

Certificate of Analysis

JNK3/SAPK1b, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-501, 14-501-K, 14-501M

Parent Lot # D7CN041U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, full length human JNK3/SAPK1b, expressed by baculovirus Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Activated with MKK4 (cat# 14-377) and MKK7β1 (cat# 14-543) and repurified using Ni²⁺-NTA agarose. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 53kDa.

Specific Activity (Parent lot# D7CN041U): 128U/mg, where one unit of JNK3, active activity is defined as 1nmol phosphate incorporated into 3μM ATF-2 (cat# 12-367) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 2.76mg/ml of 50mM Tris/HCl, pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

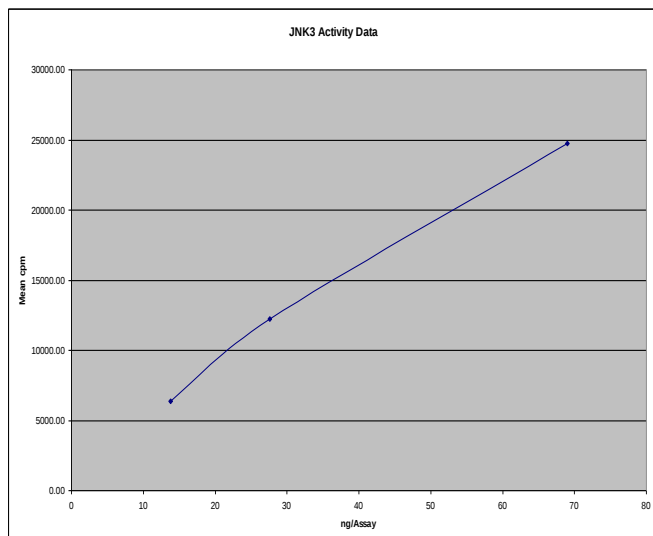
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

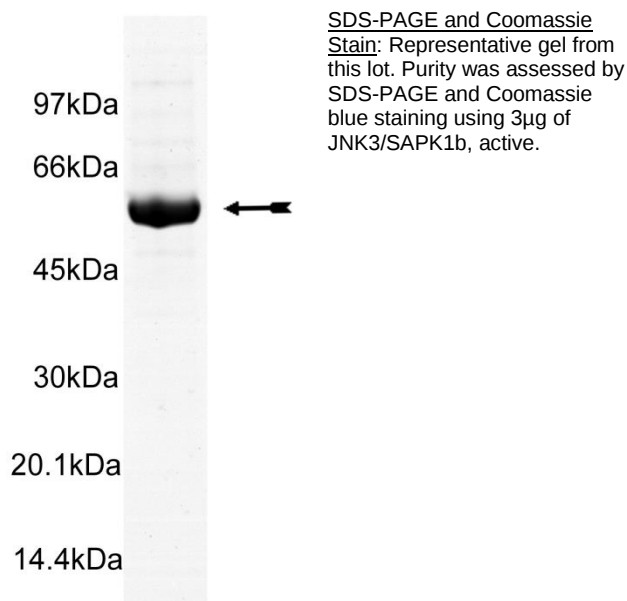
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 13–69ng of this lot of enzyme phosphorylated 3μM ATF-2 (cat# 12-367) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as JNK3/SAPK1b with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **ATF-2 (Catalogue# 12-367):** Use at a final assay concentration of 3 μ M (0.108mg/ml). Prepare a 1.08mg/ml stock and add 2.5 μ l of stock per assay point.
3. **JNK3/SAPK1b, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 13–69ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5 μ l of 10 x reaction buffer to wells.
2. Add 2.5 μ l of **ATF-2**.
3. Add **2.5 μ l (13–69ng) JNK3/SAPK1b, active**.
4. Add 7.5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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JNK3/SAPK1b Sequence Information

Protein	Human JNK3/SAPK1b
Tags	N-terminal 6His
Native sequence	M45 of the recombinant protein is equivalent to M1 of human JNK3/SAPK1b
Accession number	The JNK3 protein sequence is identical to the human JNK3/SAPK1b described in GenBank NM_138980. The DNA sequence, however, is more closely related to the rat cDNA described in GenBank NM_012806.

Recombinant JNK3/SAPK1b amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGSPG ISGVDVSSVA KHYNMSKSKV DNQFYSVEVG
61 DSTFTVLKRY QNLKPIGSGA QGIVCAAYDA VLDRNVAIKK LSRPFQNTQTH AKRAYRELVL
121 MKCVNHKNII SLNVFTFPQK TLEEFQDVYL VMELMDANLC QVIQMELDHE RMSYLLYQML
181 CGIKHLHSAG IHRDLKPSN IVVKS DCTLK ILDFGLARTA GTSFMMPYV VTRYRRAPEV
241 ILGMYKENV DIWSVGCIMG EMVRHKILFP GRDYIDQWNK VIEQLGTPCP EFMKKLQPTV
301 RNYVENRPKY AGLTFPKLFP DSLFPADSEH NKLKASQARD LLSKMLVIDP AKRISVDDAL
361 QHPYINWYD PAEVEAPPPQ IYDKQLDERE HTIEEWKELI YKEVMNSEEK TKNGVVKGQP
421 SPSGA AVNSS ESLPPSSSVN DISSMSTDQT LASD TDSSLE ASAGPLGCCR

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Recombinant JNK3/SAPK1b nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg ggcggcatggg atccccggga atttcgggtg tggatgtgtc ttctgttgcc
121 aaacattaca acatgagcaa aagcaaggta gataaccagt tctacagtgt ggaagtggga
181 gactcaacct tcacagttct aaagcgctac cagaacctga agccgatcgg ctctggggct
241 cagggaatag tttgtgctgc gtatgacgct gtcctcgaca gaaatgtggc cattaagaag
301 ctgagcagac ccttcagaa ccaactcat gccaaaggag cttaccggga gctggctctc
361 atgaagtgtg tgaaccataa aaacattatt agcttattaa atgtctttac accccagaaa
421 aactggagg agttccaaga tgtttactta gtgatggaac tgatggacgc caacttgtgt
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661 ggcacaagct tcatgatgac tccgtatgtg gtgacgagat attacagagc ccccgaggtc
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901 agaaactacg tggagaaccg gcccaagtat gcaggcctca cttcccccga gctctttcca
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1021 ttgttgtaa agatgttagt gattgaccca gcgaagagga tatcggtgga tgacgcattg
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1141 atatatgaca agcaactgga tgaaaggag cacaccatcg aagaatggaa tgaactcatc
1201 tacaaggaag taatgaactc agaagagaag actaagaacg gcgtagtcaa aggccagccc
1261 tcaccttcag gtgcagcagt gaacagcagt gagagtctcc ctccatcctc atctgtcaac
1321 gacatctcct ccatgtccac cgaccagacc ctgcgatccg acactgacag cagcctggaa
1381 gcctcggcgg gaccgctggg ttgttgacag tga

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