M5 Anti-DYKDDDDK-Tag mAb (Agarose conjugated)使用说明书

产品名称	单位	货号	Beads 含量
M5 Anti-DYKDDDDK-Tag mAb	1ml	MF098-01	0.5ml
(Agarose conjugated)	2ml	MF098-02	1ml
	5ml	MF098-05	2.5ml
	10ml	MF098-10	5ml

【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.

【BACKGROUND】
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 coimmunoprecipitation (Co-IP) of their interacting partners.

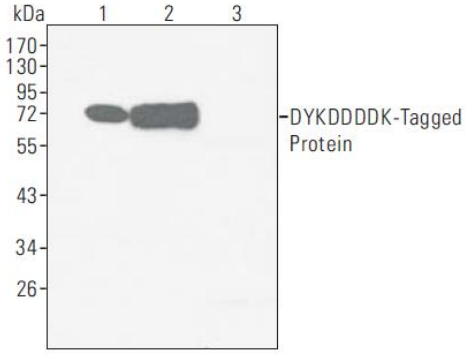
【SOURCE】
This monoclonal antibody is produced by immunizing animals with a synthetic peptide containing epitope DYKDDDDK (KLH-coupled).

【SPECIFICITY】: Anti-DYKDDDDK-Tag Mouse mAb detects transfected proteins containing the DYKDDDDK epitope tag.

【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 widebore 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 2,000x 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 1µl 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 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 2x 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.

【备注】
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