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Monoclonal Antibody to HA Epitope Tag (YPYDVPDYA) - Agarose

Alternate names: HA Tag, HA-Tag, Hemagglutinin Tag

Catalog No.: AM20783AG-N

Quantity: 0.5 ml

Background: Plasmid vectors for the expression of coding regions of eukaryotic genes in bacterial, insect and mammalian hosts are in common usage; such expression vectors are frequently used to encode hybrid fusion proteins consisting of a eukaryotic target protein and a specialized region designed to aid in the purification and visualization of the target protein. For example, the pCDM8 expression vector and derivatives thereof encode fusions between the target protein and an 11 amino acid peptide derived from the influenza protein hemagglutinin (HA). The HA epitope tag is useful in Western blotting and immunohistochemical localization of expressed fusion proteins when examined with antibodies raised specifically against the HA-epitope tag.

Host / Isotype: Mouse / IgG1

Clone: 26D11

Immunogen: Synthetic peptide containing the Infuenza Hemagglutinin epitope (YPYDVPDYA) (KLH-coupled).

Format: **State:** Liquid purified Ig fraction
Buffer System: 50% slurry in storage buffer (1xPBS, pH 7.4, containing 0.09% Sodium Azide).
Recommended Elution Buffer: 0.2 M Glycine, pH 2.2
Label: Agarose

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

Specificity: This antibody detects transfected proteins containing the HA epitope tag.
Anti-HA-Tag antibody (Agarose Conjugated) is a monoclonal anti-HA antibody covalently linked to agarose. The Agarose enables Immunoprecipitation (IP) of HA tagged proteins or Co-Immunoprecipitation (Co-IP) of their interacting partners.

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

General Readings: 1. Hopp, T.P., Prickett, K.S., Price, V.L., Libby, R.T., March, C.J., Cerretti, D.P., Urdal, D.L. and Conlon, P.J. 1988. A short polypeptide marker sequence useful for recombinant protein identification and purification. Nat. Biotechnol. 6: 1204-1210.
2. Smith DB, Johnson KS. Single-step purification of polypeptides expressed in Escherichia

coli as fusions with glutathione S-transferase. Gene. 1988 Jul 15;67(1):31-40. PubMed PMID: 3047011.

Protocols:

Immunoprecipitation Procedure:
The work can be performed in 1.5 ml micro-centrifuge tubes or in spin columns.
1. Thoroughly resuspend the Anti-HA Agarose by inverting the tube or vial several times.
2. Add 20-50µl 50% slurry of Anti-HA Agarose into cell lysate using a widebore pipette tip.
Note:The lysate should be fresh, and for a well expressed tagged protein,200 l lysate (200-500µg 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 HA tagged protein.
Option 1. Elution with elution buffer.
Add 30-50 µl elution buffer to the beads, gently tap the tube to mix well, centrifuge for 3 min, transfer the supernatant very carefully to a frsh 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 HA peptide
Add 30-50 µl HA peptide solution (100 g/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 l 2 SDS loading buffer, gently tap the tube to mix well, boil at 100 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.

Pictures:

HEK 293T cells were transfected with HA-tagged protein or not, and 100 µl cell lysate (about 100 µg total protein) was incubated with 30 µl 50% slurry of Anti-HA Agarose for 3 h at 4°C. After washing, the beads were eluted by 30 µl elution buffer twice. After neutralization of the eluant, 6 µl 6xSDS loading buffer was added. Then 20 l sample was subjected to the SDS-PAGE Blot was probed with Anti-HA-Tag Mouse mAb. Lane 1: 1st Elution with elution buffer. Lane 2: IP of untransfected HEK 293T lysate.