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ProductInformation

MONOCLONAL ANTI-MAP KINASE, ACTIVATED/ MONOPHOSPHORYLATED (Phosphothreonine ERK-1&2), CLONE ERK-PT115

Product Number **M 7802**

Product Description

Monoclonal Anti MAP Kinase, Activated/ Monophosphorylated (Phosphothreonine ERK-1&2) (mouse IgG1 isotype) is derived from the ERK-PT115 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide containing 11 amino acids HTGFLpTEYVAT, corresponding to the phosphorylated form of ERK-activation loop, conjugated to KLH.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The immunoglobulin fraction is purified from hybridoma culture supernatant fluid using protein A.

Monoclonal Anti-MAP Kinase, Activated/ Monophosphorylated (Phosphothreonine ERK-1&2) reacts specifically with the monophosphorylated (threonine) and the doubly-phosphorylated forms of MAP kinase (ERK-1 and ERK-2, 44 kDa and 42 kDa, respectively). It does not recognize the non-phosphorylated form of the MAPK molecules, or the phosphorylated forms of JNK- and p38-MAPK. The epitope recognized by the antibody contains the phosphorylated threonine residue within the regulatory site of active MAP kinase (Thr^{183} in ERK-2). The product may be used in immunoblotting of cultured cell extracts, in ELISA and in immunochem-istry. Reactivity has been observed with human and rat.

Signal transduction is the mechanism by which extracellular agents transmit their messages to intracellular target molecules. The propagation and amplification mechanism of the primary signal involves many enzymes with specialized functions. These enzymes transmit the signals by several types of post-translational modifications, the most common being phosphorylation. The mitogen-activated protein kinase (MAPK) superfamily of enzymes is involved in widespread signaling pathways.[1,2] This family includes the ERK1/2 (extracellular signal-regulated protein kinase, also termed p42/p44 MAPK), JNK (c-Jun N-terminal protein kinase, also termed stress-activated protein kinase, SAPK1), and p38 MAPK (also termed SAPK2) subfamilies, which comprise interwoven signal transduction molecules. These are the terminal enzymes in a three- or four-kinase cascade where each kinase phosphorylates and thereby activates the next member in the sequence. The terminology used for the different levels of the cascades is MAPK kinase (MAPKK) for the immediate upstream activators of the MAPK, MAPKK kinase (MAP3K), and MAP3K kinase (MAP4K) for the enzymes further upstream, respectively. Usually, the cascades are referred to by the name of the kinase in their MAPK level, although the p38 MAPK cascade is also known as the SPK cascade. Interestingly, the kinases in the MAPK level are activated by phosphorylation of both tyrosine (Y) and threonine (T) residues organized in a TXY motif. The residue in between the two phosphorylated residues determines the specificity of activation of the MAPKs, and is glutamic acid for ERK (TEY), proline for JNK and glycine for p38 MAPK. Phosphorylation of both tyrosine and threonine is essential for the full activation of all MAPKs.[3-6] This diverse family of protein kinases plays many different roles and the balance and interrelationships between the signals transmitted via the ERK, SPK and JNK cascades play important roles in the determination of signaling specificity in all eukaryotic cells. While certain stimuli are highly selective for a given cascade, other stimuli activate two or more cascades, resulting in a highly coordinated series of signaling events. However, whereas ERK generally transmits signals leading to cell proliferation, both p38 MAPK and JNK are mostly stress-responsive kinases[3] and have been implicated in cell death in several cellular systems. Several kinases with similar

functions in the MAPKK and MAP3K levels have been implicated in the ERK cascade. This cascade is initiated by the small G-protein Ras, that upon stimulation, causes membranal translocation and activation of the protein serine/threonine kinase, Raf1. Once activated, Raf1 continues the transmission of the signal by phosphorylating two regulatory serine residues located in the activation loop of MEK, thus, causing its full activation. Other kinases that can activate MEK are A-Raf, B-Raf, Mos TPL2, and MEKK2, which all seem to phosphorylate the same regulatory residues of MEK. Activated MEK is a dual specificity protein kinase that appears to be the only kinase capable of specifically phosphorylating and activating the next kinase in this cascade, which is ERK. ERK activation requires phosphorylation of two regulatory residues, threonine and tyrosine, that reside in a TEY phosphorylation motif. ERK appears to be an important regulatory molecule, which by itself can phosphorylate regulatory targets in the cytosol (phospholipase A_2 ; PLA_2), translocate into and phosphorylate substrates in the nucleus (ELK1), or can transmit the signal to the MAPKAPK level. The main MAPKAPK of the ERK cascade is RSK, which can be translocated to the nucleus upon activation and phosphorylate a set of nuclear substrates different from those phosphorylated by ERK. Another MAPKAPK is MNK, which is activated by the SPK cascade. Although the activation of the ERK cascade was initially implicated in the transmission and control of mitogenic signals, this cascade is now known to be important for differentiation, development, stress response, learning and memory, and morphology determination.

Reagents
The product is supplied as purified mouse immunoglobulin in 0.01M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration is approximately 2 mg/ml as determined by E_{280}.

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 up to one month. For extended storage freeze 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 0.5 µg/ml is determined by immunoblotting using a whole cell extract of a rat fibroblast cell line, Rat1, activated with sorbitol.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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