

Product Information

Monoclonal Anti- β -Actin, Clone AC-74
produced in mouse, purified immunoglobulin

Catalog Number **A2228**

Product Description

Monoclonal Anti- β -Actin (mouse IgG2a isotype) is derived from the AC-74 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a slightly modified synthetic β -cytoplasmic actin N-terminal peptide Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Lys, conjugated to KLH.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti- β -Actin recognizes an epitope located on the N-terminal end of the β -isoform of actin. The antibody labels specifically β -actin in a wide variety of tissues and species by various immunochemical techniques including immunoblotting (42 kDa),^{1,2} immunofluorescent staining of cultured cell lines,¹ and immunohistochemistry.^{2,3} The antibody may also be used in ELISA, immunocytochemistry,⁵ and immunoelectron microscopy using tissues or cultured cell line preparations. By immunofluorescent staining using ultra thin tissue cryosections of chicken gizzard, the antibody labels the dense bodies and the longitudinal channels linking consecutive dense bodies that are also occupied by desmin and the membrane-associated dense plaque.² The antibody does not stain adult cardiac and skeletal muscles, except for traces due to contaminations of the sample with non-muscle cells, or if embryonic tissue is being used. The antibody reacts with β -actin-expressing cells in human,⁴ bovine, sheep, pig, rabbit, cat, dog,⁵ mouse,⁶ rat,⁹ guinea pig, chicken, carp, *Hirudo medicinalis* (leech), and *Drosophila* tissues. It does not react with *Dictyostelium discoideum* amoebae. The antibody can be used for staining of acetone-fixed frozen sections and EM preparations.^{2,7} The epitope recognized by the antibody is resistant to formalin fixation and paraffin embedding. However, fixatives such as B5, ethanol, methacarn, or Bouin's may be used.

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 process 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

with at least six isoforms characterized by electrophoresis and amino acid sequence analysis.^{8,9} Four of the isoforms represent the differentiation markers of muscle tissues and two are found in almost all cells. There are three α -actins (skeletal, cardiac, and smooth muscle), one β -actin (β -nonmuscle), and two γ -actins (γ -smooth muscle and γ -nonmuscle). Actin isoforms show greater than 90% overall sequence homology, but only 50-60% homology in their 18 NH₂-terminal residues.¹⁰ The NH₂-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.

The actin in cells of various species and tissue origin is very similar in their immunological and physical properties. As a consequence, it has been difficult to produce potent antisera to this protein. Therefore the availability of monoclonal antibodies to β -actin provides a specific and useful tool in studying the intracellular distribution of β -actin and the static and dynamic aspects of the cytoskeleton.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody concentration: ~2 mg/ml

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

Store at -20 °C. For continuous use, the product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at -20 °C. 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.

Product Profile

Immunoblotting: a working antibody concentration of 0.5-1 µg/ml is recommended using total cell extracts of human or chicken fibroblasts.

Immunocytochemistry: a working antibody concentration of 10-40 µg/mL is recommended using human or chicken fibroblasts.

Note: In order to obtain best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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