

#M20018

Anti-DYKDDDDK-Tag Mouse mAb(Agarose Conjugated) (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)



Orders ■ 400-6123-828

orders@ab-mart.com

Web ■ www.ab-mart.com.cn

500 µl (10-25 immunoprecipitations)

5ml (100-250 immunoprecipitations)

DESCRIPTION

Anti-DYKDDDDK-Tag Mouse mAb (Agarose Conjugated) is a monoclonal anti-DYKDDDDK antibody covalently linked to agarose; the agarose enables immunoprecipitation (IP) of DYKDDDDK 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 epitope DYKDDDDK (KLH-coupled).

SPECIFICITY

Anti-DYKDDDDK-Tag Mouse mAb detects transfected proteins containing the DYKDDDDK 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

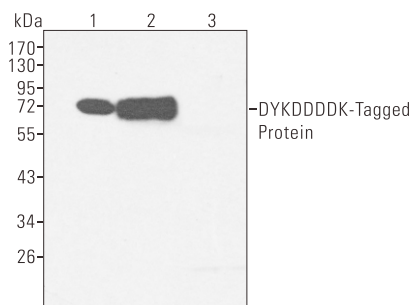
All

ISOTYPE

Mouse IgG2b

RECOMMENDED ELUTION BUFFER

0.2 M Glycine, pH 2.5



HEK 293T cells were transfected with DYKDDDDK-tagged protein or not, and 100 µl cell lysate (about 100 µg total protein) was incubated with 30 µl 50% slurry of Anti-DYKDDDDK Agarose for 3 h at 4°C. After washing, the beads were eluted by 60 µl elution buffer. After neutralization of the eluant, 12 µl 6× SDS loading buffer was added. Then 20 µl sample was subjected to the SDS-PAGE. Blot was probed with Anti-DYKDDDDK-Tag Mouse mAb. Lane 1: Total cell lysate of transfected HEK 293T cells. Lane 2: Elution with elution buffer. Lane 3: 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-DYKDDDDK Agarose by inverting the tube or vial several times.
2. Add 20-50 µl 50% slurry of Anti-DYKDDDDK 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 DYKDDDDK 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 DYKDDDDK peptide
Add 30-50 µl DYKDDDDK peptide solution (100 µg/ml DYKDDDDK 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 DYDDDDDK-Tag (3B9) Mouse mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)
- #M20012 Anti-Myc-Tag Mouse mAb (Agarose Conjugated)
- #M20013 Anti-HA-Tag Mouse mAb (Agarose Conjugated)