

Certificate of Analysis

MAP4K3, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-030, 15-030-K, 15-030M

Parent Lot # 221051

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 and C-terminal 6His tagged, recombinant, human MAP4K3 amino acids 1-291, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by glutathione agarose. Purity 86% by SDS-PAGE and Coomassie blue staining. MW = 64 kDa.

Specific Activity (Parent lot# 221051): 804 U/mg, where one unit of MAP4K3 activity is defined as 1 nmol phosphate incorporated into 250 μ M RLGRDKYKTLRQIRQ per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.44 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

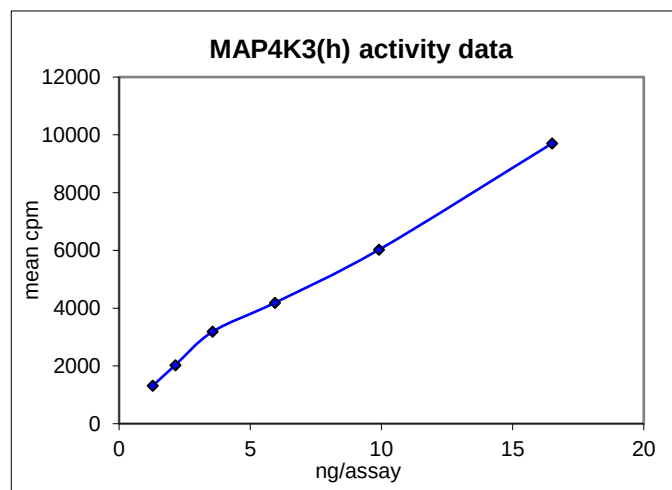
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

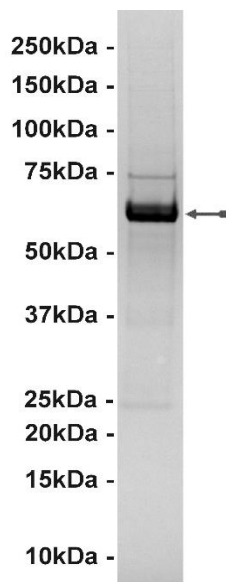
**FOR IN VITRO RESEARCH USE ONLY
 NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.3–16.5 ng of this lot of enzyme phosphorylated 250 μ M RLGRDKYKTLRQIRQ 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 identity as MAP4K3 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of MAP4K3, active.

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. RLGRDKYKTLRQIRQ:** Use at a final assay concentration of 250 μ M. Prepare a 2.5 mM stock and add 2.5 μ l of stock per assay point.
- 3. MAP4K3, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 1.3–16.5 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of 2.5 mM RLGRDKYKTLRQIRQ to wells.
3. Add **2.5 μ l (1.3–16.5 ng) MAP4K3, active.**
4. Add 5 μ l of 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 3% 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 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4 ml 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.

Certificate of Analysis

MAP4K3, active Sequence Information

<u>Protein</u>	human MAP4K3
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	M240 of the recombinant protein is equivalent to M1 of human MAP4K3
<u>Accession number</u>	GenBank NM_003618.2

Recombinant MAP4K3 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 KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSHNQT  SLYKKAGTAM
241 NPGFDLSRRN  PQEDFELIQR  IGSPTYGDVY  KARNVNTGEL  AAIKVIKLEP  GEDFAVVQQE
301 IIMMKDCKHP  NIVAYFGSYL  RRDKLWICME  FCGGSLQDI  YHVTGPLSEL  QIAYVSRETL
361 QGLYYLHSGK  KMRDIKGAN  ILLTDNGHVK  LADFGVSAQI  TATIAKRKSF  IGTPYWMAPE
421 VAAVERKGGY  NQLCDLWAVG  ITAIELAELQ  PPMFDLHPMR  ALFLMTKSNF  QPPKLKDKMK
481 WSNSFHFFVK  MALTKNPKKR  PTAEKLLQHP  FVTQHLTRSL  AIELLDKVVN  ENLYFQGEAC
541 QLGTDDYDIP  TTHHHHHH

```

Recombinant MAP4K3 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattgat
181  ggtgatgta  aattaacaca  gtctatggcc  atcatagctt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttt
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatatta  atggtgatca  tgtaaccat  cctgacttca  tgttgatatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatccca  caaccagacg  tcgtatata  aaaaagccgg  caccggccatg
721  aaccccggt  tcgatttgct  ccgccggaac  ccgcaggagg  acttcgagct  gattcagcgc
781  atcggcagcg  gcacctacgg  cgacgtctac  aaggcacgga  atgttaacac  tgggtgaatta
841  gcagcaatta  aagtaataaa  attggaacca  ggagaagact  ttgcagttgt  gcagcaagaa
901  attatcatga  tgaaagactg  taaacaccca  aatattgttg  cttatttttg  aagctatctc
961  aggcgagata  agctttggat  ttgcatggag  ttttgtggag  gtggttcttt  acaggatatt
1021  tatcacgtaa  ctggacctct  gtcagaactg  caaattgcat  atgttagcag  agaaacactg
1081  cagggattat  attatcttca  cagtaaagga  aaaatgcaca  gagatataaa  gggagctaac
1141  attctattaa  cggataatgg  tcatgtgaaa  ttggctgatt  ttggagtatc  tgcacagata
1201  acagctacaa  ttgccaacg  gaagctcttc  attggcacac  catattggat  ggctccagaa
1261  gttgcagctg  ttgagaggaa  ggggggttac  aatcaactct  gtgatctctg  ggcagtgagg
1321  atcactgcca  tagaacttgc  agagctctac  cctcctatgt  ttgacttaca  cccaattgaa
1381  gcattatttc  taatgacaaa  aagcaatttt  cagcctccta  aactaaagga  taaaatgaaa
1441  tgggtcaaata  gttttcatca  ctttgtgaaa  atggcactta  ccaaaaatcc  gaaaaaaga
1501  cctactgctg  aaaaattatt  acagcatcct  tttgtaacac  aacatttgac  acggtctttg

```

Certificate of Analysis

```
1561 gcaatcgagc tgttggataa agtaaataat gaaaacctgt attttcaggg cgaagcctgt
1621 caattgggca cggacgatta cgacatcccg actaccacc atcaccatca ccattaa
```

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.