

Recombinant Mouse Active JNK1

Certificate of Analysis

Catalog Number: 1776-KS

Lot Number: 1479930

Specifications and Use

- Source** ♦ Recombinant full-length mouse JNK1 was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM_016700.
- Molecular Mass** ♦ The approximate molecular weight is 70 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
- Formulation** ♦ Supplied in 50 mM Tris-HCl, pH 7.5, 50 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 94 nmol/min/mg using an ATF2 substrate (see activity assay protocol).
- Storage** ♦ This product is stable at ≤ -70 °C for up to 1 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.**

JNK1

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.

References

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

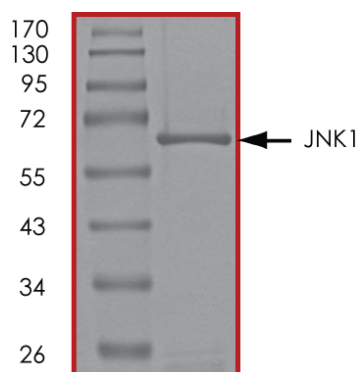


Figure 1: The approximate molecular weight is 70 kDa and the purity is > 95%.

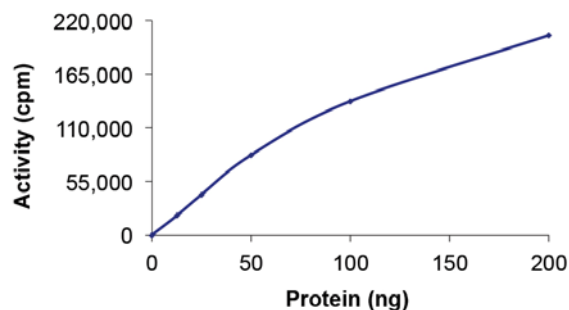


Figure 2: The specific activity of JNK1 was determined to be 94 nmol/min/mg as per activity assay protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active JNK1 (0.1 µg/µL) diluted with Kinase Dilution Buffer III and assayed as outlined in Figure 2.
Note: These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer III, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/µL of BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- **[³³P]-ATP Assay Cocktail** - Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [³³P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I.
- **Substrate** - ATF2 substrate prepared in buffer (50 mM Tris-HCl, pH 7.2, 50 mM NaCl₂, 5 mM EDTA, and 0.25 mM DTT) diluted in distilled or deionized water to a final concentration of 0.2 mg/mL.

Assay Procedure

1. Thaw the [³³P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active JNK1, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer III on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active JNK1	10 µL
Substrate (0.2 mg/mL Stock Solution)	10 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5.0 µL [³³P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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.

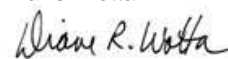
Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5.0 µL [³³P]-ATP/pmole of ATP (in 5.0 µL of a 250 µM ATP stock solution, i.e. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

Diane Wotta



Quality & Regulatory Affairs