Technical Bulletin

Anti-HA−Peroxidase antibody, Mouse monoclonal

clone HA-7, purified from hybridoma cell culture

H6533

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.[1-6] These protein tag sequences are genetically engineered away from the protein active site, by insertion at the N- or C-terminus.

Human influenza hemagglutinin (HA) is a surface glycoprotein required for infectivity of the human virus.[7] Many recombinant proteins have been engineered to express a short sequence derived from the HA molecule corresponding to amino acid residues 98-106, known as the HA Tag. The addition of a tag sequence such as the HA sequence 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.[4-6]

Monoclonal Anti-HA-Peroxidase is a lyophilized preparation of the purified immunoglobulin fraction of monoclonal Anti-HA, conjugated to horseradish peroxidase (HRP). The antibody is derived from the HA-7 hybridoma that is produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse which has been immunized with a synthetic peptide that corresponds to amino acid residues 98-106 (YPYDVPDYA) of human influenza virus hemagglutinin (HA) conjugated to KLH.

Monoclonal Anti-HA-Peroxidase recognizes native, as well as denatured-reduced, forms of HA-tagged proteins. It is reactive with N- or C-terminal HA-tagged fusion proteins that have been expressed in *E. coli* or in mammalian cells.

Reagent

This product is lyophilized from 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 0.05% MIT.

Antibody concentration: 5-11 mg/mL (exact value on Certificate of Analysis for particular lot)

Molar ratio Ab/Enzyme: 0.6-1.5 (exact value on Certificate of Analysis for particular lot)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the vial with 0.5 mL of distilled water.

Storage/Stability

- Store the lyophilized product at 2-8 °C.
- For extended storage after reconstitution, it is recommended to store working aliquots at −20 °C.
- For continuous use after reconstitution, the solution may be stored at 2-8 °C for up to 1 month.
- Working dilutions should be discarded.
- Avoid repeated freeze-thaw cycles.

Product Profile

Immunoblotting

A minimum working antibody dilution of 1:4,000 is determined using HA-tagged fusion protein expressed in bacteria, and ECL immunoblotting detection reagent.

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

Procedure for Immunoblotting

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

1. Separate HA-tagged proteins from sample extract using a standard SDS-PAGE protocol. Load adequate bacterial lysate expressing the HA fusion protein.

   Note: The amount of extract to be loaded per slab or lane depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to nitrocellulose membrane.
3. Block the membrane using a solution of 3% non-fat dry milk in phosphate buffered saline (Cat. No. P2194) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Cat. No. P3563).
5. Incubate the membrane with Monoclonal Anti-HA-Peroxidase using an optimized concentration in PBS containing 0.05% TWEEN® 20 and 1% bovine serum albumin (BSA, such as Cat. No. A9647) for 60 to 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 at room temperature.
7. Treat the membrane with a peroxidase substrate.

References

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H6533bul Rev04/22 KK, DS, KAA,PHC,RC,GCY