Technical Bulletin

Monoclonal Anti-HA Antibody produced in mouse

Clone HA-7, purified from hybridoma cell culture

H3663

Product Description

Recombinant DNA technology enables the attachment of specific sequences to genes of interest to provide "affinity handles" (tags) that are 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 (HA) 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, which thus enables 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 which react 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 which are transfected with HA fusion protein-expressing vectors. It may also be used for the immunoprecipitation of HA fusion protein.

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 (Cat. No. ISO2).

The antibody recognizes an epitope located within the sequence YPYDVPDYA (residues 98-106) of the human influenza virus hemagglutinin, 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.

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 (exact value on Certificate of Analysis for particular lot)

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.

Precautions and Disclaimer

Because of the sodium azide content, a Safety Data Sheet for this product has been sent to the attention of the safety officer of your institution. Consult the Safety Data Sheet for information regarding hazardous and safe handling practices.

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, and then fixed with methanol/acetone.

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

Procedures

Immunoblotting

Note: 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 (such as Cat. No. D8537) containing 5% non-fat dry milk (such as Cat. No. M7409) for at least 60 minutes.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20 (Cat. 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 (such as Cat. Nos. A9917, A3682 or A2304) or with Anti-Mouse IgG, alkaline phosphatase conjugate (such as Cat. 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

Note: All incubation steps should be performed at room temperature (except Step 3).

1. Grow transfected cultured cells that express the HA-tagged protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (such as Cat. No. D8537).
3. Fix the cells either:

   (a) with –20 °C methanol (10 minutes), and then with –20 °C acetone (1 minute), **or**:

   (b) with 3% or 4% paraformaldehyde (10 minutes).

   Rinse briefly with PBS. Permeabilize with 0.5% Triton™ X-100 (2 minutes).
4. Wash the coverslips twice in PBS (5 minutes each wash).
5. Incubate the coverslips cell-side-up with Anti-HA in PBS containing 1% BSA (such as Cat. No. A9647) for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate the coverslips cell-side-up with Anti-Mouse IgG, FITC conjugate (such as Cat. Nos. 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).

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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 (such as Cat. No. P3296) for 1 minute at 2000 × g. Then wash twice with 1 mL RIPA buffer (50 mM Tris Base, 0.25% w/v deoxycholate, 1% NP-40, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 °C.
2. Add Anti-HA antibody diluted in PBS. Incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge for 1 minute at 12,000 × g. Wash twice with 1 mL RIPA buffer at 4 °C by spinning.
4. Add 0.1-1.0 mL of cell extract that contains HA-tagged protein to the antibody-coupled beads (see **Note**). 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 the beads. Remove the supernatant.
6. Wash beads four times with 1 mL RIPA buffer, and then once with PBS, by vortex and short spin.
7. Resuspend the pellet in 25 µL of 2X SDS-PAGE sample buffer. Boil the sample for 5 minutes and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

References

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