#M20013

Anti-HA-Tag Mouse mAb (Agarose Conjugated)

☐ 500 µl (10-25 immunoprecipitations)

☐ 5 ml (100-250 immunoprecipitations)

Abmart

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DESCRIPTION

Anti-HA-Tag Mouse mAb (Agarose Conjugated) is a monoclonal anti-HA antibody covalently linked to agarose; the agarose enables immunoprecipitation (IP) of HA tagged proteins or co-immunoprecipitation (Co-IP) of their interacting partners.

SOURCE

This Abmart monoclonal antibody is produced by immunizing animals with a synthetic peptide containing the influenza hemagglutinin epitope (YPYDVPDYA) (KLH-coupled).

SPECIFICITY

Anti-HA-Tag Mouse mAb detects transfected proteins containing the HA epitope tag.

STORAGE

The product is supplied as a 50% slurry in storage buffer (1× PBS, pH 7.4, containing 0.1% NaN_3). Store the product at 4°C and do not freeze.

REACTIVITY

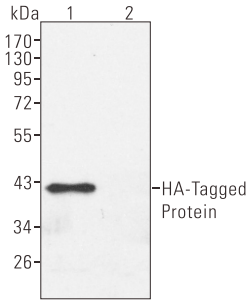
All

ISOTYPE

Mouse IgG1

RECOMMENDED ELUTION BUFFER

0.2 M Glycine, pH 2.2



HEK 293T cells were transfected with HA-tagged protein or not, and 100 µl cell lysate (about 100 µg total protein) was incubated with 30 µl 50% slurry of Anti-HA Agarose for 3 h at 4°C. After washing, the beads were eluted by 30 µl elution buffer twice. After neutralization of the eluant, 6 µl 6× SDS loading buffer was added. Then 20 µl sample was subjected to the SDS-PAGE. Blot was probed with Anti-HA-Tag Mouse mAb.
Lane 1: 1st Elution with elution buffer.
Lane 2: IP of untransfected HEK 293T lysate.

IMMUNOPRECIPITATION PROCEDURE

The work can be performed in 1.5 ml micro-centrifuge tubes or in spin columns.

1. Thoroughly resuspend the Anti-HA Agarose by inverting the tube or vial several times.
2. Add 20-50 µl 50% slurry of Anti-HA Agarose into cell lysate using a wide-bore pipette tip.
   Note: The lysate should be fresh, and for a well expressed tagged protein, 200 µl lysate (200-500 µg total protein) usually yields a good IP result.
3. Incubate with gentle mixing for 2 h to overnight at 4°C.
4. Wash the beads with 1 ml TBS buffer or lysis buffer, such as RIPA (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate), centrifuge for 3 min at 2,000× g, and discard the supernatant. Wash 3 times, avoid losing beads during washes.
5. Elution of the HA tagged protein.
   Option 1. Elution with elution buffer.
   Add 30-50 µl elution buffer to the beads, gently tap the tube to mix well, immediately centrifuge for 3 min, transfer the supernatant very carefully to a fresh tube (Avoid transferring any beads).
   Note: Neutralize the eluant immediately by add 1 µl of 1.5 M Tris, pH 9.0 per 20 µl Elution buffer.
   Option 2. Elution with HA peptide
   Add 30-50 µl HA peptide solution (100 µg/ml HA peptide in TBS buffer), gently tap the tube to mix well, incubate for 10 min, centrifuge for 3 min, and transfer the supernant to a fresh tube. TBS buffer: 50 mM Tris HCl, 150 mM NaCl, pH 7.4.
   Option 3. Elution with SDS loading buffer
   Add 30 µl 2× SDS loading buffer, gently tap the tube to mix well, boil at 100°C for 5 min, centrifuge for 3 min, transfer the supernatant to a fresh tube.
   Note: in this case, the supernatant contains not only the binding proteins, but also IgG (heavy and light chains).
6. Prepare SDS-PAGE gel for western blotting or proceed to other assays.

COMPANION PRODUCTS

#M20001 His-Tag (2A8) Mouse mAb
#M20002 Myc-Tag (19C2) Mouse mAb
#M20003 HA-Tag (26D11) Mouse mAb
#M20004 GFP-Tag (7G9) Mouse mAb
#M20007 GST-Tag (12G8) Mouse mAb
#M20008 DYDDDDDK-Tag (3B9) Mouse mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)
#M20012 Anti-Myc-Tag Mouse mAb (Agarose Conjugated)
#M20018 Anti-DYKDDDDK-Tag Mouse mAb (Agarose Conjugated) (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)