

Certificate of Analysis

MAP4K4, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-012, 15-012-K, 15-012M

Parent Lot # D15MP005N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialing runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialing run.

Product Description: N-terminal GST tagged and C-terminal 6His tagged, recombinant, human MAP4K4 amino acids 1-328, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 66 kDa.

Specific Activity (Parent lot# D15MP005N): 869 U/mg, where one unit of MAP4K4 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: 3.20 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.

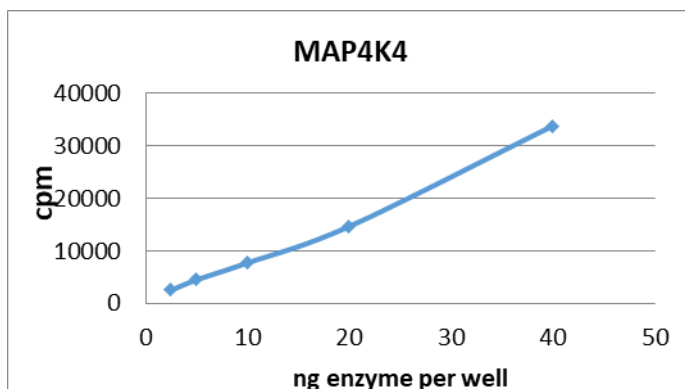
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

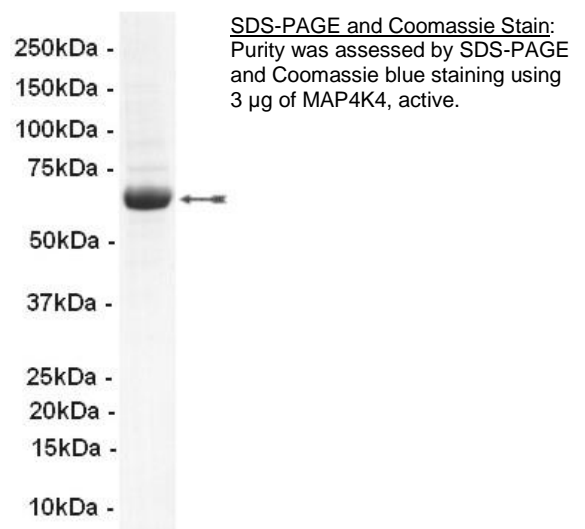
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.5–40.0 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 MAP4K4 with the translated sequence listed on page three.



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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. MAP4K4, 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 2.5–40.0 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 RLGRDKYKTLRQIRQ.
3. Add **2.5 μ l (2.5–40.0 ng) MAP4K4, 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 dried 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.

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MAP4K4, active Sequence Information

<u>Protein</u>	Human MAP4K4
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human MAP4K4
<u>Accession number</u>	GenBank NM_004834.4

Recombinant MAP4K4 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LVPRGSKEFM  ANDSPAKSLV
241  DIDLSSLRDP  AGIFELVEV  GNGTYGQVYK  GRHVKTGQLA  AIKVM DVTED  EEEEEIKLEIN
301  MLKKYSHHRN  IATYGF AFIK  KSPPGHDDQL  WLVMFCGAG  SITDLVKNTK  GNTLKEDWIA
361  YISREILRGL  AHLHIHHVIH  RDIKQNVLL  TENAEVKLVD  FGVSAQLDRT  VGRRNTFIGT
421  PYWMAPEVIA  CDENPDATYD  YRSDLWSCGI  TAIEMAEGAP  PLCDMHPMRA  LFLIPRNP PP
481  RLKSKKWSK  FFSFIEGCLV  KNYMQRPSTE  QLLKHPFIRD  QPNERQVRIQ  LKD HIDRTRK
541  KRGEKDETEY  EYSGSEEGPG  PGHHHHHH

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Recombinant MAP4K4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatt  ttgaagaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1501  aagaattaca  tgcagcggcc  ctctacagag  cagcttttga  aacatccttt  tataagggat

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1621 aagagaggcg agaaagatga aactgagtat gactacagtg ggagtgagga aggcccgggc
1681 cctggccatc accatcacca tcactaa
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