

## Product Information

### **MONOCLONAL ANTI-p38 MAP KINASE, NON-ACTIVATED Clone P38-YNP**

Purified Mouse Immunoglobulin

Product Number **M 8432**

#### **Product Description**

Monoclonal Anti-p38 MAP Kinase, Non-Activated (mouse IgG2b isotype) is derived from the P38-YNP hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide sequences, containing 13 amino acids (HTDDEMTGYVATR) corresponding to the non-phosphorylated form of p38 MAP Kinase-activation loop, conjugated to KLH. 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-p38 MAP Kinase, Non-Activated reacts specifically with the non-phosphorylated, non-activated form of p38 MAPK. Cross-reaction is observed with the threonine monophosphorylated, but not with doubly phosphorylated or tyrosine phosphorylated peptides of p38 MAPK. It does not recognize JNK and ERK1&2-MAPK. The epitope recognized by the antibody contains the regulatory site of p38 MAPK (Tyr<sup>182</sup>). Reactivity has been observed with human, rat, and mouse.

Monoclonal Anti-p38 MAP Kinase, Non-Activated may be used for immunoblotting (38-46 kDa) of cultured cells extracts, for ELISA and for immunocytochemistry.

Signal transduction is the mechanism by which extracellular agents transmit their messages to intracellular target molecules. Propagation and amplification of the primary signal involves protein-protein interactions as well as 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.<sup>1,2</sup> This family includes the ERK1/2 (extracellular signal-regulated protein kinase, also referred to as p42/p44 MAPK), JNK (c-Jun N-terminal protein kinase, also

referred to as stress-activated protein kinase, SAPK1), and p38 MAPK (also referred to as 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 terminal kinase in their MAPK level, although the p38 MAPK cascade is also known as the SPK cascade. 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.<sup>3-6</sup> 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<sup>3</sup> 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 also 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 a major role in the

activation of all SPKs. The MAPK level components of the SPK cascade are p38 MAPK (also known as RK, Hog, SAPK2a, and CSBP), SAPK2b, SAPK3 and SAPK4 (also termed p38 $\beta$ - $\delta$ ). All these kinases contain a glycine residue in their TXY activation motif.<sup>4</sup> Once these SPKs are activated, they either transmit the signal to the components of MAPKAPK level MAPKAPK 2 and 3 and MNK, or they phosphorylate regulatory molecules such as phospholipase A2 (PLA2), and the transcription factors ATF2, ELK1, CHOP and MEF2C.<sup>7,8</sup> Antibodies reacting specifically with non-activated p38 MAPK are useful tools in the study of the intracellular location of p38 MAPK enzymes, and in sorting out signal transduction pathways of the MAPK superfamily.

### Reagents

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

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

### Product Profile

The antibody concentration is approximately 2mg/ml by absorbance at 280 nm.

A working concentration of 1-5  $\mu$ g/ml is determined by immunoblotting, using a whole cell extract of rat fibroblasts cell line Rat1.

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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8. Han, J., et al., *Nature*, **386**, 296 (1997).

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