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ProductInformation

MONOCLONAL ANTI-ACTIN
CLONE AC-40
CY3 CONJUGATE
Purified Mouse Immunoglobulin

Product Number **C 5838**

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 an immunized mouse. A synthetic actin C-terminal peptide, Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe, attached to Multiple Antigen Peptide (MAP) backbone was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The product is prepared by conjugation of Cy3[1] to (Protein A) purified Monoclonal Anti-Actin antibody. The conjugate is purified by gel filtration to remove unbound Cy3 fluorophore.

Monoclonal Anti-Actin recognizes an epitope located on the C-terminus of actin, but not on the N-terminus. This epitope is conserved in all actin isoforms. The antibody specifically labels actin in a wide variety of tissues and species including human[2], bovine, sheep, goat, pig, rabbit, dog, mouse, rat, guinea pig, hamster, chicken, carp, viper, *Xenopus* and snail. The antibody can be used for staining methanol-fixed, frozen sections or formalin-fixed, paraffin-embedded sections. The Cy3 conjugated antibody may also be used for the immunofluorescent staining of cultured cells.

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.[3-5] Four of them represent the differentiation markers of muscle tissues and two are found practically in all cells. There are three α-actins (α-skeletal, α-cardiac and α-smooth muscle), one β-actin (β-nonmuscle) and two γ-actins (γ-smooth muscle and γ-nonmuscle). Actin isoforms show >90% overall sequence homology, but only 50-60% homology in their 18 NH_2-terminal residues.[6] The NH_2-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.[7] The actins in cells of various species and tissues are very similar in their immunological and physical properties. As a consequence, it has been found difficult to produce potent antisera to this protein. Therefore, the availability of monoclonal antibody to actin provides a specific and useful tool in studying actin structure and function and in probing binding sites of actin-binding proteins.

Reagents

The product is provided as a solution in 0.01M phosphate buffered saline pH 7.4 containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Cy3 Conjugated Monoclonal Anti-Actin may be used for the localization of actin using direct immunofluorescence on cultured cells and frozen or fixed tissue sections. The conjugate is suitable for dual immunofluorescent staining procedures.

Working Dilution
A minimum working dilution of at least 1:50 was determined by direct immunofluorescent labeling of cultured cells (e.g. human or chicken fibroblasts).

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

Immunofluorescence Labeling of Cultured Cells

Materials

1. Coverslips.
2. Cells (e.g., human or chicken fibroblasts) in DMEM medium (Sigma Product No. D 5546) with 10% fetal calf serum (Sigma Product F 2442) .
3. 10 mM phosphate buffered saline (PBS) pH 7.2-7.4 (Sigma Product No. P 4417).
4. Diluent: PBS containing 1% BSA (Sigma Product P 3688).
5. Absolute methanol, cooled to –20 °C.
6. Acetone, Analytical grade, cooled to –20 °C.
7. Aqueous mounting media.
8. Cy3 Conjugated Monoclonal Anti-Actin (Sigma Product No. C 5838).

Cell Growth and Fixation

1. Collect cells from tissue culture dish at a stage of almost confluency, wash with medium and seed onto coverslips. Seed 1-2 x 10^4 cells per coverslip and grow cells in incubator for 2-3 days. Do not change medium.
2. Remove coverslips from incubator, aspirate 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. Rinse cell layer twice for 10 seconds with cold acetone, aspirate.
    1. Wash 2x with PBS. Rehydrate in PBS for at least

    30 minutes prior to labeling with antibody.

Direct Immunofluorescence Labeling

1. Dilute Cy3 Conjugated Monoclonal Anti-Actin in PBS containing 1% BSA. Add enough diluted antibody to cover the cell layer and incubate coverslips for 60 minutes at room temperature.
2. Wash 3x with PBS, at least 5 minutes each.
3. Drain excess solution by touching edge of cover-slips on paper toweling.
4. Invert coverslips onto mounting media applied on glass slides.
5. Read under UV fluorescent microscope. Mounted preparations can be stored in the dark at 2-8 °C.

References

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