Technical Bulletin

Anti-HA-Biotin Antibody, Mouse Monoclonal

Clone HA-7, purified from hybridoma cell culture

B9183

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide "affinity handles" or tags. These tags are designed to enable the selective identification and purification of the protein of interest.[1-5] The tags are genetically engineered away from the protein active site, by insertion at the N- or the C-terminus.

Human influenza hemagglutinin (HA) is a surface glycoprotein required for the infectivity of the human virus.[6] The HA tag is a short sequence derived from amino acids 98-106 of the HA molecule. Many recombinant proteins have been engineered to express the HA tag, which does not appear to interfere with the bioactivity or the biodistribution of the recombinant protein. This tag facilitates the detection, isolation and purification of the proteins.[4,5]

Monoclonal Anti-HA, Biotin Conjugate is derived from the HA-7 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acid residues 98-106 (YPYDVPDYA) of human influenza virus hemagglutinin (HA), conjugated to KLH. The antibody is isolated from ascites fluid and conjugated to biotin.

Monoclonal Anti-HA, Biotin conjugate recognizes the HA tag sequence on HA-tagged fusion proteins when expressed N- or C-terminal to the fusion protein. The antibody reacts specifically with HA-tagged fusion proteins by immunoblotting and immunofluorescence. Staining of HA fusion proteins by immunoblotting is specifically inhibited by the immunizing HA peptide (Cat. No. I2149).

Monoclonal Anti-HA Biotin Conjugate may be used for the identification and characterization of HA-tagged proteins. Since avidin, streptavidin and ExtrAvidin™ interact with biotin with high affinity, the biotin-avidin system is an extremely effective tool in molecular biology, protein chemistry and immunology. Several publications cite use of this product.[7-9]

Reagent

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

Specific 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 is not recommended.
- 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

0.25-0.50 µg/mL of the antibody detects HA-tagged fusion proteins in mammalian cells extracts by immunoblotting.

Note: To obtain optimal results in different techniques and preparations, we recommend determining optimal working dilutions by titration testing.

Procedure for Immunoblotting

Note: All incubation steps should be performed at room temperature.

- Separate HA-tagged proteins from sample extract using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol:
- Load adequate bacterial lysate expressing the HA fusion protein.
- The amount of extract to be loaded per lane depends on the level of protein expression and may vary between experiments.
- Transfer proteins from the gel to a nitrocellulose membrane.
- Block the membrane for at least 60 minutes using a solution of PBS (such as Cat. No. D8537) containing 10% BSA (such as Cat. No. A7906).
- Wash the membrane three times, for 10 minutes each, in PBS containing 0.05% TWEEN® 20 (Cat. No. P3563).
- Incubate the membrane with an optimized concentration of Anti-HA Biotin Conjugate, diluted in PBS containing 1% w/v of BSA (Cat. No. P3688), and 0.05% TWEEN® 20 for 1 to 2 hours.
- Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20 at room temperature.
- Incubate the membrane with ExtrAvidin® Peroxidase conjugate (Cat. No. E2886) as the secondary reagent, at the recommended concentration in PBS containing 1% w/v of BSA and 0.05% TWEEN® 20 for 60 minutes. Adjust the concentration to maximize detection sensitivity and to minimize background.
- Treat the membrane with a peroxidase substrate (such as Cat. No. CPS1, Chemiluminescent Peroxidase Substrate).

References

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