

Mdm4/ HdmX _A 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 cycle 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
Epitope: 1-129 aa

Content	Reagent	Code	Quantity
	Mdm4/ HdmX-Trap_A	hta-20	20 reactions (0.5 ml slurry)
	Mdm4/ HdmX-Trap_A	hta -100	100 reactions (2.5 ml slurry)
	Mdm4/ HdmX-Trap_A	hta -200	200 reactions (5 ml slurry)
	Mdm4/ HdmX-Trap_A	hta -400	400 reactions (10 ml slurry)

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 **Suggested buffer composition**

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	100 mM Tris/Cl pH 7.5; 1,5 M NaCl; 50 mM EDTA; 1% SDS; 10% Triton X-100; 10% Deoxycholate
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Glycine-elution buffer	200 mM glycine pH 2.5
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

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

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

Harvest cells

For one immunoprecipitation reaction the use of $\sim 10^6 - 10^7$ 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 μ l 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 MgCl₂, 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 μ l 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.
We recommend that during incubation with the beads the final concentration of detergents does not exceed 0.2% to avoid unspecific binding to the matrix. If required, use more dilution buffer to dilute the supernatant accordingly.

Equilibrate beads

4. Vortex Mdm4/ HdmX_A beads and pipette 25 μ l bead slurry into 500 μ l 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 μ l 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 μ l supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend Mdm4/ HdmX_A beads in 500 μ l 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 μ l 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 μ l 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 μ l 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*

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