

## Product Information

### MONOCLONAL ANTI-HA BIOTIN CONJUGATE CLONE HA-7

Purified Mouse Immunoglobulin

Product Number **B 9183**

#### Product Description

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 (Product No. I 2149).

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.<sup>1-5</sup> The tags 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 the infectivity of the human virus.<sup>6</sup> 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.<sup>4,5</sup>

Monoclonal Anti-HA, Biotin Conjugate may be used for the identification and characterization of HA-tagged proteins. Since avidin, streptavidin and Extravidin<sup>™</sup> interact with biotin with high affinity, the biotin-avidin system is an extremely effective tool in molecular biology, protein chemistry and immunology.

#### 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: Approx. 1 mg/ml.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 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.

#### Product Profile

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

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

#### Procedure for Immunoblotting

All incubation steps should be performed at room temperature.

1. 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.

2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane for at least 60 minutes using a solution of PBS containing 10% BSA (PBS, Product No D 8537; BSA, Product No. A 7906).
4. Wash the membrane three times, for 10 minutes each, in PBS containing 0.05% TWEEN® 20 (Product No. P 3563).
5. Incubate the membrane with an optimized concentration of Anti-HA, Biotin Conjugate, diluted in PBS containing 1% w/v of BSA (Product No. P 3688), and 0.05% TWEEN 20 for 1 to 2 hours.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN 20 at room temperature.
7. Incubate the membrane with ExtrAvidin®, Peroxidase conjugate (Product No. E 2886) as the secondary reagent at the recommended concentration in PBS containing 1% w/v of BSA and 0.05% TWEEN 20 for 60 min. Adjust the concentration to maximize detection sensitivity and to minimize background.
8. Treat the membrane with a peroxidase substrate such as Sigma's Chemiluminescent Peroxidase Substrate, CPS-1 or use ProteoQwest kit, PQ0201 for added convenience.

#### References

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6. Wilson, I.A., et al., *Cell*, **3**, 767 (1984).

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