Technical Bulletin

Monoclonal Anti-HA Alkaline Phosphatase antibody produced in mouse

Clone HA-7, purified immunoglobulin, buffered aqueous glycerol solution

A5477

Product Description

Recombinant DNA technology enables the insertion of specific DNA sequences into genes of interest. The inserts provide 'affinity handles' (tags) designed for the selective identification and purification of the protein product of the gene.[1-5] These tags are generally inserted at the N- or C-terminus so that they will not interfere with the protein active site.

Human influenza hemagglutinin (HA) is a surface glycoprotein required for the infectivity of the human virus.[6] The short sequence derived from the HA molecule corresponding to amino acids 98-106 has been used as a tag, known as the HA-Tag. 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]

Anti-HA, Alkaline Phosphatase 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 calf intestinal alkaline phosphatase using glutaraldehyde.

Anti-HA, Alkaline Phosphatase conjugate recognizes the HA tag sequence on HA-tagged fusion proteins when expressed N-terminal or C-terminal to the fusion protein. The antibody reacts specifically with HA-tagged fusion proteins in immunoblotting.

Several research dissertations cite use of this monoclonal anti-HA alkaline phosphatase conjugate product.[7,8]

Reagent

The product is provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM $MgCl_2$, 50% glycerol, and 15 mM sodium azide as a preservative.

The specific antibody concentration is indicated on the online Certificate of Analysis (CofA) for each individual lot of this product.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. **Do not freeze.** 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

A minimum dilution of 1:4000 is determined by immunoblotting using extracts of mammalian cells expressing HA-tagged fusion proteins.

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

Procedure for Immunoblotting

Note: All incubations 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 nitrocellulose membrane.
3. Block the membrane using a solution of PBS (such as Cat. No. D8537) that contains 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 (such as Cat. No. P3563).
5. Incubate the membrane with an optimized concentration of Anti-HA, Alkaline phosphatase conjugate, diluted in PBS containing 0.05% Tween® 20 for 60 to 120 minutes.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween® 20.
7. Treat the membrane with an Alkaline phosphatase substrate (such as BCIP/NBT, Cat. No. B1911).

References

1. Jarvik, J.W., and Telmer, C.A., *Annu. Rev. Genet.*, **32**, 601-618 (1998).
2. Olins, P.O., and Lee, S.C., *Curr. Opin. Biotechnol.*, **4(5)**, 520-525 (1993).
3. Uhlen, M., and Moks, T., *Methods Enzymol.*, **185**, 129-143 (1990).
4. Kolodziej, P.A., and Young, R.A., *Methods Enzymol.*, **194**, 508-519 (1991).
5. Woychik, N.A., and Young, R.A., *Trends Biochem. Sci.*, **15(9)**, 347-351 (1990).
6. Wilson, I.A. *et al.*, *Cell*, **37(3)**, 767-778 (1984).
7. McDonnell, Barry, "Development of novel antibody-based diagnostics for the early and rapid detection of cardiac markers". Dublin City University (Dublin, Ireland), Ph.D. dissertation, pp. 68-69 (September 2010).
8. Chung, Man Wah Liliane, "Characterisation of the novel mechanism of protein-directed translation initiation used by calciviruses". Imperial College London (London, UK), Ph.D. dissertation, pp. 65, 80 (2012).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

A5477bul Rev 06/21 NV,JWM,RC,GCY