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Product Information

Anti-p38 MAP Kinase, Activated (Diphosphorylated p38) antibody, Mouse monoclonal
clone P38-TY, purified from hybridoma cell culture

Product Number **M8177**

Product Description

Anti-p38 MAP Kinase, Activated (Diphosphorylated p38) (mouse IgG2a isotype) is derived from the P38-TY hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. A synthetic peptide containing 13 amino acids HTDDEMpTGpYVATR, corresponding to the phosphorylated form of p38 MAP Kinase (MAPK)-activation loop conjugated to KLH was used as 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). The immunoglobulin fraction is purified from hybridoma culture supernatant fluid using protein A.

Anti-p38 MAP Kinase, Activated (Diphosphorylated p38) reacts specifically with the active doubly-phosphorylated form of p38 MAPK and its related isoforms. It does not recognize the non-phosphorylated and mono-phosphorylated forms of the p38 MAPK molecule, or the non-phosphorylated, monophosphorylated and diphosphorylated forms of JNK- and ERK-MAPK. The epitope recognized by the antibody contains the phosphorylated threonine and tyrosine residues within the regulatory site of active p38 MAP kinase (Thr^{180}-Gly-Tyr^{182}). The product may be used in immunoblotting of cultured cell extracts, in ELISA and in immunocytochemistry. Reactivity has been observed with human, rat and mouse.

Signal transduction is the mechanism by which extra-cellular 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. 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] 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[3] and have been implicated in cell death in several cellular systems. Many kinases in the MAPKK, MAP3K and MAP4K levels have been implicated in the SPK cascade, however their individual roles are not known. GCK1 and HPK1, and probably PAK1 belong to

the MAP4K level of SPK. At least ten distinct kinases have been implicated in the MAP3K level of this cascade (MEKK1-5, MTK1, MLK3, TPL2, DLK and TAK1). At the MAPKK level, SKK3 (SKK6, MEK6), SKK2 (MKK3), and SKK1 (MKK4, SEK1, JNKK1) seem to play the major role in the activation of all SPKs. The MAPK level components of this cascade (SPKs) are p38 MAPK (also termed RK, Hog, SAPK2a, and CSBP), SAPK2b, SAPK3 and SAPK4 (also termed p38β-δ). All these kinases contain a glycine residue in their TXY activation motif.[4] Once these SPKs are activated, they either transmit the signal to the MAPKAPK level components MAPKAPK 2 and 3 and MNK, or phosphorylate regulatory molecules such as phospholipase A_2 (PLA_2), and the transcription factors ATF2, ELK1, CHOP and MEF2C.[7,8] Antibodies reacting specifically with activated p38 MAPK are useful tools in the study of the intracellular location of p38 MAPK enzymes, and in sorting out the signal transduction pathways of the MAPK superfamily.

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 $E^{1\%}_{280}$.

Precautions and Disclaimer
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
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 1 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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