

JNK1 Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length mouse JNK1 (Met1-Gln384) kinase, supplied as a GST fusion protein.

Background: The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses, including UV and γ radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4-7, which then phosphorylate and activate the SAPK/JNK kinase (2). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (3). Both Rac1 and cdc42 mediate the stimulation of MEKs and MLKs (3). Alternatively, MKK4-7 can be activated by a pathway independent of small GTPases via stimulation of a member of the germinal center kinase (GCK) family (4). There are three SAPK/JNK genes with further diversification resulting from alternative splicing (3). SAPK/JNK, when active as a dimer, can translocate to the nucleus where it regulates transcription through its effects on c-Jun, ATF-2 and other transcription factors (3,5).

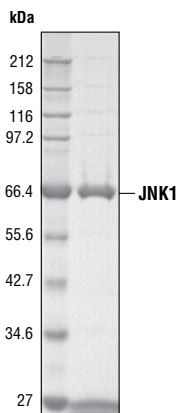


Figure 1. The purity of the GST-JNK1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length mouse JNK1 (Met1-Gln384) (GenBank Accession No. NM_016700) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-JNK1 fusion protein is 71 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. JNK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Davis, R.J. (1999) *Biochem. Soc. Symp.* 64, 1–12.
- (2) Ichijo, H. (1999) *Oncogene* 18, 6087–6093.
- (3) Kyriakis, J.M. and Avruch, J. (2001) *Physiol. Rev.* 81, 807–869.
- (4) Kyriakis, J.M. (1999) *J. Biol. Chem.* 274, 5259–5262.
- (5) Leppa, S. and Bohmann, D. (1999) *Oncogene* 18, 6158–6162.
- (6) Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Biochem. Sci.* 23, 481–485.

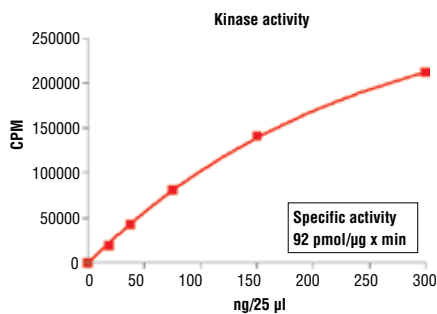


Figure 2. JNK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/ μ l BSA, 50 μ M ATP, Substrate: ATF2 200 ng/ μ l and recombinant JNK1: variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C .

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Protocol for JNK1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (10X)**
40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
40 mM $MgCl_2$
0.5 mM DTT
400 ng/ μ l BSA
- 2. ATP (10 mM) #9804**
- 3. ^{32}P - γ ATP**
- 4. ATF2 (0.5 μ g/ μ l)**

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from $-80^\circ C$ to ice. Allow enzyme to thaw on ice.
- 4.** Dilute JNK1 protein (100 ng/ μ l concentration) to 30 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted JNK1 kinase solution, 10 μ l ATF2 (0.5 μ g/ μ l) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 4 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 4 mM $MgCl_2$
 - 0.05 mM DTT
 - 40 ng/ μ l BSA
 - 200 ng/ μ L ATF2
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

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