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# Product Information

**Monoclonal Anti-Actin, Clone AC-40**
produced in mouse, ascites fluid

Catalog Number **A4700**

## 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 Mouse Monoclonal Antibody Isotyping Reagents, Catalog No. ISO2.

Monoclonal Anti-Actin recognizes an epitope located on the C-terminal end of actin, but not on the N-terminal end. This epitope is conserved in all actin isoforms. The antibody specifically labels actin in a wide variety of tissues and species in immunoblotting (band at 42 kDa), in immunofluorescent staining of cultured cell lines and in immunohistology. Cross-reaction has been observed with human, bovine, sheep, goat, pig, rabbit, dog, mouse, rat, guinea pig, hamster, chicken, carp, viper, frog, and snail. The antibody may be used in staining of methanol-fixed, frozen sections, and the epitope recognized by the antibody is resistant to formalin-fixation and paraffin-embedding. Zinc-formalin, B5, ethanol, methacarn, Brunnel's or Bouin's solution may also be used as fixatives.

Monoclonal Anti-Actin may be used for the localization of actin using ELISA, immunoblotting, dot blotting, and immunohistology.

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 nearly 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 actin in cells of various species and tissues are very similar in their immunological and physical properties. As a consequence, it is difficult to produce antisera to this protein. 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

Supplied as ascites fluid with 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/Stability

For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, the solution may be frozen 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.

## Product Profile

Indirect Immunofluorescent Staining: a minimum dilution of 1:200 was determined using cultured human or chicken fibroblasts.

Indirect Immunoblotting: a minimum dilution of 1:500 was determined using cultured human or chicken fibroblast extract.

**Note**: In order to obtain best results, it is recommended that each individual user determine working dilution by titration assay.

**Procedure for Immunoblotting**
**Note**: The whole procedure is performed at room temperature.

1. Separate proteins from sample lysates using a standard SDS-PAGE protocol; use 10%-12%gel. Load 0.5-1 confluent plate of total cell (Chicken fibroblast , Human foreskin fibroblast) lysate per slab .
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) for 60 minutes or 10% BSA in phosphate buffered saline (PBS) overnight at room temperature.
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 Monoclonal Anti-Actin (working dilution 1:500-1:1000) as the primary antibody in PBS containing 0.05% TWEEN and 0.5% non-fat dry milk , with agitation for 120 minutes.
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 (Fab specific)–Peroxidase, Catalog No. A9917, as the secondary antibody at the recommended concentration in PBS, containing 0.05% TWEEN 20. Incubate with agitation for 60 minutes. Adjust the product 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 substrate.

**References**

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4. Lessard, J., *Cell Motil. Cytoskel.*, **10**, 349 (1988).
5. Sutoh, K., and Mabuchi, I., *Biochemistry*, **25**, 6186 (1986).


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