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Product Information

MONOCLONAL ANTI-MAP KINASE, ACTIVATED (DIPHOSPHORYLATED ERK-1&2), CLONE MAPK-YT, FITC CONJUGATE

Product Number **F 7776**

Product Description

Monoclonal Anti-MAP Kinase Activated (Diphosphorylated ERK-1&2) (mouse IgG1 isotype) is derived from the MAPK-YT hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide containing 11 amino acids, HTGFLpTEpYVAT, corresponding to the phosphorylated form of ERK-activation loop.¹ The immunoglobulin fraction of antibody to Activated MAP Kinase is purified (protein A) from culture supernatant and then conjugated to fluorescein isothiocyanate (FITC) isomer I.

Anti-Map Kinase Activated (Diphosphorylated ERK-1&2) - FITC Conjugate may be used for the localization of the active, dually-phosphorylated form of MAP kinase using immunocytochemistry.

MAP kinase^{2,3} (MAPK, mitogen-activated protein kinase, also termed ERK, extracellular regulated protein kinase), consists of a family of protein kinases which are considered to play a crucial role in various signal transduction pathways leading signals of growth factor, as well as G protein-coupled receptors to their intracellular targets.^{4,5} MAP kinase was shown to regulate several cellular processes, among them proliferation, differentiation, cellular morphology and oncogenesis.^{4,5} Molecular cloning has established that MAP kinase (ERKs) consists of at least three isoforms; ERK-1 (p44^{mapk}), ERK-2 (p42^{mapk}) and ERK-5.³ Activation of ERK-1 and ERK-2 in mitogen-stimulated cells is directly mediated by MAP kinase kinase (MAPKK or MEK), a dual-specificity protein kinase, which phosphorylates both threonine and tyrosine residues in the regulatory sites of MAP kinase.^{6,7} Following activation, MAP kinase phosphorylates several nuclear targets, including transcription factors as well as membrane and cytoskeletal proteins.^{4,5}

Termination of MAP kinase signalling appears to be mediated by MAP kinase phosphatase, MKP-1, a dual specificity Thr/Tyr phosphatase which dephosphorylates and inactivates MAP kinase.⁸ MAP kinase isoforms appear to be widely expressed, in the central nervous system, thymus, spleen, heart, lung, kidney and are expressed in high levels in PC12 cells and in fibroblasts. Antibodies that react specifically with the active form of MAP kinase are useful for the study of the specific activation requirements, differential tissue expression and intracellular localization of the active form of MAP kinase in normal and neoplastic tissue.

Reagents

The conjugate is purified by gel filtration and contains no detectable free FITC. The product is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Monoclonal Anti-MAP Kinase Activated (Diphosphorylated ERK-1&2) - FITC conjugate reacts specifically with the active, dually-phosphorylated form of MAP kinase (ERK-1 and ERK-2, 44 kDa and 42 kDa, respectively). It does not recognize the non-phosphorylated or the monophosphorylated forms of the MAP kinase molecule or the diphosphorylated forms of JNK and p38 MAP¹. The epitope recognized by the antibody contains the phosphorylated threonine and tyrosine residues within the regulatory site of active MAP kinase (e.g., Thr¹⁸³ and Tyr¹⁸⁵ in ERK-2). The conjugate is useful in immunocytochemistry. It is reactive with human,¹ rat,¹ mouse,¹ insect (*Drosophila*^{7,9-11} and *Spodoptera frugiperda*¹) and yeast.¹

Specific Antibody Concentration: 0.5 - 2 mg/ml.
The F/P molar ratio of the product is 3-8.

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

For continuous use, store at 2-8 °C for a maximum of one month. For extended use store at -20 °C.

Repeated freezing and thawing is not recommended. 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

A minimum working dilution of 1:20 is determined by direct staining of paraformaldehyde-fixed, cultured human foreskin fibroblasts.

Notes:

1. In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test. The degree of activation and type of preparation under study may affect working dilution and pattern of staining.

2. It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary antibody. The ideal negative control reagent is an FITC conjugated mouse monoclonal antibody or myeloma protein. It should be isotype-matched, F/P molar ratio-matched, not specific for the tested preparation, and of the same concentration as the tested antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of preparation under study and the sensitivity of the instrument used. For fluorescent analysis of preparation expressing Fc receptors, the use of isotype-matched negative controls is mandatory.

References

1. Yung, Y., et al., FEBS Lett., **408**, 292 (1997)
2. Ray, L.B., and Sturgill, T.W., Proc. Natl. Acad. Sci. USA, **84**, 1502 (1987)
3. Boulton, T.G., et al., Cell **65**, 663 (1991)
4. Seger, R., and Krebs, E.G., FASEB J., **9**, 351 (1995)
5. Davis, R>J., J.Biol Chem., **268**, 14553 (1993)
6. Ahn, N.G., et al., Curr. Opin. Cell Biol., **4**, 992 (1992)
7. Seger, R., et al., J. Biol. Chem., **267**, 14373 (1992)
8. Sun, H., et al., Cell, **75**, 487 (1993)
9. Gabay, L., et al., Science **277**, 1103 (1997)
10. Gabay, L., et al., Development **124**, 3535(1997)
11. Gabay, L., et al., Development **124**, 4707(1997)

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