

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 Ni²⁺/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.

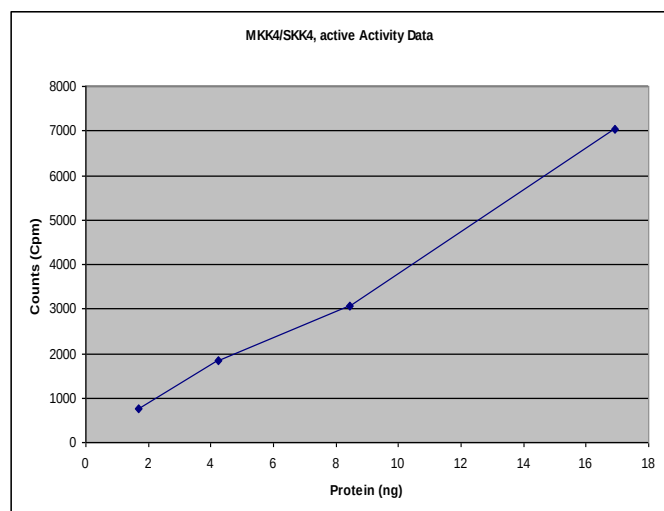
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

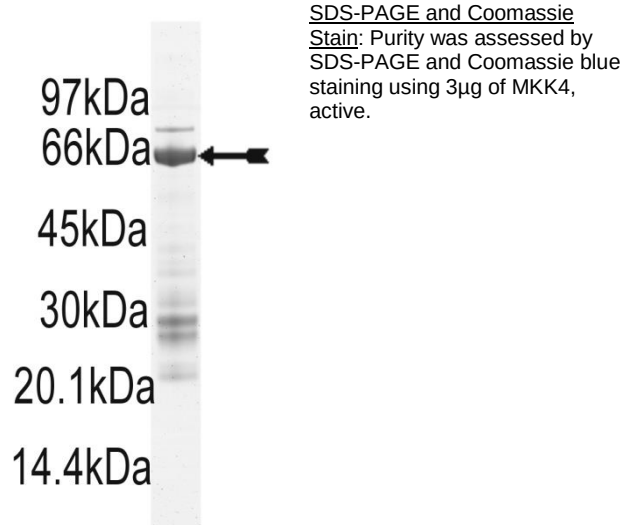
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 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.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **JNK 1α1 inactive (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 Na₃VO₄, 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-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:

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 inactive**.
3. Add **2.5μl (1.7–16.9ng) MKK4, active**. Add 2.5μl of reaction buffer to appropriate controls.
4. Add 12.5μl of dH₂O.
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 dH₂O.
5. Add 10μl of diluted [γ-³³P]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

<u>Protein</u>	murine MKK4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	S226 of the recombinant protein is equivalent to S34 of murine MKK4
<u>Accession number</u>	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  LVPRGSMQ GK  RKALKLNFAN
241  PPVKSTARFT  LNPNTTGVQN  PHIERLRTHS  IESSGKLKIS  PEQHWDFTA E  DLKDLGEIGR
301  GAYGSVNKMV  HKPSGQIMAV  KRIRSTVDEK  EQKQLLMDLD  VVMRSSDCPY  IVQFYGALFR
361  EGDCWICMEL  MSTSFDFKYK  YVYSVLDDVI  PEEILGKITL  ATVKALNHLK  ENLKIIHRDI
421  KPSNILLDRS  GNIKLCDFGI  SGQLVDSIAK  TRDAGCRPYM  APERIDPSAS  RQGYDVRS DV
481  WSLGITLYEL  ATGRFPYPKW  NSVFDQLTQV  VKGDPPQLSN  SEEREFSPSF  INFVNLCLTK
541  DESKRPKYKE  LLKHPFILMY  EERTVEVACY  VCKILDQMPA  TPSSPMYVD
  
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Recombinant MKK4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgtat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  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  gcagggttaag  cgcaaagcac  tgaagttgaa  ttttgcaaat
721  ccacctgtca  aatcgacagc  acggtttacc  ctgaatccta  atactacagg  agtcagaaac
781  ccacacatag  agagactgag  aacacacagc  attgagtcac  caggaaaact  gaagatctcc
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1381  gcacctgaaa  gaatagacct  aagtgcacat  agacaagggt  atgatgtccg  ctctgatgtc
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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 cgcattgctat gtttgtaaaa tcttgatca gatgccagcc  
1741 actcccagct cgcccatgta tgtcgactga
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Reviewed and approved by site quality representative.

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