3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-JNK, ACTIVATED (DIPHOSPHORYLATED JNK)
CLONE JNK-PT48
Purified Mouse Immunoglobulin

Product Number **J 4750**

Product Description

Monoclonal Anti-JNK, Activated (Diphosphorylated JNK) (mouse IgG1 isotype) is derived from the JNK-PT48 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide sequences containing amino acids pTPpYVVTRYYR, corresponding to the phosphorylated form of JNK-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-JNK, Activated (Diphosphorylated JNK) reacts specifically with the activated, doubly phosphorylated form of the JNK protein. It does not recognize any form of p38- or ERK-MAPK proteins. Weak cross-reaction is observed with the mono-phosphorylated threonine peptide, but not with the non-phosphorylated or monophosphorylated tyrosine peptides of JNK. The epitope recognized by the antibody contains the regulatory site of JNK (Thr^{183} and Tyr^{185}). The antibody may be used for immunoblotting (46 and 54 kDa) of activated cultured cells extracts, in ELISA, and in immuno-cytochemistry. Reactivity has been observed with human, rat and mouse.

Signal transduction is the mechanism by which extracellular agents transmit their messages to intracellular target molecules. Propagation and amplification of the primary signal, involve protein-protein interaction 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. The mitogen-activated protein kinase (MAPK) superfamily of enzymes is involved in widespread signaling pathways.[1,2] This 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 residue 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 with similar functions in the MAPKK and MAP3K and MAP4K levels have been implicated in the JNK cascade

however their individual roles are not known. As the other MAPK cascades, the JNK cascade is triggered by small GTPases that are Rac and CDC42. Next, the signals are transmitted via MAP4K and MAP3K components that are all shared with the SPK cascades. GCK1 and HPK1, and probably also PAK1 belong to the MAP4K level of JNK cascade. At least ten distinct kinases have been implicated in the MAP3K level of this cascade (MEKK1-5, MTK1, MLK3, TPL2, DLK and TAK1). Since the SPK and JNK cascades are not always simultaneously activated, the signals must be separately regulated to allow separate cascades but the mode of this regulation is unknown as yet. At the MAPKK level, the JNKs can be activated by at least three dual-specificity enzymes that are SEK1 (SKK1, MKK4); MKK7 and JNKK2. All three JNKKs seems to be able to activate the components in the MAPK level which are JNK1-3 (SAPKs) of molecular weights 46, 54, and 52 kDa respectively. No enzymes in the MAPKAPK level and no cytosolic targets have been identified for JNKs, but these enzymes appear to be major regulators of nuclear processes and in particular transcription. Shortly after activation, JNKs translocate to the nucleus where they physically associate with and activate their target transcription factors (cJun, ATF, Elk, etc.). Antibodies reacting specifically with activated JNK are useful tools in the study of the intracellular location of JNK enzymes, and in sorting out signal transduction pathways of the MAPK superfamily.

Reagents

The product is supplied as a solution 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

A working concentration of 4-20 µg/ml is determined by immunoblotting using a whole cell extract of human acute T cell leukemia cell line Jurkat, activated with anisomycin.

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

References

1. Seger, R., and Krebs, E.G., FASEB J., **9**, 351 (1995).
2. Davis, R.J., J. Biol. Chem., **268**, 14553 (1993).
3. Davis, R.J., Trends Biochem. Sci., **19**, 470 (1994).
4. Han, J., et al., Science, **265**, 808 (1994).
5. Lee, J.C., et al., Nature, **372**, 739 (1994).
6. Rouse, J., et al., Cell, **78**, 1027 (1994).

lpg 8/99