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Certificate of Analysis

MAP Kinase 1/Erk1, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-515, 14-515-K, 14-515M
Parent Lot # 1606936

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, human MAP Kinase 1/Erk1, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 70kDa.

Specific Activity (Parent lot# 1606936): Less than 1% of the value obtained with MAP Kinase 1 which has been maximally activated with saturating concentrations of MEK1 (cat# 14-429).

Formulation: 1.587mg/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.

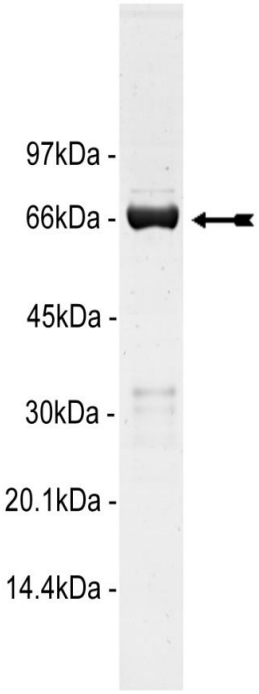
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 2µM inactive MAP Kinase 1 was activated using 0.4µM active MEK1 (cat# 14-429) diluted 25–100 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	Unactive MAP kinase 1 in activation stage 1	Mean cpm	Comments
none	3.5µg	120	Background
0.7µg	3.5µg	12150	Active MAP Kinase 1 activity.

MS Tryptic Fingerprint: Confirmed product identity as human MAP Kinase 1 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 MAPK 1, unactive.

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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 1, unactive:** Use at a final assay concentration of 2µM (0.14mg/ml). Dilute with Dilution Buffer to 1.4mg/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. **MBP (Myelin Basic Protein) 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 1 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 (3.5µg) **MAP kinase 1, 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 25 to 100fold in dilution buffer and storing on ice.

Stage Two: *Phosphorylation of MBP by MAP kinase1* (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 diluted **MAP kinase 1 (3.5ng) 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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MAPK 1 Sequence Information

Protein human MAP Kinase 1

Tags *N*-terminal GST

Native sequence M231 of the fusion protein is equivalent to M1 of human MAPK 1

Accession number Gen Bank XM_055766

Recombinant MAPK 1 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPGIP MAAAAAQGGG
241 GGEPRRTEGV GPGVPGEVEM VKGQPFDVGP RYTQLQYIGE GAYGMVSSAY DHVRKTRVAI
301 KKISPFEHQT YCQRTLREIQ ILLRFRHENV IGIRDILRAS TLEAMRDVYI VQDLMETDLY
361 KLLKSQQLSN DHICYFLYQI LRGLKYIHSA NVLHRDLKPS NLLINTTCDL KICDFGLARI
421 ADPEHDHTGF LTEYVATRWY RAPEIMLNSK GYTKSIDIWS VGCILAEMLS NRPIFPGKHY
481 LDQLNHILGI LGSPSQEDLN CIINMKARNY LQSLPSKTKV AWAKLFPKSD SKALDLLDRM
541 LTFNPNKRIT VEEALAHPYL EQYYDPTDEP VAEEPFTFAM ELDDLPKERL KELIFQETAR
601 FQPGVLEAP
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Recombinant MAPK 1 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
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 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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1681 gagcagtact atgacccgac ggatgagcca gtggccgagg agcccttcac cttcgccatg
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1741 gagctggatg acctacctaa ggagcggctg aaggagctca tcttccagga gacagcacgc
1801 ttccagcccg gagtgctgga ggccccctag
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Reviewed and approved by site quality representative.