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Certificate of Analysis

JNK1α1, 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 JNK1alpha1, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

Specific Activity (Parent lot# WAB0451): As provided, this lot demonstrated 0.24% of maximum activity. Activated by phosphorylation with MKK4 (cat# 14-377) and MKK7beta1 (cat# 14-366).

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-mercaptothanol. Liquid at -20°C**.**

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.

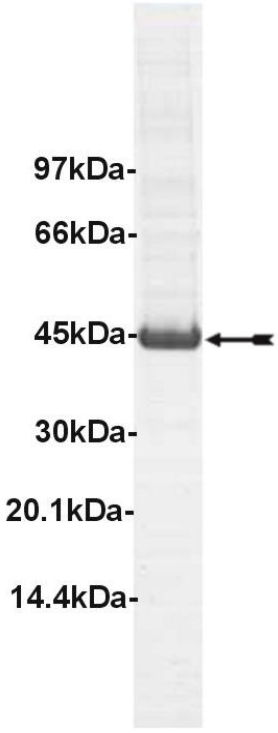
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 2µM JNK1α1, unactive was activated using 0.1µM MKK4 (cat# 14-377) and 0.1µM MKK7β1 (cat# 14-366) and the increased activity of JNK1α1 against ATF-2 determined. Assay is described on page two. Results of this assay are shown below

Active MKK4	Active MKK7β1	Unactive JNK1α1	Mean cpm	Comments
None	None	2.25µg	51	Background
169.5ng	185ng	2.25µg	8349	Kinase activity

MS Tryptic Fingerprint: Confirmed product identity as JNK1α1 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 JNK1α1, unactive.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction 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. **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.
4. **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.
5. **Magnesium/ATP Cocktail:** 50mM magnesium acetate, 0.5mM ATP.
6. **[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.)
7. **JNK1α1, unactive:** 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.
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 JNK1α1 by MKK4 and MKK7β1.*

1. Add 2.5µl of assay buffer to a microcentrifuge tube.
2. Add **2.5µl (2.25µg) of JNK1α1 unactive**.
3. Add 2.5µl (169.5ng) of **MKK4, active**.
4. Add 2.5µl (185ng) of **MKK7β1, active**.
5. Add 10µl of dH_2O.
6. Add 5µl of magnesium/ATP cocktail.
7. Run a no activator control.
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 JNK1α1*

1. Add 2.5µl of reaction buffer per assay to wells.
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 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 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 MHHHHHHMSR SKRDNNFYSV EIGDSTFTVL KRYQNLKPIG SGAQGIVCAA YDAILERNVA
  61 IKKLSRPFQN QTHAKRAYRE LVLMKCVNHK NIIGLLNVFT PQKSLEEFQD VYIVMELMDA
 121 NLCQVIQMEL DHERMSYLLY QMLCGIKHLH SAGIIHRDLK PSNIVVKSDC TLKILDFGLA
 181 RTAGTSFMMT PYVVTRYYRA 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 ttatagtgta
  61 gagattggag attctacatt cacagtcctg aaacgatatc agaatttaaa acctataggc
 121 tcaggagctc aaggaatagt atgcgcagct tatgatgcca ttcttgaaag aaatgttgca
 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 ccttctctat
 421 cagatgctgt gtggaatcaa gcaccttcat tctgctggaa ttattcatcg ggacttaaag
 481 cccagtaata tagtagtaaa atctgattgc actttgaaga ttcttgactt cggtctggcc
 541 aggactgcag gaacgagttt tatgatgacg ccttatgtag tgactcgcta ctacagagca
 601 cccgaggtca tccttggcat gggctacaag gaaaacgtgg atttatggtc tgtggggtgc
 661 attatgggag aaatggtttg ccacaaaatc ctctttccag gaagggacta tattgatcag
 721 tggaataaag ttattgaaca gcttggaaca ccatgtcctg aattcatgaa gaaactgcaa
 781 ccaacagtaa ggacttacgt tgaaaacaga cctaaatatg ctggatatag ctttgagaaa
 841 ctcttccctg atgtcctttt cccagctgac tcagaacaca acaaacttaa agccagtcag
 901 gcaagggatt tgttatccaa aatgctggta atagatgcat ctaaaaggat ctctgtagat
 961 gaagctctcc aacacccgta catcaatgtc tggtatgatc cttctgaagc agaagctcca
1021 ccaccaaaga tccctgacaa gcagttagat gaaagggaac acacaataga agagtggaaa
1081 gaattgatat ataaggaagt tatggacttg gaggagagaa ccaagaatgg agttatacgg
1141 gggcagccct ctcctttagc acaggtgcag cagtga
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