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ProductInformation

MONOCLONAL ANTI-MAP KINASE, ACTIVATED (Diphosphorylated ERK-1&2) Clone MAPK-YT Mouse Ascites Fluid

Product Number M 8159

Product Description

Monoclonal Anti-MAP Kinase, Activated (Diphosphory-lated ERK-1&2) (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c immunized mouse. A synthetic peptide containing 11 amino acids, HTGFLTpEYpVAT, corresponding to the phosphorylated form of ERK-activation loop conjugated to KLH 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 (Product Code ISO-2).

This antibody is specific for the active, dually-phosphorylated form of MAP kinase (ERK-1 and ERK-2, 44 kDa and 42 kDa, respectively). ¹ The epitope recognized by the antibody contains the phosphorylated threonine and tyrosine residues within the regulatory site of active MAP kinase (Thr¹⁸³ and Tyr¹⁸⁵ in ERK-2). It does not recognize the non-phosphorylated or the monophosphorylated forms of the MAP kinase molecule or the diphosphorylated forms of JUN kinase and p38 MAP kinase. The antibody may be used for immunoblotting^{1,9} of cultured cells and tissue extracts, in ELISA, immunocytochemistry, immunoprecipitation, and in immunohistochemistry (formalin and formaldehyde-fixed sections). Reactivity has been observed with human, bovine, rat, mouse, *Drosophila*, *Spodoptera frugiperda*, and yeast.

Monoclonal Anti-MAP Kinase, Activated (Diphosphory-lated ERK-1&2) may be used for the localization of the active, dually-phosphorylated, form of MAP kinase using various immunochemical assays.

MAP kinase (MAPK, mitogen-activated protein kinase, also termed ERK, extracellular regulated protein kinase), ^{2,3} 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 four isoforms: ERK-1 (p44^{mapk}), ERK-2 (p42^{mapk}), ERK-3, and ERK-5.² 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/Tvr 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 product is provided as ascites fluid containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

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

Storage/Stability

Store at 2-8 °C for up to one month.

For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

A minimum working dilution of 1:10,000 is determined using by immunoblotting of a rat brain extract.

In order to obtain best results in different techniques and preparations we recommend to determine optimal working dilutions by titration test.

References

- 1. Yung, Y., et al., FEBS Lett., 408, 292 (1997).
- 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).
- Seger, R., and Krebs, E.G., FASEB J., 9, 351 (1995).
- 5. Davis, R. J., J. Biol. Chem., 268, 14553 (1993).
- 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. Wappner, P., et al., Development, **124**, 4707 (1997).

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