3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-HA−Alkaline Phosphatase antibody, Mouse monoclonal
clone HA-7, purified from hybridoma cell culture

Product Number: **A5477**

TECHNICAL BULLETIN

Product Description
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- or C-terminal to the fusion protein. The antibody reacts specifically with HA tagged fusion proteins by immunoblotting.

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 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 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]

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

Specific Antibody concentration: Approx. 1 mg/ml.

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.

Storage/Stability
For continuous use and extended storage, store at 2 to 8 °C. Do not freeze. Working dilution samples should be discarded if not used within 12 hours.

Procedure
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 nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (NFDM, Product No. M 7409; PBS, Product No. D 8537) for at least 60 minutes.

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, 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 (e.g. BCIP/NBT, Product No. B 1911).

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

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

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,** 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, 347-351 (1990).
6. Wilson, I. A., et al., Cell, **37,** 767-778 (1984).

RC,NV/JWM 04/21-1

©2021 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.