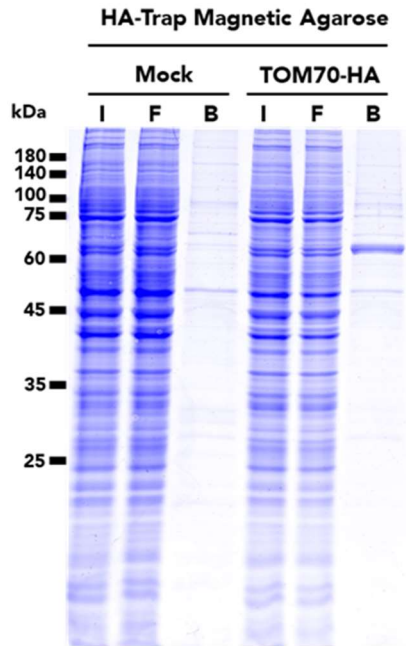
**Storage Condition:** Upon receipt store at +4°C. Do not freeze!

**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

## Kit Components

Component	Composition	Quantity
HA-Trap Magnetic Agarose	Anti-HA VHH cross-linked with magnetic agarose beads	20 rxns (500 ul slurry)
Lysis Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute, 0.09 % sodium azide	30 mL
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, 0.09 % sodium azide	30 ml
Dilution Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.018 % sodium azide	50 mL (after dilution with 40 mL water)
Wash Buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.05 % Nonidet™ P40 Substitute, 0.5 mM EDTA, 0.018 % sodium azide	50 mL (after dilution with 40 mL water)
Acidic elution buffer	200 mM glycine pH 2.5	3 x 1 mL

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*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.*

## Required Buffer Solutions

Buffer	Composition
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20% glycerol, 4% SDS, 0.04% bromophenol blue, 10% $\beta$ -mercaptoethanol
Neutralization buffer	1M Tris pH 10.4 (adjust the pH at +4°C)
Peptide elution buffer	500 $\mu$ M (0.64 mg/ml) HA-peptide (ap-1) reconstituted in PBS

## Product Sizes

Product	Product Code	Size
HA-Trap Magnetic Agarose Kit	atmak-20	20 reactions including buffers

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## Protocol at a glance

### General

- Perform all steps at 4°C
- Use your preferred cell lysis buffer and cell lysis conditions

### Cell Lysis



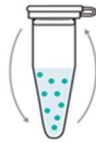
- Use  $10^6$ - $10^7$  cells and 200  $\mu$ L Lysis buffer.
- Perform cell lysis and clear lysate
- Mix 200  $\mu$ L cleared lysate with 300  $\mu$ L dilution buffer.

### Bead Equilibration



- Transfer 25  $\mu$ L bead slurry into a 1.5 mL tube
- Equilibrate beads 3x with 500  $\mu$ L dilution buffer

### Protein binding



- Add 500  $\mu$ L diluted lysate to beads
- Rotate end-over-end for 1 hour at 4°C.

### Washing



- Wash beads 3x with 500  $\mu$ L wash buffer
- Transfer beads to a new tube during the last washing step

### Elution with SDS-sample buffer



- Resuspend beads in 80  $\mu$ L 2x SDS-sample buffer
- Boil beads for 5 min at 95°C
- 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  $\sim 10^6$ - $10^7$  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.

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4. Resuspend beads in 500  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L PBS, which results in a final concentration of 500  $\mu$ M (0.64 mg/ml). Vortex for 1 min to dissolve the powder.
2. Remove the remaining supernatant.
3. Add 80  $\mu$ L HA-peptide (500  $\mu$ M) 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.

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7. Repeat this step at least once to increase elution efficiency.

**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 Agarose Kit	atak
HA-Trap Magnetic Agarose	atma
HA-Trap Magnetic Particles M-270	atd
HA-Trap Magnetic Particles M-270 Kit	atdk

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