Recombinant Mouse Active JNK1 Protein, CF

Certificate of Analysis

Catalog Number: 1776-KS-010

Lot Number: 1685690

SPECIFICATIONS AND USE

Source	Recombinant full-length mouse JNK1 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_016700. This mouse JNK1 product matches 100% of human protein sequence in accession number NP_002741.
Molecular Mass	The approximate molecular weight is 70 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of JNK1 was determined to be 104 nmol/min/mg as per activity assay protocol and was equivalent to 122 nmol/min/mg as per radiometric assay.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

JNK1 is a member of the MAP kinase group that is activated by dual phosphorylation at Threonine and Tyrosine residues during exposure to stress such as UV irradiation. JNK1 binds to the c-Jun transactivation domain and phosphorylates it on S63 and S73 (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.

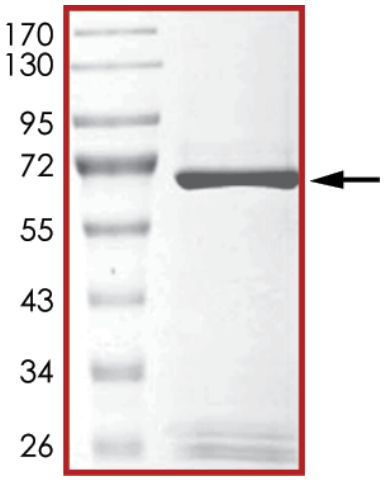

Figure 1: The purity was determined to be > 90% by densitometry. Approximately MW ~70kDa.

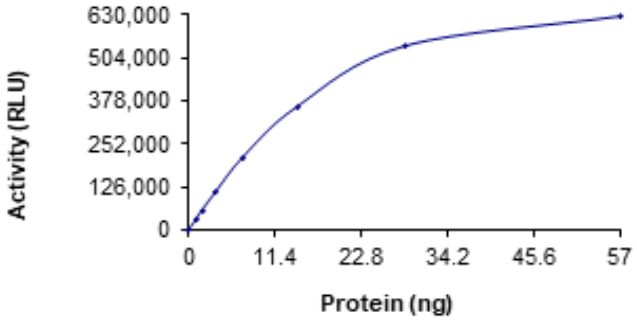

Figure 2: Enzymatic assay results. The specific activity of JNK1 was determined to be 104 nmol/min/mg as per activity assay protocol.

REFERENCES

1. Derijard, B. *et al.* (1994) Cell **76**:1025.
2. Kolodziejczyk, S.M. *et al.* (2001) Curr Biol. **11**:1278.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active JNK1 (0.1 µg/µL) diluted with Kinase Dilution Buffer IX 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 JNK for optimal results.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$ and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold, distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit- 10 mM of ATP Solution, 10 mM of ADP Solution, ADP-Glo™ Reagent, and Kinase Detection Reagent.

Substrate - ATF2 Substrate prepared in buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT) to a final concentration of 0.2 mg/mL.

ASSAY PROCEDURE

The JNK1 assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the JNK1 reaction. The ADP-Glo Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active JNK1, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution using Kinase Dilution Buffer IX (1X), at the desired concentration, in a pre-chilled 96-well plate.
2. Prepare a Substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	79 µL
Substrate at 0.2 mg/mL	80 µL

3. Transfer the following reaction components prepared in Steps 1 and 2 to a 384-well opaque plate, bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active JNK1.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Then add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)
From ATP-ADP conversion curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)

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