Mdm4/ HdmX _A Kit for Immunoprecipitation of Mdm4/ HdmX from mammalian cell extract

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

Introduction

Double minute 4 protein (Mdm4/ HdmX/ MdmX) plays a role in cell cylce control and apoptosis by inhibition of p53 and p73.
Mdm4/ HdmX-Trap is excellent for fast and efficient one-step isolation of Mdm4/ HdmX and its interacting factors from cellular extract. Isolated Mdm4/ HdmX protein may be used further for immunoblot analysis or mass spectrometry.
Mdm4/ HdmX-Trap utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of agarose beads.

Specificity

Species-Reactivity: tested on human, mouse and hamster
Epitope: 1-129 aa

Content

Reagent	Code	Quantity
Mdm4/ HdmX-Trap_A kit	htak-20	20 reactions (0.5 ml resin)
Lysis buffer (CoIP)		30 ml
RIPA buffer		30 ml
5x Wash / Dilution buffer		20 ml
Elution buffer		3 ml

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH

Stability and Storage

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

Required solutions

Buffer composition (as provided in the kit)

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40
10x 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

Related products

Mdm4/ HdmX Toolbox	Code
Mdm4/ HdmX-Trap protein	ht-250
Mdm4/ HdmX-Trap_A	hta-20
Blocked agarose beads	bab-20
Spin columns	sct-10; sct-20; sct-50

Support

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

Protocol for Immunoprecipitation of Mdm4/ HdmX-Fusion Proteins using Mdm4/ HdmX-Trap_A

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) 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 500 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 inhibitors and 1 mM PMSF (not included).

   optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$, protease inhibitors and 1 mM PMSF (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 to lysate. Discard pellet.

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

   optional: Add 1 mM PMSF and protease inhibitors (not included) to dilution buffer.

Equilibrate beads

4. Vortex Mdm4/ HdmX_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 Mdm4/ HdmX_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 Mdm4/ HdmX_A beads in 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

   optional: Increase salt concentration in the second washing step up to 500 mM.

Elute proteins

8. Resuspend Mdm4/ HdmX_A beads in 100 µl 2x SDS-sample buffer.
9. Boil resuspended Mdm4/ HdmX_A beads for 10 min at 95°C to dissociate immunocomplexes from Mdm4/ HdmX_A beads. Mdm4/ HdmX_A beads can be collected by centrifugation at 2.500x g for 2 min at 4°C and SDS-PAGE is performed with the supernatant.
10. *optional instead of steps 8 and 9: elute bound proteins by adding 50 µl 0.2 M glycine pH 2.5 (incubation time: 30 sec under constant mixing) followed by centrifugation. Transfer the supernatant to a new tube and add 5 µl 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*