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# Product Information

**Monoclonal Anti-HA−Peroxidase antibody produced in mouse**
clone HA-7, purified immunoglobulin, lyophilized powder

Catalog Number **H6533**

## Product Description

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 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 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 and is reactive with N- or C-terminal HA-tagged fusion proteins expressed in *E. coli* or in mammalian cells.

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 sequences of tails or tags are genetically engineered away from the protein active site, by insertion at the N- or C-terminus. 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. 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. This tag facilitates the detection, isolation, and purification of the proteins.[4-6]

## Reagent

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
Molar ratio Ab/Enzyme: 0.6-1.5

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

## 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**: In order to obtain best results in various techniques and preparations, we recommend determining optimal working dilution by titration.

## Procedure

Immunoblotting

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, Catalog Number P2194, for at least 60 minutes.

4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number 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, Catalog Number A 9647) 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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