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# Certificate of Analysis

## MKK4/SKK1, active

**(Recombinant enzyme expressed in *E.coli* cells)**
**Item # 14-377, 14-377-K, 14-377M**
**Parent Lot # D8PN048U**

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 GST-tagged, murine MKK4 residues 34–end, expressed in *E.coli* cells. Purified using glutathione agarose. Activated by incubation with MEKK and then re-purified using $Ni^{2+}$/NTA agarose. Purity 63% by SDS-PAGE and Coomassie blue staining. MW = 67.7kDa.

**Specific Activity (Parent lot# D8PN048U):** 607U/mg, where one unit of MKK4 activity is defined as the amount of MKK4 which activates 2µM unactive JNK 1α1 (cat# 14-328) by 1 unit per minute at 30°C using 100µM ATP. One unit of JNK 1α1 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: 1.553mg/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.

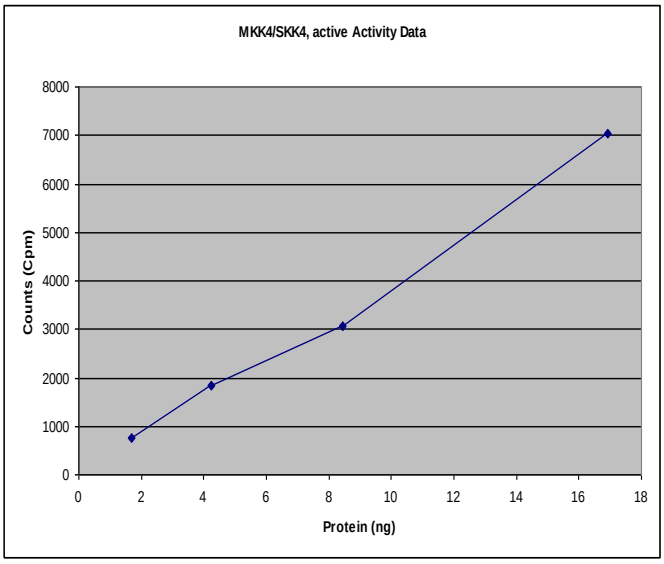
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

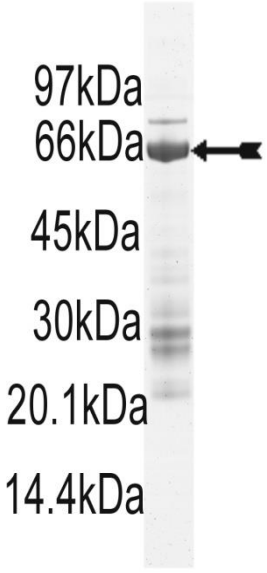
**FOR IN VITRO RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

Kinase Assay: 1.7–16.9ng of this lot of enzyme phosphorylated 2µM JNK 1α1 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 MKK4 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 MKK4, active.

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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. **JNK 1α1 unactive (Catalogue# 14-328):** Use at a final assay concentration of 2µM (0.09mg/ml). Prepare 0.9mg/ml stock and add 2.5µl of stock per assay point.
3. **ATF-2 (Catalogue# 12-367):** Use at a final assay concentration of 3µM (0.108mg/ml). Prepare a 1.08mg/ml stock and add 2.5µl of stock per assay point.
4. **MKK4, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM $Na_3VO_4$, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.7–16.9ng per assay point.
5. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 0.5mM ATP.
6. **[γ-$^{33}$P]ATP:** 2.5 x magnesium acetate/[γ-$^{33}$P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-$^{33}$P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure:

*Stage One: Activation of JNK 1α1 by MKK4.*

1. Add 2.5µl of 10 x reaction buffer to a microcentrifuge tube.
2. Add 2.5µl of **JNK 1α1 unactive**.
3. Add **2.5µl (1.7–16.9ng) MKK4, active**. Add 2.5µl of reaction buffer to appropriate controls.
4. Add 12.5µl of $dH_2O$.
5. Add 5µl of stage one 5 x Mg/ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Immediately transfer 2.5µl into **Stage Two**.

*Stage Two: Assay procedure.*

1. Add 2.5µl of 10 x reaction buffer to a microcentrifuge tube.
2. Add 2.5µl of **ATF-2**
3. Add 2.5µl of **Stage One** reaction product**.**
4. Add 7.5µl of $dH_2O$.
5. Add 10µl of diluted [γ-$^{33}$P]ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Transfer a 20µl aliquot onto the centre of a 2cm x 2cm **P81** paper square.
8. Wash the assay squares twice for 5 minutes with 75mM phosphoric acid.
9. Wash the assay squares once for 2 minutes with acetone.
10. Transfer the assay squares to a scintillation vial and add 1ml of scintillation cocktail.
11. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all relevant assay components plus 1µl of 30% phosphoric acid.

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**MKK4 Sequence Information**

| | |
|---|---|
| **Protein** | murine MKK4 |
| **Tags** | *N*-terminal GST |
| **Native sequence** | S226 of the recombinant protein is equivalent to S34 of murine MKK4 |
| **Accession number** | EMBL U18310 |

***Recombinant MKK4 amino acid sequence:***

```
   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSMQGK RKALKLNFAN
 241 PPVKSTARFT LNPNTTGVQN PHIERLRTHS IESSGKLKIS PEQHWDFTAE DLKDLGEIGR
 301 GAYGSVNKMV HKPSGQIMAV KRIRSTVDEK EQKQLLMDLD VVMRSSDCPY IVQFYGALFR
 361 EGDCWICMEL MSTSFDKFYK YVYSVLDDVI PEEILGKITL ATVKALNHLK ENLKIIHRDI
 421 KPSNILLDRS GNIKLCDFGI SGQLVDSIAK TRDAGCRPYM APERIDPSAS RQGYDVRSDV
 481 WSLGITLYEL ATGRFPYPKW NSVFDQLTQV VKGDPPQLSN SEEREFSPSF INFVNLCLTK
 541 DESKRPKYKE LLKHPFILMY EERTVEVACY VCKILDQMPA TPSSPMYVD
```

***Recombinant MKK4 nucleotide sequence:***

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccat gcagggtaag cgcaaagcac tgaagttgaa ttttgcaaat
 721 ccacctgtca aatcgacagc acggtttacc ctgaatccta atactacagg agtccagaac
 781 ccacacatag agagactgag aacacacagc attgagtcat caggaaaact gaagatctcc
 841 cctgaacaac actgggattt cactgcagag gacttgaaag accttggaga aattggacga
 901 ggagcttatg gttctgtcaa caaaatggtc cacaaaccaa gtgggcagat aatggcagtt
 961 aaaagaattc ggtcaactgt ggatgaaaaa gaacaaaaac aacttcttat ggatttggat
1021 gtagtaatgc ggagtagtga ttgcccatac attgttcagt tctatggtgc actcttcaga
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1141 tatgtatata gtgtgttaga tgacgttatt ccggaagaga tcttaggcaa aatcacttta
1201 gcaactgtga aagcactaaa ccacttaaaa gaaaacttga aaattattca cagagacatc
1261 aaaccttcca atattcttct ggacagaagt ggaaatataa agctctgtga tttcggcatc
1321 agtggacagc ttgtggactc tattgccaag acaagagatg ctgggtgtag gccgtatatg
1381 gcacctgaaa gaatagaccc aagtgcatca agacaagggt atgatgtccg ctctgatgtc
1441 tggagtttgg ggatcacatt gtacgagttg gccacaggcc gatttcctta tccaaagtgg
1501 aatagtgtat ttgatcagct aacacaagtg gtgaaaggag accctccgca gctgagtaat
1561 tctgaagaaa gggagttctc ccccagtttc atcaactttg tcaacttgtg ccttacgaag
1621 gatgaatcca aaaggccaaa gtataaagag cttctgaaac atccctttat tttgatgtat
```

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```
1681 gaagaacgta ctgtagaggt cgcatgctat gtttgtaaaa tcctggatca gatgccagcc
1741 actcccagct cgcccatgta tgtcgactga
```

Reviewed and approved by site quality representative.