HaloTag-Trap_A for Immunoprecipitation of HaloTag-Fusion Proteins from mammalian cell extract

For research applications only. Not for diagnostic or therapeutic use.

Introduction

The HaloTag is a modified variant of the bacterial haloalkane dehalogenase enzyme from *Rhodococcus rhodochrous* which is designed to form a covalent bond with reactive chloralkane-based ligands. HaloTag-Trap_A utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of agarose beads. The HaloTag-Trap_A enables the purification of any protein of interest fused to the HaloTag even after ligand binding. The interaction between the HaloTag-Trap and the HaloTag is reversible.

Specificity

HaloTag

Size

Reagent	Code	Quantity
HaloTag-Trap_A	ota-20	20 reactions (0.5 ml slurry)
HaloTag-Trap_A	ota-100	100 reactions (2.5 ml slurry)
HaloTag-Trap_A	ota-200	200 reactions (5 ml slurry)
HaloTag-Trap_A	ota-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH
Binding capacity: 10 µl HaloTag-Trap® _A slurry binds 3 - 4 µg of recombinant HaloTag

Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 1 year. Do not freeze.

Required buffers

Suggested buffer compositions for mammalian cells

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% Nonidet P40 Substitute
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
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Elution buffer	200 mM glycine pH 2.5, or 100 mM citrate acid pH 3.0
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

Note

In case you are experiencing a low binding efficiency when working with RIPA buffer, try using a modified lysis buffer without SDS.

In case you want to apply harsh washing conditions, you can add the following substances to the wash buffer (not the dilution buffer):

Substance	Concentration
NaCl	1 M
Nonidet P40 Substitute	2%
Triton X-100	1%
DTT	10 mM
Urea	4 M
Tested up to specified value. Higher concentrations may be compatible.	

Related products

HaloTag Toolbox	Code
HaloTag-binding protein (uncoupled)	ot-250
HaloTag-Trap_A Kit	otak-20
Binding control agarose beads	bab-20
Spin columns	sct-10; sct-20; sct-50

Protocol for Immunoprecipitation of HaloTag-Fusion Proteins using HaloTag-Trap®_A

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a HaloTag fusion protein of interest is recommended. To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500x g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS, gently resuspending the cells. After washing:

Lyse cells

1. Resuspend cell pellet in 200 µl ice-cold Lysis buffer by pipetting or using a syringe.

   note: Supplement lysis buffer with protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included).

   optional for nuclear proteins: Resuspend cell pellet in 200 µl ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 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 with extensively pipetting every 10 min.

3. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer lysate to a pre-cooled tube. Add 300 µl Dilution buffer supplemented with PMSF (f.c. 1 mM) and protease inhibitor cocktail (not included) to lysate. Discard pellet.

   note: At this point cell lysate may be put at -80°C for long-term storage.

Equilibrate beads

4. Resuspend HaloTag-Trap_A beads and pipette 25 µl bead slurry into 500 µl ice-cold Dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

Bind proteins

5. Add diluted lysate (step 3) to equilibrated HaloTag-Trap_A beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Tumble end-over-end for 1 hour at 4°C.
6. Centrifuge at 2.500x g for 2 min at +4°C. If required, save 50 µl supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend HaloTag-Trap_A beads in 500 µl ice-cold Wash buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

Elute proteins

Elution with SDS-sample Buffer (Laemmli)

a. Resuspend beads in 80 µl 2x SDS-sample buffer.
b. Boil beads for 5 min at 95°C to dissociate immunocomplexes from beads.
c. Spin down beads for 2 min at 2,500x g at room temperature. Collect supernatant and analyze samples by SDS-PAGE and Western blot.

Elution with Elution Buffer (low pH)

- Resuspend beads in 50–100 µl Elution buffer and permanently pipet up and down for 30-60 seconds at room temperature.
- Spin down beads for 2 min at 2,500x g at room temperature.
- Transfer supernatant to new tube.
- Immediately neutralize solution with 5-10 µl 1 M Tris pH 10.4.
- To increase elution efficiency, steps a-d may be repeated.

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