

Certificate of Analysis

MAP kinase 1, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-439, 14-439-K, 14-439M

Parent Lot # 2492972

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: Recombinant, full-length, human MAP kinase 1 containing an N-terminal GST-tag. Expressed in *E.coli* cells and purified by glutathione-agarose. Activated with MEK1 and re-purified on Ni²⁺/NTA agarose. Purity = 88.7% by SDS-PAGE and Coomassie blue staining. MW = 69.9kDa.

Formulation: 3.414mg/ml of enzyme in 20mM MOPS/NaOH pH7.2, 25mM sodium-β-glycerophosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol, 50 % glycerol. Liquid at -20°C.

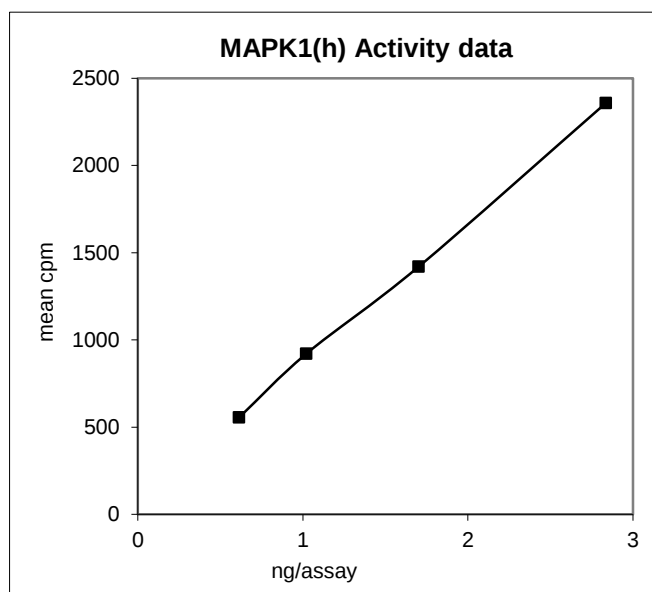
Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Specific Activity (Parent lot# 2492972) 896U/mg, where one unit of MAP kinase 1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100μM.

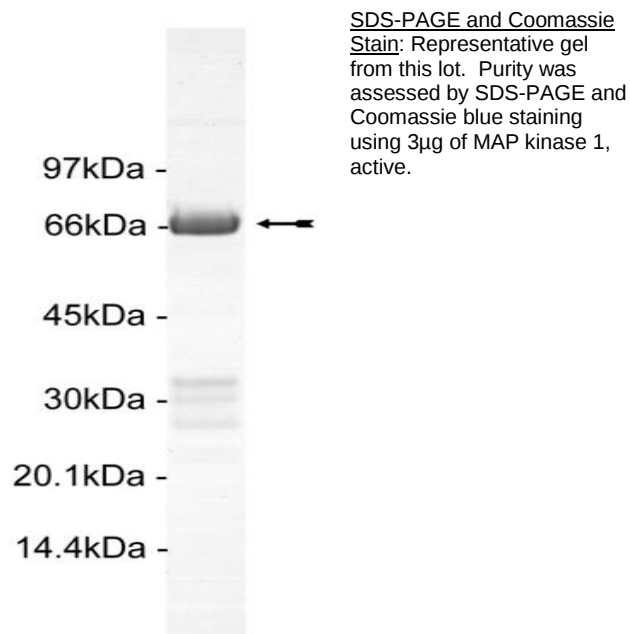
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.6–2.8ng of this lot of enzyme phosphorylated 0.33mg/ml MBP 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 MAPK1 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
- 2. Myelin Basic Protein (MBP) substrate:** Use a final assay concentration of 0.33mg/ml. Make up a 3.3 mg/ml stock. Use 2.5µl of stock per assay point.
- 3. MAP kinase 1, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.6–2.8ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **MBP substrate**.
3. Add **2.5µl (0.6–2.8ng) MAP kinase 1, active**.
4. Make up to 15µl with dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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MAP kinase 1 Sequence Information

<u>Protein</u>	Human MAP kinase 1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of the fusion protein is equivalent to M1 of human MAP kinase 1
<u>Accession number</u>	GenBank XM_055766

Recombinant MAP kinase 1 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPGIP MAAAAAQGGG
241 GGEPRRTEGV GPGVPGEVEM VKGQPFVGP RYTQLQYIGE GAYGMVSSAY DHVRKTRVAI
301 KKISPFHQYT YCQRTLREIQ ILLRFRHENV IGIRDILRAS TLEAMRDVYI VQDLMETDLY
361 KLLKSQQLSN DHICYFLYQI LRGLKYIHS NVLHRDLKPS NLLINTTCDL KICDFGLARI
421 ADPEHDHTGF LTEYVATRWY RAPEIMLNSK GYTKSIDIWS VGCILAEMLS NRPIFP GKHY
481 LDQLNHILGI LGSPSQEDLN CIINMKARNY LQSLPSKTKV AWAKLFPKSD SKALDLLDRM
541 LTFNPNKRIT VEEALAHPYL EQYYDPTDEP VAEFPFTFAM ELDDL PKERL KELIFQETAR
601 FQPGVLEAP

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Recombinant MAP kinase 1 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tataagtaaag actttgaaac tctcaaagtt
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1561 gcttgggcca agcttttccc caagtcagac tccaaagccc ttgacctgct ggaccggatg
1621 ttaaccttta accccaataa acggatcaca gtggaggaag cgctggctca cccctacctg
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1741 gagctggatg acctacctaa ggagcggctg aaggagctca ttttccagga gacagcacgc
1801 ttccagcccg gagtgtgga ggccccctag

Reviewed and approved by site quality representative.

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