

Certificate of Analysis

JNK 2α2/SAPK1a, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-329, 14-329-K, 14-329M

Parent Lot # D14CP010N

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, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Activated using MKK4 (cat# 14-377) and MKK7 (cat# 14-366) and repurified using Ni²⁺/NTA agarose. Purity 95.5% by SDS-PAGE and Coomassie blue staining. MW = 49.2kDa.

Specific Activity (Parent lot# D14CP010N): 117U/mg, where one unit of JNK 2 alpha 2, active activity is defined as 1nmol phosphate incorporated into 3μM ATF-2 (19-96) (cat# 12-367) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.337mg/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.

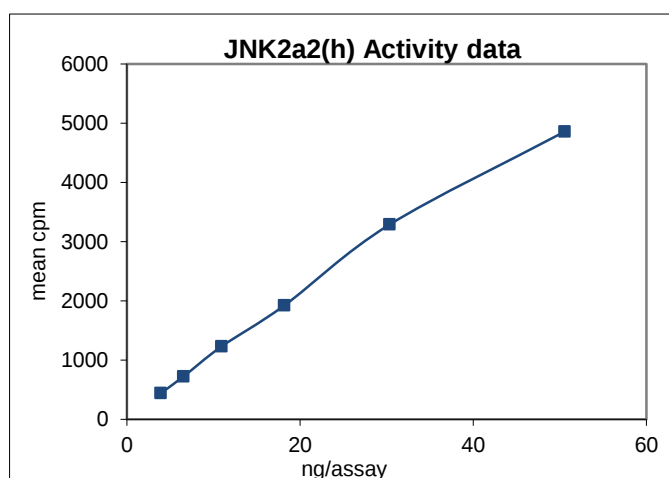
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

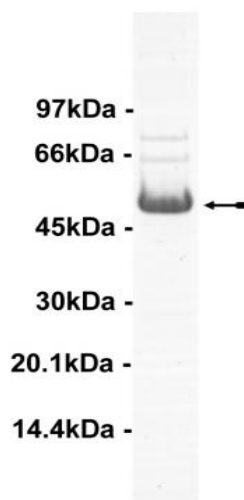
Quality Control Testing

Kinase Assay: 4–51ng of this lot of enzyme phosphorylated 3μM ATF-2 (19-96) 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 JNK 2α2 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 JNK 2α2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **ATF-2 (19-96) (Catalogue# 12-367):** Use at a final assay concentration of 3 μ M (0.108mg/ml). Prepare a 1.08mg/ml stock and add 2.5 μ l of stock per assay point.
3. **JNK 2 α 2, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol. Use 4–51ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5 μ l of 10 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **ATF-2**.
3. Add **2.5 μ l (4–51ng) JNK 2 α 2, active**.
4. Make up to 15 μ l with dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 30% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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JNK 2α2 Sequence Information

<u>Protein</u>	Human JNK 2α2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human JNK 2α2
<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 MHHHHHMSD SKCDQFYSV QVADSTFTVL KRYQQLKPIG SGAQGIVCAA FDTVLGINVA
61 VKKLSRPFQN QTHAKRAYRE LVLLKCVNHNK NIISLLNVFT PQKTLEEFQD VYLVMEI LMDA
121 NLCQVIHME L DHERMSYLLY QMLCGIKHLH SAGIIHRDLK PSNIVKSDC TLKILDFGLA
181 RTACTNFM MT PYVVTRYRA PEVILGMGYK ENVDIWSVGC IMGELVKGCV IFQGTDHIDQ
241 WNKVIEQLGT PSAEFMKKLQ PTVRNVVENR PKYPGIKFEE LFPDWIFPSE SERDKIKTSQ
301 ARDLLSKMLV IDPDKRISVD EALRHPYITV WYDPAEAEAP PPQIYDAQLE EREHAIEEWK
361 ELIYKEVMDW EERSKNGVVK DQPSDAAVSS NATPSQSSSI NDISSMSTEQ TLASDTSDDL
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 aagggtattgt ttgtgctgca tttgatacag ttcttgggat aaatgttgca
181 gtcaagaaac taagccgtcc ttttcagaac caaactcatg caaagagagc ttatcgtgaa
241 cttgtcctct taaaaatgtgt caatcataaa aatataatta gtttgtttaa tgtgtttaca
301 ccacaaaaaa ctctagaaga atttcaagat gtgtatttgg ttatggaatt aatggatgct
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1141 gatcagcctt cagatgcagc agtaagtagc aacgccactc cttctcagtc ttcacgatc
1201 aatgacattt catccatgtc cactgagcag acgctggcct cagacacaga cagcagtctt
1261 gatgcctcga cgggaccctt tgaaggctgt cgatga

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