Halo-Trap Magnetic Agarose

Product code: otma

Introduction

The ChromoTek Halo-Trap Magnetic Agarose consists of an anti-Halo-tag Nanobody (VHH), which is covalently bound to magnetic agarose beads. Halo-Trap Magnetic Agarose is used to immunoprecipitate Halo-tagged fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc. in the presence or absence of a covalently bound ligand. The interaction between Halo-Trap and the Halo-tagged fusion protein is reversible.

Properties

Ligand: Anti-Halo-tag single domain antibody fragment (VHH, Nanobody)

Reactivity: Specifically binds to Halo-tag (modified variant of the bacterial haloalkane dehalogenase enzyme from *Rhodococcus rhodochrous*) in the absence or presence of covalently bound chloralkane-based ligands.

Binding capacity: 12.5 µg of recombinant Halo-tag per 25 µL bead slurry

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

Buffer compatibility: See *Wash buffer compatibility table*.

Storage buffer: 20 % ethanol

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

Stability: Stable for 1 year upon receipt.

Shipment: Shipped at ambient temperature.

RRID: AB_2892995

Suggested buffer compositions

Required buffer solutions

NEW: Update of Wash buffer components.

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 or 100 mM citric acid pH 3.0 (adjust the pH at +4°C)
Neutralization buffer	1 M Tris pH 10.4 (adjust the pH at +4°C)

Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria.

Note: Consider using a Wash buffer without detergent for co-immunoprecipitation.

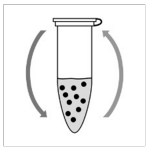
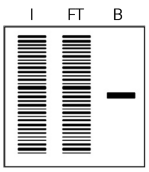
Wash buffer compatibility table

Buffer ingredients	Max. concentration
DTT	10 mM
NaCl	2 M
Nonidet™ P40 Substitute	tested up to 2 %
SDS	0 %
TCEP	10 mM
Triton™ X-100	tested up to 2 %
Urea	2 M

Product sizes

Product	Product code	Size
Halo-Trap Magnetic Agarose	otma-10	10 reactions (250 µL slurry)
	otma-20	20 reactions (500 µL slurry)
	otma-100	100 reactions (2.5 mL slurry)
	otma-200	200 reactions (5 mL slurry)
	otma-400	400 reactions (10 mL slurry)
Halo-Trap Magnetic Agarose Kit	otmak-20	20 reactions (500 µL slurry) including buffers

Protocol at a glance

General		• Perform all steps at +4°. • 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.

Immunoprecipitation protocol

Cell material

The following protocol describes the preparation of 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 to add 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:
   - 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).
   - For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (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 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 table* 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 Halo antibody [28A8] (28a8-20; -100).

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.

Product overview and related products

Halo-tag toolbox	Product code
Halo-Trap Agarose	ota-10; -20; -100
Halo-Trap Agarose Kit	otak-20
Halo-Trap Magnetic Agarose	otma-10; -20; -100
Halo-Trap Magnetic Agarose Kit	otmak-20
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin columns	sct-10; sct-20; sct-50
Halo VHH, recombinant binding protein	ot-250
Halo antibody [28A8]	28a8-20; -100

For product details, information, and ordering visit www.chromotek.com.

Contact

support@chromotek.com

ChromoTek GmbH
Am Klopferspitz 19
82152 Planegg-Martinsried
Germany
phone: +49 89 124 148 80
fax: +49 89 124 148 811

ChromoTek Inc.
62-64 Enter Lane
Islandia, NY 11749
USA
phone: 631 501 1058
fax: 631 501 1060

Disclaimer

Only for research applications, not for diagnostic or therapeutic use!