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Product Information

Monoclonal Anti-HA antibody produced in mouse
clone HA-7, purified from hybridoma cell culture

Catalog Number **H3663**

Product Description

Monoclonal Anti-HA, Clone HA-7 (mouse IgG1 isotype) is derived from the HA-7 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide corresponding to amino acid residues YPYDVPDYA (98-106) of the human influenza virus hemagglutinin (HA), conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog No. ISO2.

The antibody recognizes an epitope located within the sequence YPYDVPDYA (residues 98-106) of the human influenza virus hemagglutinin (HA), known as the HA tag. The product is reactive with HA-tagged fusion proteins expressed at either the amino or the carboxy terminus of the fusion protein. Applications include ELISA, immunoblotting, immunocytochemistry, and immunoprecipitation.

Recombinant DNA technology enables the attachment of specific sequences to genes of interest to provide "affinity handles" (tags) designed to enable the selective identification and purification of the protein of interest.[1-6] The addition of a tag to a given gene creates a stable fusion product that may not interfere with the bioactivity of the protein, or with the biodistribution of the tagged product.

HA peptide is a nonapeptide derived from the hemagglutinin protein of the human influenza virus, which is a major spike membrane glycoprotein The nucleic acid sequence encoding this peptide (amino acids 98-106 of influenza HA) has been incorporated into various expression plasmids adjacent to the cloning site, thus enabling the cloning and expression of HA-tagged fusion proteins. Such fusion proteins may be expressed in cells of various organisms: bacteria, yeast, insects and mammals. In the fusion protein, the HA sequence may serve as a recognition target for specific antibodies. This enables detection, subcellular localization, characterization, quantification, functional analysis and affinity purification of the HA-tagged protein and associated bound proteins.[4] Insertion of the HA epitope in different regions of a cellular protein followed by examination of the immunoreactivity of the epitope in intact and permeabilized cells is useful for studying the cellular expression levels, topology and functional activity of the tagged protein.[7]

Monoclonal antibodies reacting specifically with HA may be useful in various immunotechniques, to identify the expression of an HA fusion protein *in situ* and by immunoblotting, in bacteria, bacterial lysates of cells and tissue transfected with HA fusion protein expressing vectors. It may also be used for the immunoprecipitation of HA fusion protein.

Reagent

The product is provided as purified IgG in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Antibody concentration: ~1 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 0.25-1 μg/ml is determined using cell extracts expressing N-terminal HA fusion proteins.

Immunoprecipitation: 0.5-2.0 μg of the antibody can immunoprecipitate a C-terminal HA fusion protein from transfected mammalian cell lysates.

Indirect immunofluorescence: a working concentration of 1.0-2.0 μg/ml is determined using 293-T cells transfected with N-terminal HA-fusion proteins, fixed with methanol/acetone.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

Procedures

Immunoblotting

All incubation steps should be performed at room temperature

1. Separate HA tagged proteins from sample lysates using a standard SDS-PAGE protocol. Load 2.5-20 μg total lysate protein per lane.

   Note: The amount of lysate to be loaded depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (PBS, Catalog No. D8537; non-fat dry milk, Catalog No. M7409) for at least 60 min.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20, Catalog No. P3563.

   Note: blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibody.
5. Incubate the membrane with anti-HA antibody as the primary antibody in PBS containing 0.05% TWEEN 20, with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Mouse IgG, peroxidase conjugate (Catalog Nos. A9917, A3682 or A2304) or with Anti-Mouse IgG, alkaline phosphatase conjugate (e.g. Catalog Nos. A1293, A2179 or A1682) as the secondary antibody, at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with a peroxidase substrate.

Indirect Immunofluorescent staining of cultured cells

All incubation steps should be performed at room temperature (except step 3).

1. Grow transfected cultured cells expressing HA-tagged protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (Catalog No.D8537).
3. Fix the cells with –20 °C methanol (10 minutes) and then with –20 °C acetone (1 minute), **or** fix with 3% or 4% paraformaldehyde (10 minutes), rinse briefly with PBS and permeabilize with 0.5% Triton™ X-100 (2 minutes).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips cell-side-up with Anti-HA in PBS containing 1% BSA, Catalog No. A9647, for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate coverslips cell-side-up with Anti-Mouse IgG, FITC conjugate (e.g. Catalog No. F4018 or F8771) as the secondary antibody, at the recommended dilution, in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with appropriate filters.

Immunoprecipitation

1. Centrifuge 20 µl of a 1:1 suspension of protein G-agarose beads, Catalog No.P3296, for 1 minute at 2000 x g, and then wash twice with 1 ml RIPA buffer (50 mM Tris Base, 0.25% w/v deoxycholate, 1% NP40, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 °C.
2. Add anti-HA antibody diluted in PBS, and incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge for 1 minute at 12,000 x g, and wash twice with 1 ml RIPA buffer at 4 °C by spinning.
4. Add 0.1-1.0 ml of cell extract containing HA tagged protein to the antibody-coupled beads (see Note), and incubate from 2 hours to overnight at 4 °C, while swinging head-over tail.

Note: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.

5. Spin down beads; remove supernatant.
6. Wash beads four times with 1ml RIPA buffer and once with PBS by vortex and short spin.
7. Resuspend the pellet in 25 µl of 2X SDS-PAGE sample buffer. Boil sample for 5 minutes and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

References

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