

Technical Bulletin

Monoclonal Anti-HA-TRITC antibody produced in mouse

Clone HA-7, purified immunoglobulin, buffered aqueous solution

H9037

Product Description

Recombinant DNA technology enables the insertion 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.¹⁻⁵ These sequences or 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 virus.⁶ The HA-Tag consists of a short sequence that corresponds to amino acids 98-106 of HA. 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 recombinant proteins.^{4,5}

Monoclonal Anti-HA is derived from the HA-7 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. A synthetic peptide corresponding to amino acid residues 98-106 (YPYDVPDYA) of human influenza virus hemagglutinin (HA) conjugated to KLH was used as the immunogen. The antibody is isolated from ascites fluid and conjugated to crystalline tetramethylrhodamine isothiocyanate (TRITC). The conjugate is then further purified to remove the unbound free TRITC.

Monoclonal Anti-HA recognizes the HA tag sequence on HA-tagged fusion proteins by immunofluorescence and by immunocytochemistry.⁷ The antibody reacts specifically with N- and C-terminal HA-tagged fusion proteins. The immunofluorescent staining of HA fusion proteins is specifically inhibited by the immunizing HA peptide (Cat. No. I2149).

Reagent

This product is supplied 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: ~1.0 mg/mL (exact value on Certificate of Analysis for particular lot)

F/P Molar Ratio: 1-5 (exact value on Certificate of Analysis for particular lot)

Storage/Stability

- **Store product protected from light.**
- 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.
- 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

Immunocytochemistry: 1-5 µg/mL of the antibody detects HA-tagged proteins in mammalian cells.

Immunofluorescence: 10-15 µg/mL of the antibody detects HA-tagged fusion proteins in mammalian cells.

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

Procedure for Direct Immunofluorescent Staining of Cultured Cells

1. Grow transfected cultured cells expressing the HA-fusion protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS.
3. Fix the cells for 10 minutes with 3% paraformaldehyde. Immediately permeabilize with 0.5% Triton® X-100.
4. Wash the coverslips twice in PBS (5 minutes each wash).
5. Incubate the coverslips cell-side-up with Monoclonal Anti-HA-TRITC at working dilutions in PBS, at room temperature for 1 hour.
6. Wash three times in PBS (5 minutes each wash).
7. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles. Examine using a fluorescence microscope with appropriate filters.

References

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