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# 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

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

**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.

**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**

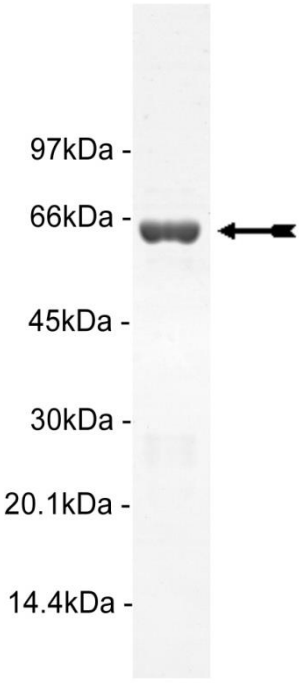
## Quality Control Testing

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.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of unactive MAP Kinase 2




**Eurofins Pharma Discovery Services UK Limited**
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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## Kinase Assay Procedure

Stock Solutions:

1. **10 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM $Na_3VO_4$, 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 $Na_3VO_4$, 1mg/ml BSA.
4. **MAP kinase 2, unactive:** 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-$^{33}$P]ATP:** 2.5 x magnesium acetate/[γ-$^{33}$P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-$^{33}$P]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 $dH_20$.
3. Add **2.5µl (3.4µg) MAP kinase 2, unactive**.
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 $dH_2O$.
5. Add 10µl of the diluted [γ$^{33}$P]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:***

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSNMAA AAAAGPEMVR
241 GQVFDVGPRY TNLSYIGEGA YGMVCSAYDN LNKVRVAIKK ISPFEHQTYC QRTLREIKIL
301 LRFRHENIIG INDIIRAPTI EQMKDVYIVQ DLMETDLYKL LKTQHLSNDH ICYFLYQILR
361 GLKYIHSANV LHRDLKPSNL LLNTTCDLKI CDFGLARVAD PDHDHTGFLT EYVATRWYRA
421 PEIMLNSKGY TKSIDIWSVG CILAEMLSNR PIFPGKHYLD QLNHILGILG SPSQEDLNCI
481 INLKARNYLL SLPHKNKVPW NRLFPNADSK ALDLLDKMLT FNPHKRIEVE QALAHPYLEQ
541 YYDPSDEPIA EAPFKFDMEL DDLPKEKLKE LIFEETARFQ PGYRS
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***Recombinant MAP Kinase 2 nucleotide sequence:***

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccaa catggcggcc gcagcagcgg ccggcccgga gatggtccgc
 721 gggcaggtgt ttgacgtagg gccgcgctac accaacctct cgtacatcgg agaaggcgcc
 781 tacggcatgg tttgctctgc ttatgataat ctcaacaaag ttcgagttgc tatcaagaaa
 841 atcagtcctt ttgagcacca gacctactgt caaagaaccc taagagagat aaaaatctta
 901 ctgcgcttca gacatgagaa catcattggc atcaatgaca tcatccgggc accaaccatt
 961 gagcaaatga aagatgtata tatagtacag gacctcatgg agacggacct ttacaagctc
1021 ttgaagacac agcacctcag caatgaccac atctgctatt ttctttatca gatcctgaga
1081 gggctaaagt atatccattc agctaacgtt ctgcaccgtg acctcaagcc ttccaacctc
1141 ctgctgaaca ccacttgtga tctcaagatc tgtgactttg gccttgcccg tgttgcagat
1201 ccagatcatg atcacacagg gttcttgaca gagtacgtag 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 acaaaaataa ggtgccatgg
1501 aacaggttgt tcccaaatgc tgactccaaa gctctggatt tactggataa aatgttgaca
1561 tttaaccctc acaagaggat tgaagttgaa caggctctgg cccacccata cctggagcag
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```
1621 tattatgacc caagtgatga gcccattgct gaagcgccat tcaagtttga catggagttg
1681 gacgacttac ctaaggagaa gctcaaagaa ctcatttttg aagagactgc tagattccag
1741 ccaggataca gatcttaa
```

Reviewed and approved by site quality representative.