

Certificate of Analysis

JNK 2 α 2/SAPK1a, unactive

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-330, 14-330-K, 14-330M

Parent Lot # 2423252

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 JNK 2 α 2/SAPK1a unactive, expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 99.3% by SDS-PAGE and Coomassie blue staining. MW = 49.2kDa

Formulation: 2.453mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Specific Activity (Parent lot# 2423252): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MKK4, active (cat# 14-377) and MKK7 beta 1, active (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**

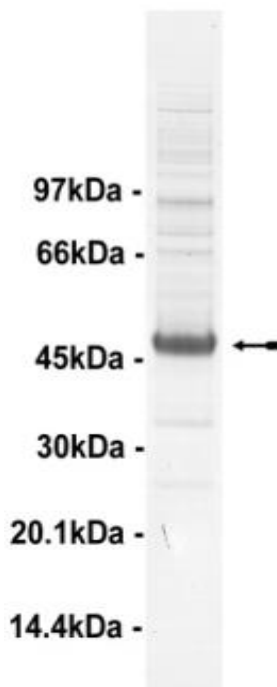
Quality Control Testing

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

Active MKK4	Active MKK7	Unactive JNK 2alpha2	Mean cpm	Comments
None	None	679ng	9	Background
8.47ng	12.14ng	679ng	3135	Kinase activity

MS Tryptic Fingerprint: Confirmed identity as JNK 2 alpha 2 with the translated sequence listed on page three

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of JNK2 α 2/SAPK1a, unactive.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Assay Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **Enzyme Dilution Buffer:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1 % 2-mercaptoethanol.
3. **JNK 2 alpha 2, inactive:** Use at a final assay concentration of 2μM (0.0984mg/ml). Prepare a 0.984mg/ml stock and add 2.5μl per assay point
4. **MKK4, active (Catalogue#14-377):** Use at a final assay concentration of 0.1μM (6.78μg/ml). Prepare a 67.8μg/ml stock and add 2.5μl of stock per assay point.
5. **MKK7 beta 1, 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.
6. **Magnesium/ATP Cocktail:** 50mM magnesium acetate, 0.5mM ATP.
7. **[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.)
8. **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 JNK 2 alpha 2 by MKK4 and MKK7 beta 1.*

1. Add 2.5μl of assay buffer to a microcentrifuge tube.
2. Add **2.5μl (679ng) JNK 2 alpha 2 inactive.**
3. Add 2.5μl (8.47ng) of **MKK4, active.**
4. Add 2.5μl (12.14ng) of **MKK7 beta 1, active.**
5. Add 10μl of dH₂O.
6. Add 5μl of magnesium/ATP cocktail.
7. Run a no activator control also.
8. Incubate for 30 minutes at 30°C.
9. Dilute assay tubes in enzyme dilution buffer 20-fold to stop reaction and incubate on ice.

Stage Two: *Phosphorylation of ATF-2 by JNK 2 alpha 2.*

1. Add 2.5μl of reaction buffer to a microcentrifuge tube.
2. Add 5μl of **ATF-2.**
3. Add **5μl** of diluted **Stage One** reaction product.
4. Add 2.5μl of dH₂O.
5. Add 10μl of the diluted [γ-³³P]ATP.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5μl of 3% phosphoric acid.
8. Transfer a 10μl 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 1μl of 30% phosphoric acid.

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JNK 2 alpha 2 Sequence Information

<u>Protein</u>	Human JNK 2 alpha 2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the fusion protein is equivalent to M1 of human JNK 2 alpha 2
<u>Accession number</u>	EMBL L31951. Recombinant protein contains the amino acid residue substitution S51N with respect to EMBL L31951. This conflict is reported in GenBank U09759 and the following GenBank ESTs, AL532378, AL550761, BI333490.

Recombinant JNK 2α2 amino acid sequence:

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1  MHHHHHHMSD SKCDSQFYVS QVADSTFTVL KRYQQLKPIG SGAQGIVCAA FDTVLGINVA
61  VKKLSRPFQN QTHAKRAYRE LVLLKCVNHK NIISLLNVFT POKTLEEFQD VYLVMEI LMDA
121 NLCQVIHMEL DHERMSYLLY QMLCGIKHLH SAGIIHRDLK PSNIVVKSDC TLKILDFGLA
181 RTACTNFMMT PYVTRYRYRA PEVILGMGYK ENVDIWSVGC IMGELVKGCV IFQGTDHIDQ
241 WNKVIEQLGT PSAEFMKKLQ PTVRNYVENR PKYPGIKFEE LFPDWIFPSE SERDKIKTSQ
301 ARDLLSKMLV IDPDKRISVD EALRHPYITV WYDPAAEAEAP PPQIYDAQLE EREHAIEEWK
361 ELIYKEVMDW EERSKNGVVK DQPSDAAVSS NATPSQSSSI NDISSMSTEQ TLASDTDSSL
421 DASTGPLEGC R

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Recombinant JNK 2α2 nucleotide sequence:

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1  atgcaccatc accatcacca tatgagcgac agtaaatgtg acagtcagtt ttatagtgtg
61  caagtggcag actcaacctt cactgtccta aaacgttacc agcagctgaa accaattggc
121 tctggggccc aagggtattg ttgtgctgca tttgatacag ttcttgggat aaatgttgca
181 gtcaagaaac taagccgtcc ttttcagaac caaactcatg caaagagagc ttatcgtgaa
241 cttgtcctct taaaatgtgt caatcataaa aatataatta gtttggttaa tgtgtttaca
301 ccacaaaaaa ctctagaaga atttcaagat gtgtatttgg ttatggaatt aatggatgct
361 aacttatgtc aggttattca catggagctg gatcatgaaa gaatgtccta ccttctttac
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481 cctagcaaca ttgttgtgaa atcagactgc accctgaaga tccttgactt tggcctggcc
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1141 gatcagcctt cagatgcagc agtaagtagc aacgccactc cttctcagtc ttcacatgatc
1201 aatgacattt catccatgtc cactgagcag acgctggcct cagacacaga cagcagtctt
1261 gatgcctcga cgggaccctt tgaaggctgt cgatga

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