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Certificate of Analysis

MAP Kinase 2/Erk2, unactive

(Recombinant enzyme expressed in *E.coli* cells.)
Item # 14-536, 14-536-K, 14-536M
Parent Lot # 1606930

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, human, full length MAP Kinase 2, expressed in *E.coli* cells. Purified using glutathione agarose. Purity 97.6% by SDS-PAGE and Coomassie blue staining. MW = 67.8kDa.

Specific Activity (Parent lot# 1606930): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MEK1(h) (cat# 14-429.)

Formulation: 1.463mg/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.

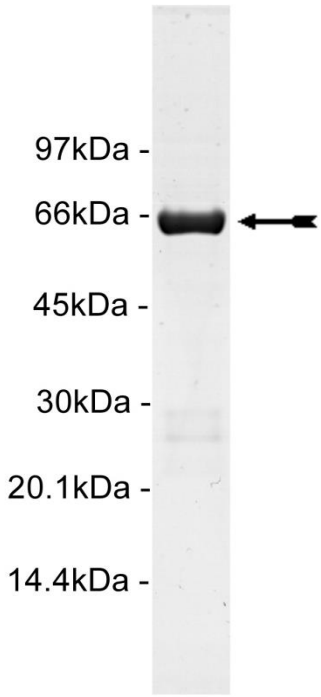
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2µM unactive MAP Kinase 2 was activated using 0.4µM MEK1(h) (cat# 14-429) diluted 250-fold, and the increased activity against myelin basic protein 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	17243	Kinase Activity
None	3.4µg	44	Background

MS Tryptic Fingerprint: Confirmed product identity as MAP Kinase 2, unactive with the translated sequence listed on pages three and four.



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

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 (0.136 mg/ml). Dilute in 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 distilled H_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 well.
2. Add 2.5µl of MBP substrate.
3. Add 2.5µl of diluted **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 scintillation cocktail.
12. Read in scintillation counter. Compare cpm of enzyme samples with cpm of control samples containing all components and 1µl of 30% phosphoric acid.

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MAP Kinase 2 Sequence Information

Protein human MAP Kinase 2

Tags *N*-terminal GST

Native sequence M227 of the recombinant protein is equivalent to M1 of human MAP Kinase 2

Accession number GenBank NM_002745. The cDNA sequence corresponds to a humanised version of the mouse MAP Kinase 2 coding sequence describ4ed in GenBank D10939. At the protein level the sequence is identical to GenBank NM_002745.

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 LVPRGSMAAA AAAGAGPEMV
241 RGQVFDVGPR YTNLSYIGEG AYGMVCSAYD NVNKVRVAIK KISPFEHQTY CQRTLREIKI
301 LLRFRHENII GINDIIRAPT IEQMKDVYIV QDLMETDLYK LLKTQHLSND HICYFLYQIL
361 RGLKYIHSAN VLHRDLKPSN LLLNTTCDLK ICDFGLARVA DPDHDHTGFL TEYVATRWYR
421 APEIMLNSKG YTKSIDIWSV GCILAEMLSN RPIFPGKHYL DQLNHILGIL GSPSQEDLNC
481 IINLKARNYL LSLPHKNKVP WNRLFPNADS KALDLLDKML TFNPHKRIEV EQALAHPYLE
541 QYYDPSDEPI AEAPFKFDME LDDLPKEKLK ELIFEETARF QPGYRS
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Recombinant MAP Kinase 2 nucleotide sequence:

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   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
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 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccat ggcggccgca gcagcggccg gcgctggacc ggagatggtc
 721 cgcgggcagg tgtttgacgt agggccgcgc tacaccaacc tctcgtacat cggagaaggc
 781 gcctacggca tggtttgctc tgcttatgat aatgtcaaca aagttcgagt tgctatcaag
 841 aaaatcagtc cttttgagca ccagacctac tgtcaaagaa ccctaagaga gataaaaatc
 901 ttactgcgct tcagacatga gaacatcatt ggcatcaatg acatcatccg ggcaccaacc
 961 attgagcaaa tgaaagatgt atatatagta caggacctca tggagacgga cctttacaag
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1561 acatttaacc ctcacaagag gattgaagtt gaacaggctc tggcccaccc atacctggag
1621 cagtattatg acccaagtga tgagcccatt gctgaagcgc cattcaagtt tgacatggag
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1681 ttggacgact tacctaagga gaagctcaaa gaactcattt ttgaagagac tgctagattc
1741 cagccaggat acagatctta a
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Reviewed and approved by site quality representative.