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Product Information

Anti-MAP Kinase, Monophosphorylated Tyrosine antibody ,Mouse monoclonal
clone ERK-PY193, purified from hybridoma cell culture

Product Number **M3682**

Product Description

Monoclonal Anti-MAP Kinase, Mono-phosphorylated Tyrosine (pY-ERK) (mouse IgG1 isotype) is derived from the ERK-PY193 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide sequences containing 11 amino acids HTGFLTEpYVAT, corresponding to the phosphorylated form of ERK-activation loop, conjugated to KLH.[1] 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). The antibody is purified from culture supernatant of hybridoma cells, grown in a bioreactor.

Monoclonal Anti-MAP Kinase, Mono-phosphorylated Tyrosine (pY-ERK) reacts specifically with the monophosphorylated tyrosine form of MAP kinase (ERK-1 and ERK-2, 44 kDa and 42 kDa, respectively).[1] It does not recognize the non-phosphorylated, the doubly-phosphorylated, and the monophosphorylated threonine forms of the MAPK molecules, nor the doubly-phosphorylated forms of JNK- or p38-MAPK. The epitope recognized by the antibody contains the phosphorylated tyrosine residue within the regulatory site of MAP kinase (e.g.,Tyr^{185} in ERK-2).[1] Cross-reactivity has been observed with the monophosphorylated tyrosine peptide of JNK. The product may be used for immunoblotting[1] of cultured cells extracts, in ELISA,[1] dot-blot[1] and in immunocytochemistry[1]. Reactivity has been observed with human and rat.

Signal transduction is the mechanism by which extra-cellular agents transmit their messages to intracellular target molecules. The propagation and amplification mechanisms of the primary signal involve 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) super-family of enzymes is involved in widespread signaling pathways.[1-3] 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.[4-7] It appears that this diverse family of protein kinases plays many different roles, and that 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, p38 MAPK and JNK are both mostly stress-responsive kinases[4] 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, which 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 also activate MEK are A-Raf, B-Raf, Mos TPL2, and MEKK2, all of which 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 ERK, the next kinase in this cascade. ERK appears to be an important regulatory molecule, which by itself can phosphorylate regulatory targets in the cytosol (phospholipase A_2 ; PLA_2), translocated 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 also translocate 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 also by the SPK cascade. The inactivation of ERK may occur by removal of either tyrosine, threonine or both residues by phosphatases. The process of ERK inactivation in the early stages of mitogenic stimulation involves separate threonine and tyrosine phosphatases, which may react differently in different cellular compartment and in different cell types.[1] 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. Antibodies that specifically recognize phosphate incorporation into the regulatory tyrosine residues of ERK, are important tools in the study of activation/inactivation processes of ERK.

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

Antibody Concentration: Approx. 2mg/ml

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses.

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. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A working concentration of 1-5 μg/ml is determined by immunoblotting, using a whole cell extract of rat fibroblasts 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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