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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M34-10BG-05	5 µg
M34-10BG-10	10 µg

JNK2, Active

Full-length human protein expressed in Sf9 cells

Catalog # M34-10BG

Lot # M2851-2

Product Description

Recombinant full-length human JNK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_139069.

Gene Aliases

MAPK9, SAPK, p54a, JNK2A, JNK2B, PRKM9, JNK-55, JNK2BETA, p54aSAPK, JNK2ALPHA

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 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.

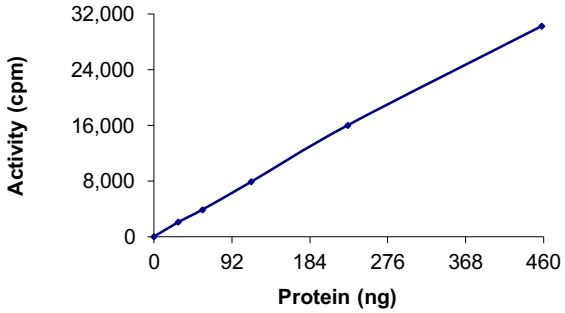
Scientific Background

JNK2 is a member of the JNK family that acts as an integration point for multiple biochemical signals, and is involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. JNK2 targets specific transcription factors, and mediates immediate-early gene expression in response to various cell stimuli. Both UV radiation and the proinflammatory cytokine TNFα can induce JNK2. JNK2 play a main role in the regulation of regional specific apoptosis (1) during early brain development and providing protection against autoimmune diabetes (2).

References

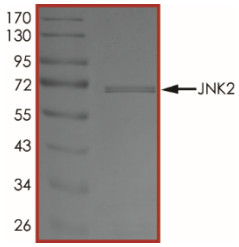
1. Kuan, C. et.al: The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron 22: 667-676, 1999.
2. Jaeschke, A. et.al: Disruption of the Jnk2 (Mapk9) gene reduces destructive insulitis and diabetes in a mouse model of type I diabetes. Proc. Nat. Acad. Sci. 102: 6931-6935, 2005.

Specific Activity



The specific activity of JNK2 was determined to be **11 nmol /min/mg** as per activity assay protocol.

Purity



The purity of JNK2 was determined to be **>95%** by densitometry. Approx. MW **~70kDa**.

JNK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	M34-10BG
Specific Activity	11 nmol/min/mg
Lot #	M2851-2
Purity	>95%
Concentration	0.1µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M34-10BG)

Active JNK2 (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 JNK2 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: A10-55G)

ATF2 protein substrate prepared in buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.25mM DTT, 0.1mM PMSF) to a final concentration of 0.2 μg/μl.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active JNK2, Kinase Assay Buffer, Substrate and Kinase 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 JNK2 (Catalog #M34-10BG)
- **Component 2.** 10μl of 0.2mg/ml ATF2 substrate (Catalog #A10-55G)

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 [^{33}P]-ATP Assay Cocktail bringing 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^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{33}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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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