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
produced in rabbit, affinity isolated antibody

Catalog Number **H6908**

Product Description

Anti-HA is produced in rabbit using a synthetic peptide corresponding to amino acid residues 98-106 (Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala) of the human Influenza hemagglutinin (HA), conjugated to KLH. The antibody is affinity-purified on the immobilized immunizing peptide

Anti-HA may be used for immunoblotting of recombinant proteins tagged with HA at the amino or carboxy terminus. The antibody may recognize cross-reacting bands in some mammalian cells. Staining of the fusion protein band is specifically inhibited with the immunizing HA peptide, Catalog Number I2149. The antibody is also useful for detection of HA-tagged fusion proteins by immunoprecipitation and by immunofluorescent staining of transfected cells.

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes, which can provide 'affinity handles' (tags) designed to enable the selective identification and purification of the protein of interest.[1-6] The addition of a tag to a given gene creates a stable fusion product that does not appear to interfere with the bioactivity of the protein, or with the biodistribution of the tagged product.

Influenza hemagglutinin protein is a nonapeptide derived from the major spike membrane glycoprotein of the human influenza virus. This strain specific glycoprotein is a homotrimer of 84 kDa monomers, each containing two disulfide-linked subunits: HA1 and HA2. The nucleic acid sequence encoding the HA-peptide has been incorporated into various expression plasmids adjacent to the cloning site thus enabling the cloning and expression of HA-tagged fusion protein. Such fusion proteins may be expressed in cells of various organisms: bacteria, yeast, insects and mammals. In the fusion protein, the HA sequence may serve as a recognition target for specific antibodies thus enabling detection, subcellular localization, characterization, quantification, functional analysis and affinity purification of the HA-tagged protein and associated bound proteins.[4] Insertion of the HA epitope in different regions of a cellular protein followed by examination of the immunoreactivity of the epitope in intact and in permeabilized cells is useful for studying the cellular expression levels, topology and functional activity of the tagged protein.[7]

Antibody mediated detection of the HA tag obviates the need for time consuming generation of antibody specific for newly identified, low abundance, unstable, difficult to purify, cross-reactive or poorly immunogenic proteins.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1 % BSA and 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards 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. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a minimum working concentration of 0.5-0.8 µg/ml is determined using a whole extract of transfected cells expressing HA-tagged protein.

Immunoprecipitation: a minimum working concentration of 2.5-4.0 µg/test is determined using a whole lysate of transfected cells expressing HA-tagged protein.

Indirect immunofluorescence: a minimum working concentration of 10-20 µg/ml is determined using transfected cells expressing HA-tagged protein.

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

Procedures

Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate HA-tagged proteins from sample lysates using a standard SDS-PAGE protocol. Load 2.5 to 20 µg of total lysate protein per lane. The amount of lysate 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 using a solution of 5-10% non-fat dry milk in PBS (PBS, Catalog No. D8537 for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN® 20, Catalog No. P3563.
5. Incubate the membrane with Anti-HA antibody as the primary antibody in PBS containing 0.05 % TWEEN 20 for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN 20.
7. Incubate the membrane with Anti-Rabbit IgG-Peroxidase, Catalog No. A0545, or Alkaline-Phosphatase conjugate, Catalog No. A9919, as the secondary antibody at the recommended dilution in PBS containing 0.05 % TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN 20.
9. Treat the membrane with a peroxidase or an alkaline-phosphatase substrate as appropriate.

Indirect Immunofluorescent Staining of Cultured Cells

1. All incubation steps should be performed at room temperature.
2. Grow transfected cultured cells expressing HA-tagged fusion protein of choice on sterile coverslips at 37 °C.
3. Wash the cells briefly in PBS.
4. Fix the cells with 3 % paraformaldehyde (10 minutes) and then with 0.5 % Triton™ X-100 (10 minutes) at room temperature.
5. Wash specimens twice, 5 minutes each wash in PBS. Note: Blocking with PBS containing 1 % BSA for 10 minutes at room temperature followed by draining prior to step 6 may minimize non-specific adsorption of the antibody.
6. Incubate specimens cell-side-up with anti-HA antibody as primary antibody in PBS containing 1% BSA, Catalog No. A9647. Incubate for 60 minutes.
7. Wash three times in PBS (5 minutes each wash).
8. Incubate specimens cell-side-up with Anti-Rabbit IgG-FITC, Catalog No. F9887, as the secondary antibody at the recommended dilution in PBS containing 1 % BSA. Incubate for 30 minutes.
9. Wash three times in PBS (5 minutes each wash).
10. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
11. Examine using a fluorescence microscope with appropriate filters.

Immunoprecipitation

Note: The amount of cell lysate to be used for immunoprecipitation depends on the level of expression of the tagged protein and the specific application.

1. To 0.1-1.0 ml of cell lysate containing HA-tagged protein add Anti-HA antibody and incubate on a rotator for 2 hours to overnight at 4 °C (see Note above).
2. Centrifuge 20 µL Protein A-agarose beads, Catalog No. P3476, for 1 min 12,000 x *g*, and then wash twice with 1 ml RIPA buffer (50 mM Tris Base, 0.25 % w/v Deoxycholate, 1 % IGEPAL, 150 mM NaCl, 1mM EDTA, pH 7.4) at 4 °C.
3. Add the mixture from step 1 to the beads and incubate on a rotator for 2 hours at 4 °C.
4. Spin down beads; remove supernatant.
5. Wash beads four times with 1 ml RIPA buffer and one time with PBS by vortex and short spin.
6. Resuspend pellet in 25 µL 2X SDS-PAGE sample buffer. Boil sample for 5 minutes and spin down. The sample is ready to be loaded on a SDS-PAGE gel.

References

1. Narayanan, S.R., *J. Chromatogr.*, **658**, 237-258 (1994).
2. Olins, P.O., and Lee, S.C., *Curr. Opin. Biotechnol.*, **4**, 520-525 (1993).
3. Uhlen, M., and Moks, T., *Meth. Enzymol.*, **185**, 129-143 (1990).
4. Kolodziej, P.A., and Young, R.A., *Meth. Enzmol.*, **194,** 508-519 (1991).
5. Pines, J. and Hunter, T., *J. Cell Biol.*, **115,** 1-17 (1991).
6. Antebi, A., and Fink, G.R., *Mol. Biol. Cell*, **3,** 633-654 (1992).
7. Canfield, V.A., and Levenson, R., *Biochemistry*, **32**, 13782-13786 (1993).

DS,PHC 03/15-1