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Product Specification

JNK1, Active

Full-length recombinant protein expressed in Sf9 cells

Cat.#	7757-5
Lot#	_____
Aliquot Size:	5 µg in 50 µl/vial
Concentration:	0.1 µg/µl
Purity:	>90%
Storage:	-80°C
Shipping:	in Dry ice
Shelf Life:	6-12 months from shipping date
Specific Activity:	124 nmol/min/mg

Product Description

Recombinant full-length mouse JNK1 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_016700

Gene Aliases

JNK; JNK1; PRKM8; SAPK1; JNK1A2; JNK21B1/2

Formulation

Recombinant protein in stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

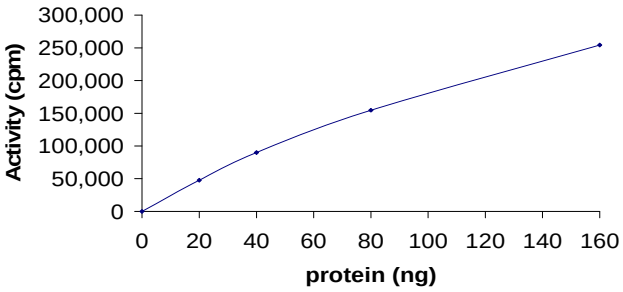
Scientific Background

JNK1 is a member of the MAP kinase group that is activated by dual phosphorylation at thr and tyr residues during exposure to stress such as UV irradiation. JNK1 binds to the c-Jun transactivation domain and phosphorylates it on Ser-63 and Ser-73 (1). JNK1 has been shown to play an important role in disease processes. Activation of JNK1 results in defects in myotube viability and integrity leading to dystrophic myofiber destruction (2). JNK1 activity is also abnormally elevated in obesity and removal of JNK1 results in decreased adiposity and significantly improved insulin sensitivity.

References

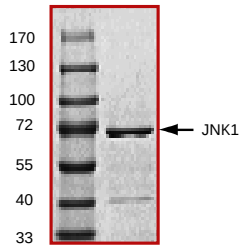
1. Derijard, B. et al: JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell. 1994 Mar 25;76(6):1025-37.
2. Kolodziejczyk, S M. et al: Activation of JNK1 contributes to dystrophic muscle pathogenesis. Curr Biol. 2001 Aug 21;11(16):1278-82.

Specific Activity



The specific activity of JNK1 was determined to be **124 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry.
Approx. MW **~71kDa**.

Activity Assay Protocol

Reaction Components

Active Kinase

Active JNK1 (0.1μg/μl) diluted with Kinase Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active JNK1 for optimal results).

Kinase Dilution Buffer, pH 7.2

Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer I, pH 7.2

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution, 100μl [^{32}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer. Store 1ml aliquots at –20°C.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200μl aliquots at –20°C.

Substrate

ATF2 substrate prepared in buffer (50mM Tris-HCl, pH 7. 2, 50mM $NaC1_2$, 5mM EDTA and 0.25mM DTT) to a final concentration of 0.5mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active JNK1, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active JNK1.
- **Component 2.** 10μl of 0.5mg/ml ATF2 substrate.

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{32}P]-ATP Assay Cocktail bring the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{32}P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmols/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]