

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

产品名称	单位	货号
M5 Anti-Myc-Tag mAb (Agarose conjugated)	1ml	MF096-01
M5 Anti-Myc-Tag mAb (Agarose conjugated)	5ml	MF096-05

[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.

[BACKGROUND]

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]

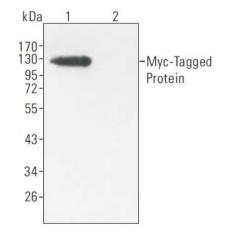
This monoclonal antibody is produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to residues 410-419 of human c-Myc (EQKLISEEDK).

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

[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 μ l cell lysate (about 100 μ g total protein) was incubated with 30 μ l 50% slurry of Anti-Myc Agarose for 3 h at 4°C. After washing, the beads were eluted by 30 μ l elution buffer twice. After neutralization of the eluant, 6 μ l 6× SDS loading buffer was added. Then 20 μ l 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 μ l elution buffer to the beads, gently tap the tube to mix well, 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 μ l Myc peptide solution (100 μ g/ml Myc 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 μ l 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).

Prepare SDS-PAGE gel for western blotting or proceed to other assays.