

## Certificate of Analysis

### JNK1 $\alpha$ 1/SAPK1c, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-327, 14-327-K, 14-327M

Parent Lot # 1684883

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 JNK1 $\alpha$ 1/SAPK1c, expressed in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Activated using MKK4 (cat# 14-377) and MKK7 (cat# 14-366) and repurified using Ni<sup>2+</sup>/NTA agarose. Purity 100% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

**Specific Activity (Parent lot# 1684883):** 141U/mg, where one unit of JNK1 $\alpha$ 1/SAPK1c, active activity is defined as 1nmol phosphate incorporated into 3 $\mu$ M ATF-2 (cat# 12-367) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 2.039mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

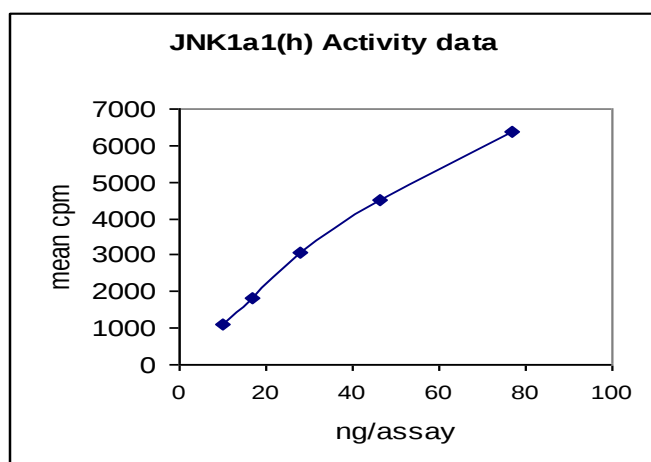
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

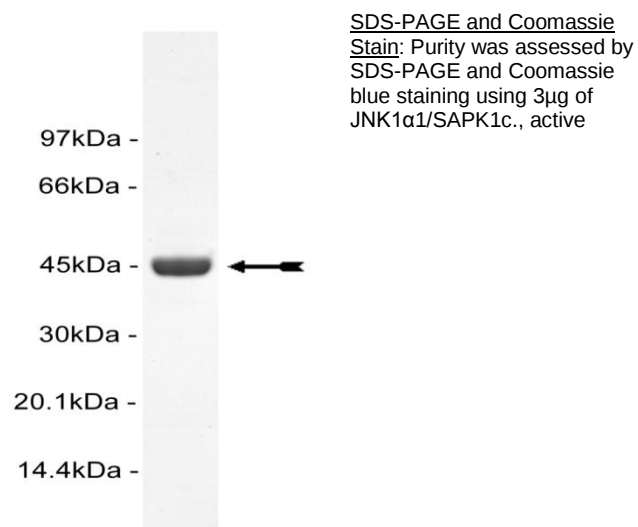
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 10–77ng of this lot of enzyme phosphorylated 3 $\mu$ M ATF-2 (cat# 12-367) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



**MS Tryptic Fingerprint:** Confirmed product identity as JNK1 $\alpha$ 1 with the translated sequence listed on page three.



## Certificate of Analysis

### Kinase Assay Protocol

#### Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **ATF-2 (Catalogue# 12-367):** Use at a final assay concentration of 3 $\mu$ M. Prepare a 1.08mg/ml stock. Add 2.5 $\mu$ l of stock per assay point.
3. **JNK1 $\alpha$ 1, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 10–77ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 2.5 $\mu$ l of 10 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **ATF-2**.
3. Add **2.5 $\mu$ l (10–77ng) JNK1 $\alpha$ 1, active**.
4. Make up to 15 $\mu$ l with dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat twice 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 1 $\mu$ l of 30% phosphoric acid.

# Certificate of Analysis

## JNK1α1 Sequence Information

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

### Recombinant JNK1α1 amino acid sequence:

```

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

```

### Recombinant JNK1α1 nucleotide sequence:

```

1 atgcaccatc accatcacca tatgagcaga agcaagcgtg acaacaattt ttatagtgtg
61 gagattggag attctacatt cacagtcctg aaacgatata 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 gcaccttcac tctgctggaa ttattcatcg ggacttaaa
481 cccagtaata tagtagtaaa atctgattgc actttgaaga ttcttgactt cggctctggc
541 aggactgcag gaacgagttt tatgatgacg cttatgttag tgactcgcta ctacagagca
601 cccgagggtc tccttggtcat gggctacaag gaaaacgtgg atttatggtc tgtgggggtg
661 attatgggg aaatgggttg ccacaaaatc ctctttccag gaagggacta tattgatcag
721 tggaataaag ttattgaaca gcttggaaac ccatgtcctg aattcatgaa gaaactgcaa
781 ccaacagtaa ggacttacgt tgaaaacaga cctaaatatg ctggatatag ctttgagaaa
841 ctcttccttg atgtcctttt cccagctgac tcagaacaca acaaaactta agccagtcag
901 gcaagggatt tggtatccaa aatgctggta atagatgcat ctaaaaggat ctctgtagat
961 gaagctctcc aacacccgta catcaatgtc tggatgatc cttctgaagc agaagctcca
1021 ccaccaaaga tccctgacaa gcagtttagt gaaaggggaa acacaataga agagtggaaa
1081 gaattgatat ataaggaagt tatggacttg gaggagagaa ccaagaatgg agttatacgg
1141 gggcagccct ctcttttagc acaggtgcag cagtga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.