chromotek® His Fab-Trap® Magnetic Particles M-270

Product Code: hfd

Product Information

Description: The ChromoTek His Fab-Trap® Magnetic Particles M-270 consists of an anti-His-tag Fab-fragment, which is coupled to magnetic particles. It can be used for the immunoprecipitation of His-fusion proteins from cell extracts of various organisms. It is highly recommended when very large proteins/complexes are being investigated.

Applications: IP, Co-IP

Specificity/Target: Binds specifically to the His-tag (also known as hexahistidine or His_6-tag) fused to a protein of interest at N-, C- or internal position. Please note that the affinity is highest for a C-terminal fusion (dissociation constant K_D of 10 nM for a C-terminal His-tag and 220 nM for an N-terminal His-tag).

Binding capacity: 1 µg of recombinant His-tagged protein (~30 kDa) per 25 µL bead slurry

Bead Size: Magnetic Particles M-270; size: 2.8 µM

Elution Buffer: 2x SDS-sample buffer (Lämmli), 200 mM glycine pH 2.5, 100 µM His-Peptide

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

Ligand: Anti-His_6-Fab-fragment

Clone: Fab fragment of monoclonal mouse IgG1 1B7G5

Host: Mouse

Shipment: Shipped at ambient temperature

Storage Buffer: PBS pH 7.4. Preservative: 0.09 % sodium azide

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

Stability: Stable for 1 year upon receipt

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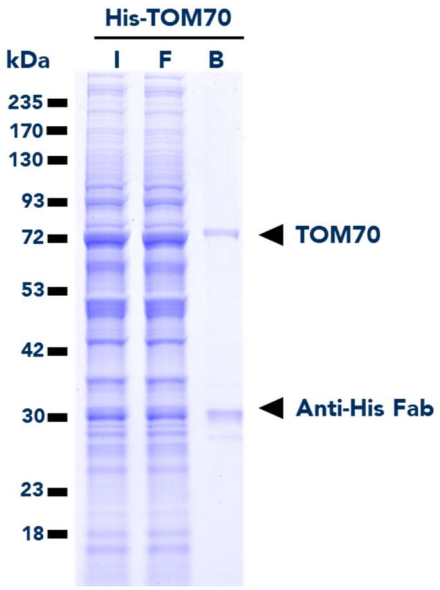
Selected Validation Data



Immunoprecipitation of His-TOM70 fusion protein using His Fab-Trap® Magnetic Particles M-270. 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	100 µM His-Peptide (hp-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.

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Product Sizes

Product	Product Code	Size
His Fab-Trap® Magnetic Particles M-270	hfd-10	10 reactions
	hfd-20	20 reactions
	hfd-100	100 reactions
	hfd-200	200 reactions
	hfd-400	400 reactions

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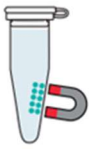
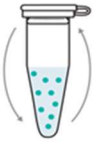
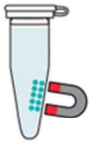
Protocol at a glance

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.

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.

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 His-Peptide

1. Reconstitute 1 mg His-Peptide (hp-1) in 1190 µl PBS, which results in a final concentration of 1 mM. Vortex for 1 min to dissolve the powder and dilute to 100 µM for the working solution.
2. Remove the remaining supernatant.
3. Add 80 µL His-Peptide (100 µm) and mix using a pipette.
4. Incubate at 4-37 °C for 5 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.

Note: Elution will be most efficient for N-terminal and internal His-tag fusions even at 4 °C. For C-terminal His-tag fusions, elute at 37 °C for up to 10 min.

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Related Products

Product	Code
His Fab-Trap® Agarose	hfa
His Fab-Trap® Agarose Kit	hfak
His Fab-Trap® Magnetic Agarose	hfma
His Fab-Trap® Magnetic Agarose Kit	hfmak
His Fab-Trap® Magnetic Particles M-270 Kit	hfdk
His-Peptide	hp-1

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