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AM20780AG-N Monoclonal Antibody to DYKDDDDK Epitope Tag - Agarose

Alternate names: D-tag, ECS Epitope Tag, ECS-tag, FLAG Epitope Tag, FLAG-tag

Quantity: 0.5 ml

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 co-immunoprecipitation (Co-IP) of their interacting partners.

Host / Isotype: Mouse / IgG2b

Clone: 3B9

Immunogen: Synthetic peptide containing epitope DYKDDDDK (KLH-coupled).

Format:
State: Liquid purified Ig fraction
Buffer System: 50% slurry in storage buffer (1× PBS, pH 7.4, containing 0.09% sodium azide).
Recommended elution buffer: 0.2 M Glycine, pH 2.5
Label: Agarose

Applications:
Immunoprecipitation.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This antibody reacts to DYKDDDDK Epitope Tag.
Species: Human.
Other species not tested.

Storage:
Store the antibody undiluted at 2-8°C.
DO NOT FREEZE!
Shelf life: one year from despatch.

Protocols:
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 g 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 g/ml DYKDDDDK 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