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Certificate of Analysis

JNK 2α2/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 2α2/SAPK1a unactive, expressed in Sf21 insect cells. Purified using Ni^{2+}/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 6 months 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.

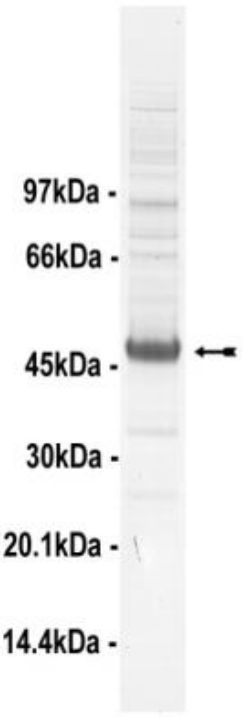
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 2µM unactive JNK 2α2 was activated using 0.1µM active MKK4 (cat# 14-377) and 0.1µM 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 3µg of JNK2α2/SAPK1a, unactive.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Assay Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol.
2. **Enzyme Dilution Buffer:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1 % 2-mercaptoethanol.
3. **JNK 2 alpha 2, unactive:** 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-^{33}P]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 unactive**.
3. Add 2.5µl (8.47ng) of **MKK4, active**.
4. Add 2.5µl (12.14ng) of **MKK7 beta 1, active**.
5. Add 10µl of dH_2O.
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 dH_2O.
5. Add 10µl of the diluted [γ-^{33}P]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

Protein Human JNK 2 alpha 2

Tags *N*-terminal 6His

Native sequence M8 of the fusion protein is equivalent to M1 of human JNK 2 alpha 2

Accession number 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:

```
  1 MHHHHHHMSD SKCDSQFYSV QVADSTFTVL KRYQQLKPIG SGAQGIVCAA FDTVLGINVA
 61 VKKLSRPFQN QTHAKRAYRE LVLLKCVNHK NIISLLNVFT PQKTLEEFQD VYLVMELMDA
121 NLCQVIHMEL DHERMSYLLY QMLCGIKHLH SAGIIHRDLK PSNIVVKSDC TLKILDFGLA
181 RTACTNFMMT PYVVTRYYRA PEVILGMGYK ENVDIWSVGC IMGELVKGCV IFQGTDHIDQ
241 WNKVIEQLGT PSAEFMKKLQ PTVRNYVENR PKYPGIKFEE LFPDWIFPSE SERDKIKTSQ
301 ARDLLSKMLV IDPDKRISVD EALRHPYITV WYDPAEAEAP PPQIYDAQLE EREHAIEEWK
361 ELIYKEVMDW EERSKNGVVK DQPSDAAVSS NATPSQSSSI NDISSMSTEQ TLASDTDSSL
421 DASTGPLEGC R
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Recombinant JNK 2α2 nucleotide sequence:

```
   1 atgcaccatc accatcacca tatgagcgac agtaaatgtg acagtcagtt ttatagtgtg
  61 caagtggcag actcaacctt cactgtccta aaacgttacc agcagctgaa accaattggc
 121 tctggggccc aagggattgt ttgtgctgca tttgatacag ttcttgggat aaatgttgca
 181 gtcaagaaac taagccgtcc ttttcagaac caaactcatg caaagagagc ttatcgtgaa
 241 cttgtcctct taaaatgtgt caatcataaa aatataatta gtttgttaaa tgtgtttaca
 301 ccacaaaaaa ctctagaaga atttcaagat gtgtatttgg ttatggaatt aatggatgct
 361 aacttatgtc aggttattca catggagctg gatcatgaaa gaatgtccta ccttctttac
 421 cagatgcttt gtggtattaa acatctgcat tcagctggta taattcatag agatttgaag
 481 cctagcaaca ttgttgtgaa atcagactgc accctgaaga tccttgactt tggcctggcc
 541 cggacagcgt gcactaactt catgatgacc ccttacgtgg tgacacggta ctaccgggcg
 601 cccgaagtca tcctgggtat gggctacaaa gagaacgttg atatctggtc agtgggttgc
 661 atcatgggag agctggtgaa aggttgtgtg atattccaag gcactgacca tattgatcag
 721 tggaataaag ttattgagca gctgggaaca ccatcagcag agttcatgaa gaaacttcag
 781 ccaactgtga ggaattatgt cgaaaacaga ccaaagtatc ctggaatcaa atttgaagaa
 841 ctctttccag attggatatt cccatcagaa tctgagcgag acaaaataaa aacaagtcaa
 901 gccagagatc tgttatcaaa aatgttagtg attgatcctg acaagcggat ctctgtagac
 961 gaagctctgc gtcacccata catcactgtt tggtatgacc ccgccgaagc agaagcccca
1021 ccacctcaaa tctatgatgc ccagttggaa gaaagagaac atgcaattga agaatggaaa
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1141 gatcagcctt cagatgcagc agtaagtagc aacgccactc cttctcagtc ttcatcgatc
1201 aatgacattt catccatgtc cactgagcag acgctggcct cagacacaga cagcagtctt
1261 gatgcctcga cgggacccct tgaaggctgt cgatga
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Reviewed and approved by site quality representative.