

Certificate of Analysis

JNK1 α 1, unactive

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-328, 14-328-K, 14-328M

Parent Lot # WAB0451

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, human, full-length JNK1 α 1, unactive, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

Formulation: 1.33mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Specific Activity (Parent lot# WAB0451): As provided, this lot demonstrated 0.24% of maximum activity. Activated by phosphorylation with MKK4 (cat# 14-377) and MKK7 β 1 (cat# 14-366).

Storage and Stability: On receipt of material store at -20°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.

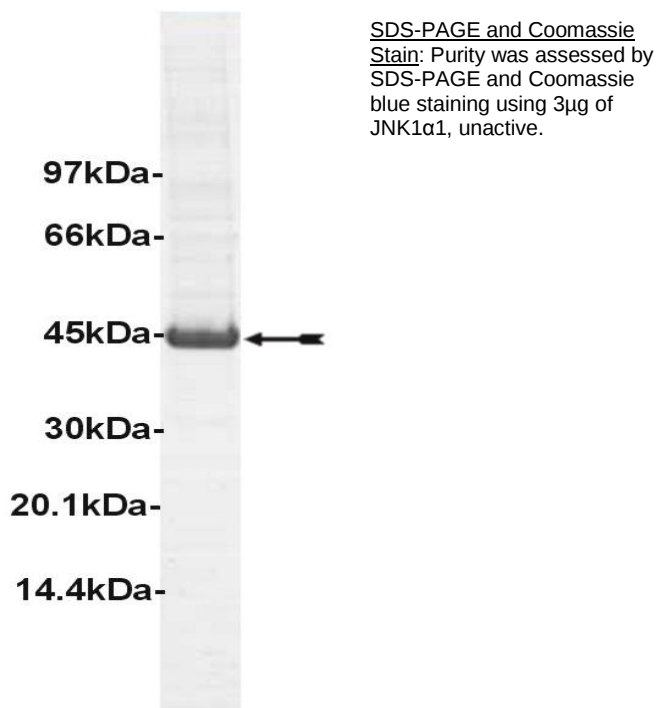
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Activation Assay: 2 μ M JNK1 α 1, unactive was activated using 0.1 μ M MKK4 (cat# 14-377) and 0.1 μ M MKK7 β 1 (cat# 14-366) and the increased activity of JNK1 α 1 against ATF-2 determined. Assay is described on page two. Results of this assay are shown below

Active MKK4	Active MKK7 β 1	Unactive JNK1 α 1	Mean cpm	Comments
None	None	2.25 μ g	51	Background
169.5ng	185ng	2.25 μ g	8349	Kinase activity

MS Tryptic Fingerprint: Confirmed product identity as JNK1 α 1 with the translated sequence listed on page three



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Kinase Assay Protocol

Stock Solutions:

- 10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
- Enzyme Dilution Buffer:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol.
- MKK4, active (Catalogue# 14-377):** Use at a final concentration of 0.1μM (6.78μg/ml). Prepare a 67.8μg/ml stock and add 2.5μl of stock per assay point.
- MKK7β1, active (Catalogue# 14-366):** Use at a final assay concentration of 0.1μM (7.4μg/ml). Prepare a 74μg/ml stock and add 2.5μl of stock per assay point.
- Magnesium/ATP Cocktail:** 50mM magnesium acetate, 0.5mM ATP.
- [γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
- JNK1α1, inactive:** Use at a final assay concentration of 2μM (0.09mg/ml). Prepare a 0.9mg/ml stock and add 2.5μl of stock per assay point.
- ATF-2:** Use at a final assay concentration of 3μM (0.108mg/ml). Prepare a 0.54mg/ml stock and add 5μl of stock per assay point.

Assay Procedure:

Stage One: *Activation of JNK1α1 by MKK4 and MKK7β1.*

- Add 2.5μl of assay buffer to a microcentrifuge tube.
- Add **2.5μl (2.25μg) of JNK1α1 inactive.**
- Add 2.5μl (169.5ng) of **MKK4, active.**
- Add 2.5μl (185ng) of **MKK7β1, active.**
- Add 10μl of dH₂O.
- Add 5μl of magnesium/ATP cocktail.
- Run a no activator control.
- Incubate for 30 minutes at 30°C.
- Dilute assay tubes in enzyme dilution buffer 20-fold to stop reaction and incubate on ice.

Stage Two: *Phosphorylation of ATF-2 by JNK1α1*

- Add 2.5μl of reaction buffer per assay to wells.
- Add 5μl of **ATF-2.**
- Add **5μl** of diluted **Stage One** reaction product.
- Add 2.5μl of dH₂O.
- Add 10μl of the diluted [γ-³³P]ATP.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5μl 3% phosphoric acid.
- Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat.**
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
- Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all enzyme components plus 1μl 30% phosphoric acid.

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JNK1α1 Sequence Information

Protein	human JNK1α1
Tags	N-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human JNK1α1
Accession number	EMBL L26318

Recombinant JNK1α1 amino acid sequence:

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1  MHHHHHMSR SKRDNNFYVS EIGDSTFTVL KRYQNLKPIG SGAQGIVCAA YDAILERNVA
61 IKKLSRPFQN QTHAKRAYRE LVLMKCVNHK NIIGLLNVFT PQKSLEEFQD VYIVMELMDA
121 NLCQVIQMEI DHERMSYLLY QMLCGIKHLH SAGIIHRDLK PSNIVVKSDC TLKILDFGLA
181 RTAGTSFMMT PYVVTRYRYR PEVILGMGYK ENVDLWSVGC IMGEMVCHKI LFPGRDYIDQ
241 WNKVIEQLGT PCPEFMKKLQ PTVRTYVENR PKYAGYSFEK LFPDVLFPAD SEHNKLKASQ
301 ARDLLSKMLV IDASKRISVD EALQHPYINV WYDPSEAEAP PPKIPDKQLD EREHTIEEWK
361 ELIYKEVMDL EERTKNGVIR GQPSPLAQVQ Q

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Recombinant JNK1α1 nucleotide sequence:

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1  atgcaccatc accatcacca tatgagcaga agcaagcgtg acaacaattt ttatagtgtgta
61 gagattggag attctacatt cacagtcctg aaacgatatc agaatttaaa acctataggc
121 tcaggagctc aaggaatagt atgcgcagct tatgatgcca ttcttgaaaag aaatggttgca
181 atcaagaagc taagccgacc atttcagaat cagactcatg ccaagcgggc ctacagagag
241 ctagttctta tgaaatgtgt taatcacaaa aatataattg gccttttgaa tgttttcaca
301 ccacagaaat ccctagaaga atttcaagat gtttacatag tcatggagct catggatgca
361 aatctttgcc aagtgattca gatggagcta gatcatgaaa gaatgtccta cttctcttat
421 cagatgctgt gtggaatcaa gcacctcat tctgctggaa ttattcatcg ggacttaaaag
481 cccagtaata tagtagtaaa atctgattgc actttgaaga ttcttgactt cggctctggcc
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601 cccgaggtca tccttgatc gggctacaag gaaaacgtgg atttatggtc tgtgggggtgc
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961 gaagctctcc aacacccgta catcaatgtc tggatgatc cttctgaagc agaagctcca
1021 ccaccaaaaga tccctgacaa gcagttagat gaaagggaac acacaataga agagtggaaa
1081 gaattgatat ataaggaagt tatggacttg gaggagagaa ccaagaatgg agttatacgg
1141 gggcagccct ctccttttagc acaggtgacg cagtga

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