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

**Monoclonal Anti-HA, clone HA-7**
produced in mouse, ascites fluid

Catalog Number **H9658**

## Product Description

Monoclonal Anti-HA (mouse IgG1 isotype) 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 YPYDVPDYA (98-106) of the human Influenza virus Hemagglutinin (HA), conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO-2.

Monoclonal Anti-HA recognizes HA tag, applying ELISA, immunoblotting, immunocytochemistry, and immunoprecipitation. The product 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, 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. This enables 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] Monoclonal antibody reacting specifically with HA may be useful in various immunotechniques, to identify the expression of a HA fusion protein *in situ* and by immunoblotting, in bacteria, bacterial lysates or cells and tissues transfected with HA fusion protein expressing vectors. It may also be used for the immunoprecipitation of HA fusion proteins.

## Reagent

Supplied as ascites fluid with 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, or storage in "frost-free" freezers, 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 dilution of 1:20,000 is determined using a whole extracts of transfected 293T (human embryonal kidney) cells, expressing HA-tagged fusion protein.

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

## References

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3. Uhlen, M., and Moks, T., *Methods Enzymol.*, **185**, 129-143 (1990).
4. Kolodziej, P. A., and Young, R. A., *Methods 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).

## Procedures

Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate HA-tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (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 % non-fat dry milk in phosphate buffered saline (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). Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibody.
5. Incubate the membrane with Anti-HA antibody as the primary antibody using an optimized concentration in PBS containing 0.05 % TWEEN 20 and 1 % bovine serum albumin (BSA, Catalog No. A9647) 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-mouse IgG Peroxidase conjugate (e.g. Catalog No. A9917, A3682, or A2304) or with Anti-mouse Alkaline Phosphatase conjugate (e.g. Catalog No. A1293, A2179 or A1682) as the secondary antibody at the recommended concentration 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 either a peroxidase or an alkaline phosphatase substrate as appropriate.

Indirect Immunofluorescent Staining of Cultured Cells

All incubation steps should be performed at room temperature (except step 3).

1. Grow transfected cultured cells expressing HA-tagged protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (Catalog No. D8537).
3. Fix the cells with –20 °C methanol (10 minutes) and then with –20 °C acetone (1 minute), **or** fix with 3 % to 4 % paraformaldehyde (10 minutes), rinse briefly with PBS and permeabilize with 0.5 % Triton™ X-100 (2 minutes).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips cell-side-up with Anti-HA in PBS containing 1 % BSA (Catalog No. A9647) for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate coverslips cell-side-up with Anti-mouse FITC conjugate (e.g. Catalog No. F4018 or F8771) as the secondary antibody, at the recommended dilution, in PBS containing 1 % BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
10. 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 to 1.0 ml of cell lysate containing HA-tagged protein add Anti-HA antibody and incubate on a rotator 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, 1 mM EDTA, pH 7.4) at 4 °C.
3. Add the mixture from step 1 to the beads and incubate on a rocker or 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 (Catalog No. D8537) 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.



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