

Certificate of Analysis

MAP Kinase 2/Erk2, unactive

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

Item # 14-198, 14-198-K, 14-198M

Parent Lot # 1684881

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 mouse full length MAP Kinase 2, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 99.5% by SDS-PAGE and Coomassie blue staining. MW = 67.8kDa

Formulation: 3.779mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Specific Activity: As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MEK1(h) (cat# 14-429).

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.

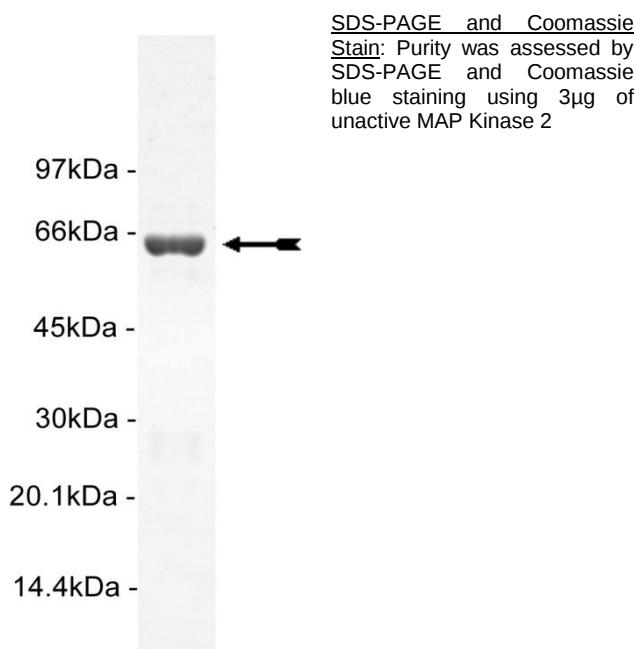
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Activation Assay: 2µM unactive MAP Kinase 2 was activated using 0.4µM active MEK1(h) (cat# 14-429), diluted 100-fold, and the increased activity against MBP determined. The activation and assay are described on page two. Results of this assay are shown below.

Active MEK1(h)	Unactive MAP Kinase 2	Mean cpm	Comments
0.7µg	3.4µg	19067	Kinase activity
None	3.4µg	1673	Background

MS Tryptic Fingerprint: Confirmed identity as MAP Kinase 2 with the translated native sequence listed on page three.



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Kinase Assay Procedure

Stock Solutions:

1. **10 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol, 0.3% Brij-35.
2. **5 x Reaction buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
3. **Dilution Buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.1mM Na₃VO₄, 1mg/ml BSA.
4. **MAP kinase 2, inactive:** Use at a final assay concentration of 2μM (0.136mg/ml). Dilute with Dilution Buffer to 1.36mg/ml. Use 2.5μl per assay point.
5. **MEK1, active (Catalogue# 14-429):** Use at a final assay concentration of 0.4μM (0.028mg/ml). Dilute with Dilution Buffer to 0.28mg/ml. Use 2.5μl per assay point.
6. **Stage One Mg/ATP:** 50mM MgAc, 0.5mM ATP.
7. **[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.)
8. **Myelin Basic Protein (MBP) substrate:** Use a final assay concentration of 330μg/ml. Make up a 3.3mg/ml stock. Add 2.5μl of stock per assay point.

Assay Protocol:

Stage One: Activation of MAP kinase 2 by MEK1

1. Add 2.5μl of 10 x activation buffer to a microcentrifuge tube.
2. Add 12.5μl dH₂O.
3. Add **2.5μl (3.4μg) MAP kinase 2, inactive.**
4. Add 2.5μl (0.7μg) **MEK1, active.**
5. Add 5μl of stage one 5 x Mg/ATP.
6. In appropriate controls, add dilution buffer to a final volume of 25μl.
7. Incubate at 30°C for 30 minutes.
8. Stop the reaction by diluting 100-fold in dilution buffer and storing on ice.

Stage Two: Phosphorylation of MBP by MAP kinase 2 (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 of activated **MAP kinase 2 (3.4ng) from Stage One.**
4. Make up to 15μl with dH₂O.
5. Add 10μl of the 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. Spot 10μl 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 scintillation counter. Compare cpm of enzyme samples with cpm of control samples containing all components plus 1μl of 30 % phosphoric acid.

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MAP Kinase 2 Sequence Information

Protein	mouse MAP Kinase 2
Tags	N-Terminal GST
Native sequence	M228 of the recombinant protein is equivalent to M1 of mouse MAP Kinase 2
Accession number	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 KYLKSSKYIA WPLQGWQATF GGDHPPKSD LVPRGSNMAA AAAAGPEMVR
241 GQVFDVGPY TNLSYIGEGA YGMVCSAYDN LNKVRVAIKK ISPFHQTYC QRTLREIKIL
301 LRFRHENIIG INDIIRAPTI EQMKDVYIVQ DLMETDLYKL LKTQHLSNDH ICYFLYQILR
361 GLKYIHSANV LHRDLKPSNL LLNTTCDLKI CDFGLARVAD PDHDTGFLT EYVATRKYRA
421 PEIMLSKGY TKSIDIWSVG CILAEMLSNR PIFPGKHYLD QLNHILGILG SPSQEDLNCI
481 INLKARNYLL SLPHKNKVPW NRLFPNADSK ALDLLDKMLT FNPBKRIEVE 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 catttgtatg agcgcatga aggtgataaa
121 tggcgaaaca aaaagtattga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaa
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatatta atggtgatca tgtaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtgggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccaa catggcggcc gcagcagcgg ccggcccggg gatggtccgc
721 ggcaggtgtt ttgacgtagg gccgcgtac accaactctc cgtacatcgg agaaggcgcc
781 tacggcatgg tttgctctgc ttatgataat ctcaacaaag ttcgagttgc tatcaagaaa
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901 ctgcgcttca gacatgagaa catcattggc atcaatgaca tcatccgggc accaaccatt
961 gagcaaatga aagatgtata tatagtacag gacctcatgg agacggacct ttacaagctc
1021 ttgaagacac agcacctcag caatgaccac atctgctatt ttctttatca gatcctgaga
1081 gggctaaagt atatccattc agctaactgt ctgcaccgtg acctcaagcc ttccaacctc
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1201 ccagatcatg atcacacagg gttcttgaca gactacgtag ccacacgttg gtacagagct
1261 ccagaaatta tgttgaattc caagggttat accaagtcca ttgatatttg gtctgtgggc
1321 tgcatcctgg cagagatgct atccaacagg cctatcttcc caggaaagca ttaccttgac
1381 cagctgaatc acatcctggg tattcttgga tctccatcac aggaagatct gaattgtata
1441 ataaatttaa aagctagaaa ctatttgctt tctctcccgc aaaaaataa ggtgccatgg
1501 aacaggttgt tcccaaatgc tgactccaaa gctctggatt tactggataa aatgttgaca
1561 tttaaccttc acaagaggat tgaagttgaa caggctctgg ccacccata cctggagcag

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1621 tattatgacc caagtgatga gccattgct gaagcgccat tcaagtttga catggagttg
1681 gacgacttac ctaaggagaa gctcaaagaa ctcatttttg aagagactgc tagattccag
1741 ccaggatata gatcttaa
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