

AM20780AG-L**Monoclonal Antibody to DYKDDDDK Epitope Tag - Agarose**

Alternate names:	D-tag, ECS Epitope Tag, ECS-tag, FLAG Epitope Tag, FLAG-tag
Quantity:	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 μ l 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 μ l of 1.5 M Tris, pH 9.0 per 20 μ l Elution buffer.

Option 2. Elution with DYKDDDDK peptide

Add 30-50 μ l 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 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 μ l 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