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Product Information

Monoclonal Anti-Actin-FITC, clone AC-40
produced in mouse, purified immunoglobulin

Catalog Number **F3046**

Product Description

Monoclonal Anti-Actin (mouse IgG2a isotype) is derived from the AC-40 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. A synthetic actin C-terminal peptide (Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe), attached to a Multiple Antigen Peptide (MAP) backbone, was used as the immunogen. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. Monoclonal Anti-Actin is purified using Protein A, then conjugated to fluoroscein isothiocyanate isomer I (FITC). It is further purified by gel filtration and contains no detectable free FITC.

Monoclonal Anti-Actin-FITC recognizes an epitope located on the C-terminal end of actin but not on the N-terminal. This epitope is conserved in all actin isoforms. The antibody specifically labels actin in a wide variety of tissues and species, using immunofluorescent staining of cultured cell lines and immunohistochemistry. Cross-reaction has been observed with human, bovine, sheep, goat, pig, rabbit, dog, mouse, rat, guinea pig, hamster, chicken, carp, viper, *Xenopus* and snail. The antibody can be used for staining of methanol-fixed, frozen sections. The epitope recognized by the antibody is resistant to formalin-fixation and paraffin-embedding.

The antibody conjugate may be used for the localization of actin using direct immunofluorescent staining of frozen or fixed tissue sections and cultured cells. It is suitable for dual immunofluorescent staining procedures.

The two major cytoskeletal proteins implicated in cell motility are actin and myosin. Actin and myosin are constituents of many cell types and are involved in a myriad of cellular processes including locomotion, secretion, cytoplasmic streaming, phagocytosis and cytokinesis. Although actin is one of the most conserved eukaryotic proteins, it is expressed in mammals and birds as at least six isoforms characterized by electrophoresis and amino acid sequence analysis.[1-3] Four of them represent the differentiation markers of muscle tissues and two are found in practically all cells. There are three α-actins (α-skeletal, α-cardiac and α-smooth muscle), one β-actin (β-non-muscle) and two γ-actins (γ-smooth muscle and γ-non-muscle). Actin isoforms show >90% overall sequence homology, but only 50-60% homology in their 18 N-terminal residues.[4] The N-terminal region of actin appears to be a major antigenic region, and may be involved in the interaction of actin with other proteins such as myosin.[5] The actins in cells of various species and tissues are very similar in their immunological and physical properties. As a consequence, it is difficult to produce potent antisera to actin. Therefore, the availability of monoclonal antibody to actin provides a specific and useful tool in studying actin structure and function and probing binding sites of actin-binding proteins.

Reagent

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.

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

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. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Antibody concentration: 2-4 mg/ml
F/P Molar Ratio: 3 - 8

Direct immunofluorescence: a working dilution of at least 1:75 was determined using cultured human or chicken fibroblasts.

Note: n order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References
1. Herman, I., *Curr. Opin. Cell Biol*., **5**, 48 (1993).
2. Vandekerckhove, J., and Weber, K., *Eur. J. Biochem.*, **90**, 451 (1978).
3. Drew, J., et al., *Amer. J. Physiol*., **260**, C1332 (1991).
4. Lessard, J., *Cell Motil. Cytoskel*., **10**, 349 (1988).
5. Sutoh, K., and Mabuchi, I., *Biochemistry*, **25**, 6186 (1986).

Direct Immunofluorescent Labeling of Cultured Cells

Materials
1. Coverslips.
2. Cells, e.g., human or chicken fibroblasts, in DMEM medium, Catalog No. D5546, with 10% fetal bovine serum, Catalog No. F2442.
3. 10 mM phosphate buffered saline (PBS), pH 7.2-7.4, without preservative, Catalog No. P4417
4. PBS containing 1% BSA (diluent).
5. Absolute methanol cooled to –20 °C.
6. Acetone, Analytical grade, cooled to –20 °C (1:1 v/v).
7. Aqueous mounting medium.
8. Test antibody conjugate

Cell Growth and Fixation
1. Collect cells from tissue culture dish at a stage of almost confluency, wash with medium and seed on to coverslips. Seed 1-2 x 10^4 cells per cover slip and grow cells in incubator for 2-3 days. Do not change medium**.**
2. Remove coverslips from incubator, discard medium.
3. Wash twice with PBS, remove solution by aspiration.
4. Add enough cold methanol to cover the cell layer. Incubate 10 minutes at –20 °C. Aspirate solution.
5. Cover cell layer with cold acetone. Incubate 1 minute at –20 °C. Aspirate solution.
6. Wash twice with PBS. Rehydrate in PBS for at least 30 minutes prior to labeling with antibody.

Test
1. Dilute Monoclonal Anti-Actin-FITC 1:50 in PBS containing 1% BSA (diluent). Add enough diluted antibody to cover the cell layer and incubate coverslip for 60 minutes at room temperature.
2. Wash 3 times with PBS, 5 minutes each.
3. Drain excess solution by touching edge of coverslip on paper toweling.
4. Invert onto mounting medium applied on glass slides.
5. Read under the UV fluorescence microscope. Mounted preparations can be stored in the dark at 2-8 °C.

Note
1. Do not allow cell layer to dry out at any time during the procedure.
2. In case of excessive background staining, remove aggregates from the labeled reagent (conjugate) by centrifuging for 15 minutes immediately prior to use.

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