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Certificate of Analysis

JNK3/SAPK1b, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-523, 14-523-K, 14-523M
Lot # 33280U

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 unactive, expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 97.2% by SDS-PAGE and Coomassie blue staining. MW = 53kDa.

Specific Activity (Parent lot# 33280U): As provided, this lot demonstrated <3.5% of maximum activity. Activated by phosphorylation with MKK4, active (cat# 14-377) and MKK7β1, active (cat# 14-543).

Formulation: 3.224mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 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.

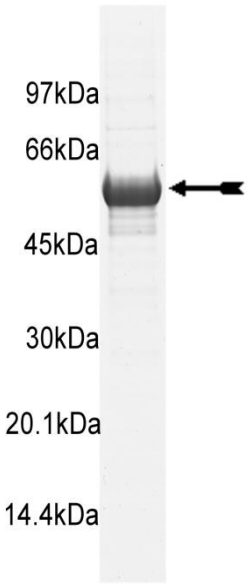
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 3µM unactive JNK3/SAPK1b was activated using 0.1µM active MKK4 (cat# 14-377) and 0.1µM active MKK7β1 (cat# 14-543) and the increased activity of JNK3/SAPK1b against (RRELVEPLTPSGEAPNQALL) determined. Activation and subsequent assay is described on page two. Results of this assay are shown below

Active MKK4	Active MKK7	Unactive JNK3/SAPK1b	Mean cpm	Comments
169.5ng	185ng	4µg	161876	Kinase activity
None	None	4µg	6115	Background

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



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of JNK3/SAPK1b, 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, 1mg/ml BSA.
3. **JNK3/SAPK1b, unactive:** Use at a final assay concentration of 3µM (0.16mg/ml). Prepare a 1.6mg/ml stock and add 2.5µl of stock 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. **MKK7β1, active (Catalogue#14-543):** 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:** 100mM magnesium acetate, 1mM 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. **(RRELVEPLTPSGEAPNQALLR):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of JNK3 by MKK4 and MKK7β1.*

1. Add 2.5µl of assay buffer to a microcentrifuge tube.
2. Add **2.5µl (4µg) JNK3/SAPK1b, unactive.**
3. .Add 2.5µl (169.5ng) of **MKK4, active.**
4. Add 2.5µl (185ng) of **MKK7β1, active**.
5. Add 12.5µl of dH_2O.
6. Add 2.5µl 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 JNKtide by JNK3/SAPK1b* (96 well plate format)

1. Add 2.5 µl of reaction buffer per assay to wells.
2. Add 2.5µl of **(RRELVEPLTPSGEAPNQALLR)**.
3. Add **5µl** of diluted **Stage One** reaction product.
4. Add 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 scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all appropriate assay components plus 1µl 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 LSRPFQNQTH AKRAYRELVL
121 MKCVNHKNII SLLNVFTPQK TLEEFQDVYL VMELMDANLC QVIQMELDHE RMSYLLYQML
181 CGIKHLHSAG IIHRDLKPSN IVVKSDCTLK ILDFGLARTA GTSFMMTPYV VTRYYRAPEV
241 ILGMGYKENV DIWSVGCIMG EMVRHKILFP GRDYIDQWNK VIEQLGTPCP EFMKKLQPTV
301 RNYVENRPKY AGLTFPKLFP DSLFPADSEH NKLKASQARD LLSKMLVIDP AKRISVDDAL
361 QHPYINVWYD PAEVEAPPPQ IYDKQLDERE HTIEEWKELI YKEVMNSEEK TKNGVVKGQP
421 SPSGAAVNSS ESLPPSSSVN DISSMSTDQT LASDTDSSLE ASAGPLGCCR
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Recombinant JNK3/SAPK1b nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atccccggga atttccggtg tggatgtgtc ttctgttgcc
 121 aaacattaca acatgagcaa aagcaaggta gataaccagt tctacagtgt ggaagtggga
 181 gactcaacct tcacagttct aaagcgctac cagaacctga agccgatcgg ctctggggct
 241 cagggaatag tttgtgctgc gtatgacgct gtcctcgaca gaaatgtggc cattaagaag
 301 ctcagcagac ccttccagaa ccaaactcat gccaagaggg cttaccggga gctggtcctc
 361 atgaagtgtg tgaaccataa aaacattatt agcttattaa atgtctttac accccagaaa
 421 acactggagg agttccaaga tgtttactta gtgatggaac tgatggacgc caacttgtgt
 481 caggtgattc agatggagct ggaccacgag cggatgtcgt acttgctgta ccagatgctg
 541 tgcggcatca aacacctcca ctccgctggg atcatccaca gggacttaaa acccagtaac
 601 atcgtagtca agtctgattg cacactgaaa atcctggact ttggactggc caggacagcg
 661 ggcacaagct tcatgatgac tccgtatgtg gtgacgagat attacagagc ccccgaggtc
 721 atcctgggca tgggctacaa ggagaacgtg gacatatggt ctgtgggctg catcatggga
 781 gaaatggttc gtcacaaaat cctctttccc ggaagggact atattgacca gtggaacaaa
 841 gtcatagagc agctaggaac tccgtgtcca gaattcatga agaaattgca gcccaccgtc
 901 agaaactacg tggagaaccg gcccaagtat gcaggcctca ccttccccaa gctctttcca
 961 gattccctct tcccagcgga ttccgagcac aataaactta aagccagcca agccagggac
1021 ttgttgtcaa agatgttagt gattgaccca gcgaagagga tatcggtgga tgacgcattg
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1141 atatatgaca agcaactgga tgaaagggag cacaccatcg aagaatggaa agaactcatc
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1261 tcaccttcag gtgcagcagt gaacagcagt gagagtctcc ctccatcctc atctgtcaac
1321 gacatctcct ccatgtccac cgaccagacc ctcgcatccg acactgacag cagcctggaa
1381 gcctcggcgg gaccgctggg ttgttgcagg tga
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Reviewed and approved by site quality representative.