chromotek® SUMO1-Trap Agarose, Kit for Immunoprecipitation

Product Code: s1tak

Product Information

Description: The ChromoTek SUMO1-Trap Agarose, Kit for Immunoprecipitation consists of an anti-SUMO-Tag NANOBODY®/VHH, which is coupled to agarose beads. It also contains lysis, wash, and elution buffers that can be used for the immunoprecipitation of SUMO1 proteins from cell extracts of various organisms.

Applications: IP, Co-IP

Specificity/Target: Binds specifically to human SUMO1 (Small Ubiquitin-like Modifier 1). The ChromoTek SUMO1-Trap Agarose will immunoprecipitate both monovalent SUMO1 and SUMO1 chains formed by the process of SUMOylation. In addition to human SUMO1, it will also bind SUMO1 homologs from other mammals.

Binding capacity: 25 µg SUMO1 fusion protein (40 kDa) per 25 µl bead slurry

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

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

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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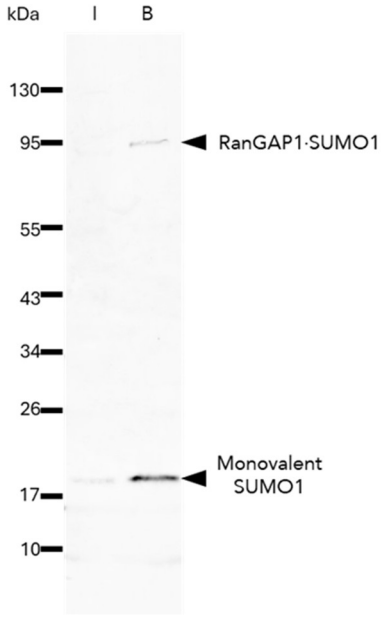
Selected Validation Data



The SUMO1-Trap Agarose (s1ta) was used to immunoprecipitate endogenous SUMO1 and SUMO1ylated proteins from HEK293T cells. Samples of the input lysate (I), and bound (B) fraction were analyzed using Western blot using 10329-1-AP.
SUMO1 and the commonly SUMO1ylated protein RanGAP1 are highly enriched using the SUMO1-Trap.

Kit Components

Component	Composition	Quantity
SUMO1-Trap Agarose	Anti-SUMO1 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
SUMO-Tag-Trap Agarose, Kit for Immunoprecipitation	s1tak-20	20 reactions including buffers

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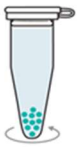
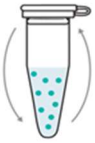
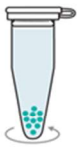
Protocol at a glance

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 types of cells, we recommend using 500 µg of cell extract and start the protocol with step Bead equilibration.

Note: Precipitation of SUMOylated proteins may be facilitated by treating cells with proteasome inhibitors or other stressors prior to harvesting. Commonly used stressors are MG-132 (also known as MG132), arsenic trioxide or interferon α, for example. Exact conditions must be optimized for each cell type etc.

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, SUMO proteases may be inhibited by the addition of inhibitors such as PR-619, and either 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.

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.

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

Related Products

Product	Code
SUMO1-Trap Agarose	s1ta
SUMO1-Trap Magnetic Agarose	s1tma
SUMO1-Trap Magnetic Agarose, Kit for Immunoprecipitation	s1tmak

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

Proteintech Germany

Fraunhoferstr. 1
82152, Planegg-Martinsried
Germany

+49 89 124 148 850
germany@ptglab.com

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