

#M20012

Anti-Myc-Tag Mouse mAb (Agarose Conjugated)

- 500 µl (10-25 immunoprecipitations)
- 5 ml (100-250 immunoprecipitations)



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DESCRIPTION

Anti-Myc-Tag Mouse mAb (Agarose Conjugated) is a monoclonal anti-Myc antibody covalently linked to agarose; the agarose enables immunoprecipitation (IP) of Myc 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 (KLH-coupled) corresponding to residues 410-419 of human c-Myc (EQKLISEEDK).

SPECIFICITY

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

STORAGE

The product is supplied as a 50% slurry in storage buffer (1× PBS, pH 7.4, containing 0.1% NaN₃). Store the product at 4°C and do not freeze.

REACTIVITY

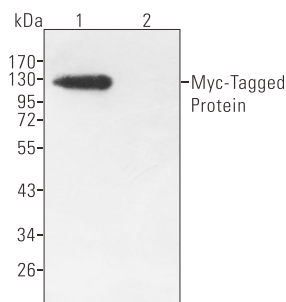
All

ISOTYPE

Mouse IgG1

RECOMMENDED ELUTION BUFFER

0.2 M Glycine, pH 2.5



HEK 293T cells were transfected by Myc-tagged protein, and 100 µl cell lysate (about 100 µg total protein) was incubated with 30 µl 50% slurry of Anti-Myc 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-Myc-Tag Mouse mAb.

Lane 1: 1st Elution with elution buffer.
Lane 2: 2nd Elution with elution buffer.

IMMUNOPRECIPITATION PROCEDURE

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

1. Thoroughly resuspend the Anti-Myc Agarose by inverting the tube or vial several times.
2. Add 20-50 µl 50% slurry of Anti-Myc 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 Myc 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 Myc peptide
Add 30-50 µl Myc peptide solution (100 µg/ml Myc peptide in TBS buffer), gently tap the tube to mix well, incubate for 10 min, centrifuge for 3 min, and transfer the supernatant 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 DYDDDDK-Tag (3B9) Mouse mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)
- #M20013 Anti-HA-Tag Mouse mAb (Agarose Conjugated)
- #M20018 Anti-DYKDDDDK-Tag Mouse mAb (Agarose Conjugated) (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)