

## Product Information

### Anti- $\beta$ -Actin-FITC antibody, Mouse monoclonal Clone AC-15, purified from hybridoma cell culture

Catalog Number **F3022**

#### Product Description

Monoclonal Anti- $\beta$ -Actin (mouse IgG1 isotype) is derived from the AC-15 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A slightly modified  $\beta$ -cytoplasmic synthetic actin N-terminal peptide Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Lys conjugated to KLH was used as the immunogen.<sup>1</sup> The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of fluorescein isothiocyanate isomer I to Protein A purified Monoclonal Anti- $\beta$ -Actin. It is purified by gel filtration and contains no detectable free FITC.

Monoclonal Anti- $\beta$ -Actin<sup>1,2</sup> recognizes an epitope located on the N-terminal end of the  $\beta$ -isoform of actin. The antibody labels specifically  $\beta$ -actin in a wide variety of tissues and species using immunoblotting (42 kDa),<sup>1,2</sup> immunofluorescent staining of cultured cell lines,<sup>1</sup> and immunohistochemistry.<sup>2,3</sup> In immunofluorescent staining of chicken gizzard ultrathin tissue cryosections, 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.<sup>2</sup> It does not stain adult cardiac and skeletal muscles (besides traces due to contaminations of the sample with nonmuscle cells or if embryonic tissue is being used). The antibody cross-reacts with  $\beta$ -actin expressing cells in human, bovine, sheep, pig, rabbit, cat, dog, mouse, rat, guinea pig, chicken, carp, and *Hirudo medicinalis* (leech) tissues, but not in *Dictyostelium discoideum* amoebae nor *Drosophila*. The epitope recognized by the antibody is resistant to formalin-fixation and paraffin-embedding. B5, ethanol, methacarn, or Bouin's solutions may also be used as fixatives.

Anti- $\beta$ -Actin-FITC antibody, Mouse monoclonal may be used for the localization of  $\beta$ -actin using direct immunofluorescent staining of frozen or fixed tissue sections and cultured cells. The conjugate is suitable for dual immuno-fluorescent 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 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 as at least six isoforms characterized by electrophoresis and amino acid sequence analysis.<sup>4,5</sup> Four of them represent the differentiation markers of muscle tissues and two are found practically in all cells. There are three  $\alpha$ -actins ( $\alpha$ -skeletal,  $\alpha$ -cardiac, and  $\alpha$ -smooth muscle), one  $\beta$ -actin ( $\beta$ -nonmuscle), and two  $\gamma$ -actins ( $\gamma$ -smooth muscle and  $\gamma$ -nonmuscle). Actin isoforms show >90% overall sequence homology, but only 50–60% homology in their 18 NH<sub>2</sub>-terminal residues.<sup>6</sup> The NH<sub>2</sub>-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 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  $\beta$ -actin provides a specific and useful tool in studying the intracellular distribution of  $\beta$ -actin and the static and dynamic aspects of the cytoskeleton.

#### Reagents

The product is provided 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/Stability

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. Protect from prolonged exposure to light.

### Product Profile

F/P Molar Ratio: 3–8

Direct immunofluorescence: a minimum working dilution of at least 1:250 is determined by labeling of cultured human and chicken fibroblasts.

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

### References

1. Gimona, M., et al., *Cell Motil. Cytoskel.*, **27**, 108 (1994).
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3. North, A., et al., *J. Cell Sci.*, **107**, 437 (1994).
4. Vandekerckhove, J., and Weber, K., *Eur. J. Biochem.*, **90**, 451 (1978).
5. Drew, J., et al., *Amer. J. Physiol.*, **260**, C1332 (1991).
6. Lessard, J., *Cell Motil. Cytoskel.*, **10**, 349 (1988).

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