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ProductInformation

c-Jun N-Terminal Kinase Rat, Recombinant Expressed in *E. coli*

Product Number **C 3108** Storage Temperature: –70 °C

Synonyms: JNK3, SAPK-β, p54β

Product Description

c-Jun N-terminal kinases (JNK) or stress-activated protein kinases (SAPK) are proline-directed, serine/threonine-specific kinases that require phosphorylation on tyrosine for phosphotransferase activity.^{1,2,3} JNK/SAP kinases were originally shown to phosphorylate microtubule-associated protein-2, but c-Jun appears to be an important physiological target of this kinase.^{2,3} Phosphorylation of Ser⁶³ and Ser⁷³ of c-Jun by JNK/SAP kinases promotes activation of the transcription factor AP-1.^{4,5,11} cDNA cloning studies with rat and human libraries have shown that there are several isoforms of the SAP Kinase family.^{6-8,11} Many stimuli activate JNK/ SAP kinase, including heat shock,⁷ hyperosmotic shock,^{8,9} UV irradiation, exposure to protein synthesis inhibitors such as cycloheximide and anisomycin,^{7,9,10} and treatment with proinflammatory cytokines such as tumor necrosis factor-α.^{7,8}

JNK1 and JNK2 are expressed ubiquitously, while JNK3 is expressed primarily in brain, heart, and testis.¹¹ JNK3 activation appears to be in a common pathway leading to neuronal apoptosis induced by β -amyloid¹², arsenic¹³, and kainic acid receptor (GluR6) activation¹⁴.

This c-Jun kinase was cloned from a rat skeletal muscle cDNA library and expressed in *E. coli*. The expression vector encoded a fusion protein comprising, from the amino- to the carboxyl-terminus, 1) a calmodulin binding peptide, 2) the thrombin cleavage site, and 3) JNK3. The recombinant protein was purified by affinity chromatography on a calmodulin resin. The molecular weight of the fusion protein is 52 kDa. JNK3 has a calculated molecular weight of 48.1 kDa.

JNK3 may be used to study enzyme regulation and kinetics and to phosphorylate target substrates.

Reagent

JNK3 is supplied as a solution containing 0.4 mg/ml protein in 50 mM Tris HCl, pH 7.5, 150 mM sodium chloride, 1,mM magnesium acetate, 1 mM imidazole, 0.5 μ M ATP, 10 mM 2-mercaptoethanol and 10% glycerol.

Precautions and Disclaimer

Please consult the Material Safety Data Sheet for handling recommendations before working with this product.

Product Profile

Purity is >90% by SDS-PAGE.

The enzyme activity is >30 nmol [32 P] incorporated per min per mg enzyme at 30 °C using GST-c-Jun fragment 1-79 (C 3233) as substrate. The standard reaction contains 0.1 µg JNK and 2 µg c-Jun kinase substrate in 25 mM HEPES, pH 7.4, 10 mM magnesium acetate, and 50 µM ATP (2 µCi [32 P]-ATP) in a final volume of 40 µl. Allow the reaction to proceed for 30 min at 30 °C.

Note: Optimal assay conditions must be determined by the user for different substrates and systems.

Storage/Stability

The product should be stored at -70 °C. After initial thawing, store the solution in working aliquots at -70 °C. Avoid repeated freeze/thaw cycles.

References

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