chromotek® Ubiquitin-Trap Agarose Kit

Product Code: utak

Product Information

Description: The ChromoTek Ubiquitin-Trap Agarose Kit consists of an anti-Ubiquitin Nanobody/VHH, which is coupled to agarose beads. It also contains lysis, wash, and elution buffers that can be used for the immunoprecipitation of ubiquitin and ubiquitinylated proteins from the cell extracts of various species including human, mouse, hamster, dog, yeast, and plants.

Applications: IP, Co-IP

Specificity/Target: Binds to monomeric ubiquitin, ubiquitin chains, and ubiquitinylated proteins. Ubiquitin chains may be linked via lysines; linkages via lysines such as K11, K48 and K63 are compatible with binding by the Ubiquitin-Trap (further linkage types also likely to be compatible, but not tested).

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

Wash Buffer Compatibility: 2M NaCl, 5 mM DTT, 0 mM β-mercaptoethanol, 5 mM TCEP, 2% NP40, 2% Triton X-100, 0% SDS, 2-3 M Urea

Type: Nanobody

Class: Recombinant

Host: Camelid

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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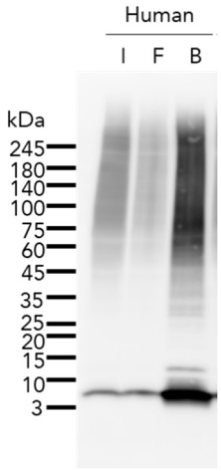
Selected Validation Data



Immunoprecipitation of Ubiquitin from HEK293T cell extracts (treated with MG-132) using Ubiquitin-Trap Agarose beads.

Kit Components

Component	Composition	Quantity
Ubiquitin-Trap Agarose	Anti-ubiquitin VHH cross-linked with 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

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.

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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% β-mercaptoethanol
Neutralization buffer	1M Tris pH 10.4 (adjust the pH at +4°C

Product Sizes

Product	Product Code	Size
Ubiquitin-Trap Agarose Kit	utak-20	20 reactions including buffers

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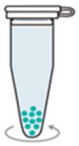
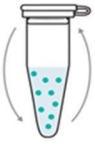
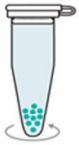
Protocol at a glance

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 µL Lysis buffer. • Perform cell lysis and clear lysate • Mix 200 µl cleared lysate with 300 µL dilution buffer.
Bead Equilibration		• Transfer 25 µL bead slurry into a 1.5 mL tube • Equilibrate beads 3x with 500 µL dilution buffer
Protein binding		• Add 500 µL diluted lysate to beads • Rotate end-over-end for 1 hour at 4°C.
Washing		• Wash beads 3x with 500 µL wash buffer • 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 • 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.

Note: Precipitation of ubiquitinylated proteins may be facilitated by treating cells with proteasome inhibitors prior to harvesting. A commonly used inhibitor is MG-132 (also known as MG132 or carbobenzoxy-Leu-Leu-leucinal), for example. Exact conditions must be optimized for each cell type etc.; a good starting point is to incubate cells with f.c. 5-25 µM MG-132 for 1-2 h. Over-exposure to MG-132 can lead to cytotoxic effects, however.

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.

Note: If necessary, deubiquitylates (DUBs), i.e. ubiquitin hydrolases, may be inhibited by the addition of either EDTA or EGTA and either f.c. 5-10 mM iodoacetamide (IAA) or N-ethylmaleimide (NEM). Concentrations may need to be optimized for each cell type, growth conditions etc.

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.

Protein Binding

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

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 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 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 Ubiquitin Recombinant antibody (80992-1-RR) or Ubiquitin Polyclonal antibody (10201-2-AP) in combination with HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (SA00001-2)

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. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
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.

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

Related Products

Product	Code
Ubiquitin-Trap Agarose	uta
Ubiquitin-Trap Magnetic Agarose	utma
Ubiquitin-Trap Magnetic Agarose Kit	utmak

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