

Certificate of Analysis

MAP Kinase 2, active

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

Item # 14-173, 14-173-K, 14-173M

Parent Lot # D8NN028U

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, recombinant, full-length, mouse MAP Kinase 2, expressed in *E.coli* cells. Purified using glutathione agarose. Activated using MEK1 (cat# 14-429 and repurified using Ni²⁺/NTA agarose). Purity 96.1% by SDS-PAGE and Coomassie blue staining. MW = 67.8kDa.

Specific Activity (Parent lot# D8NN028U): 1900U/mg, where one unit of MAP Kinase 2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

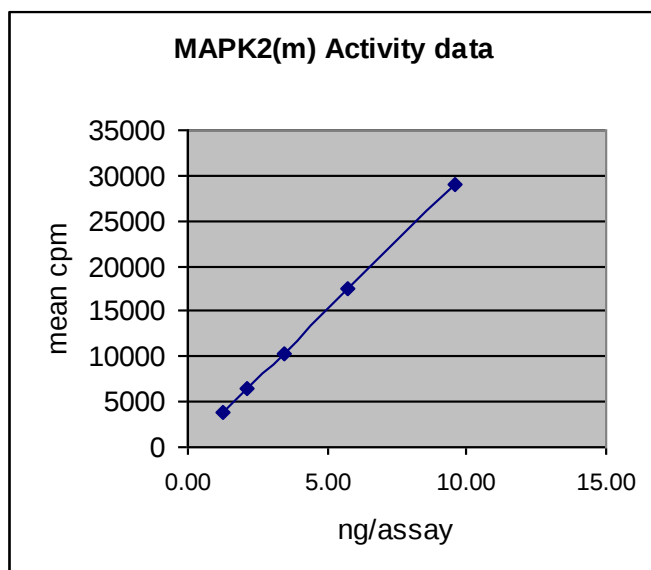
Formulation: 0.7665mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Liquid at -20°C.

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.

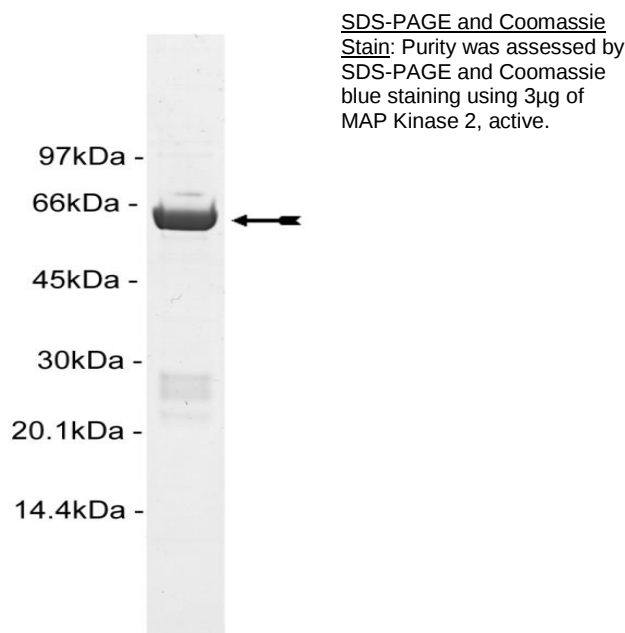
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.3–9.6ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein 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 MAP Kinase 2 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):** Use at a final concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **MAP Kinase 2, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.3–9.6ng 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 **myelin basic protein**.
3. Add **2.5µl (1.3–9.6ng) MAP Kinase 2/Erk2, active**.
4. Add 5µl of 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 2 Sequence Information

<u>Protein</u>	Mouse MAP Kinase 2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M228 of the recombinant protein is equivalent to M1 of mouse MAP Kinase 2
<u>Accession number</u>	EMBL D10939

Recombinant MAP Kinase 2 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSNMAA AAAAGPEMVR
241 GQVFDVGPY TNLSYIGEGA YGMVCSAYDN LNKVRVAIKK ISPFHQTYC QRTLREIKIL
301 LRFRHENIIG INDIIRAPTI EQMKDVYIVQ DLMETDLYKL LKTQHLSNDH ICYFLYQILR
361 GLKYIHSANV LHRDLKPSNL LLNTTCDLKI CDFGLARVAD PDHDHTGFLT EYVATRKYRA
421 PEIMLNSKGY TKSIDIWSVG CILAEMLSNR PIFPGKHLYD QLNHILGILG SPSQEDLNCI
481 INLKARNYLL SLPHKNKVPW NRLFPNADSK ALDLLDKMLT FNPHKRIEVE QALAHPLYEQ
541 YYDPSDEPIA EAPFKFDMEL DDLPEKELKE LIFEETARFQ PGYRS
  
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Recombinant MAP Kinase 2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtgtga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acggtgtttc gagaattgca tatagtaaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
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541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccaa catggcggcc gcagcagcgg ccggcccggg gatggtccgc
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781 tacggcatgg tttgctctgc ttatgataat ctcaacaaag ttcgagttgc tatcaagaaa
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1381 cagctgaatc acatcctggg tattcttgga tctccatcac aggaagatct gaattgtata
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1501 aacaggttgt tcccaaatgc tgactccaaa gctctggatt tactggataa aatgttgaca
1561 ttaaaccttc acaagaggat tgaagttgaa caggctctgg cccaccata cctggagcag
1621 tattatgacc caagtgatga gccattgct gaagcgccat tcaagtttga catggagttg
  
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1681 gacgacttac ctaaggagaa gctcaaagaa ctcatttttg aagagactgc tagattccag
1741 ccaggataca gatcttaa

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