eurofins | Discovery Services

Certificate of Analysis

MEK1, active

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-429, 14-429-K, 14-429M
Parent Lot # 2055150

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 and *C*-terminal 6His-tagged, recombinant full-length human MEK1 expressed in *E.coli* cells. Purified using glutathione agarose followed by Ni^{2+}/NTA agarose, activated using c-Raf and then re-purified using Ni^{2+}/NTA agarose. Purity 86.8% by SDS-PAGE and Coomassie blue staining. MW = 71kDa.

Specific Activity (Parent lot# 2055150): 12754U/mg, where one unit of MEK1, active activity is defined as the amount of MEK1 which activates 1µM unactive MAPK2 by 1 unit per minute at 30°C using 100µM ATP. One unit of MAPK2 activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.824mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

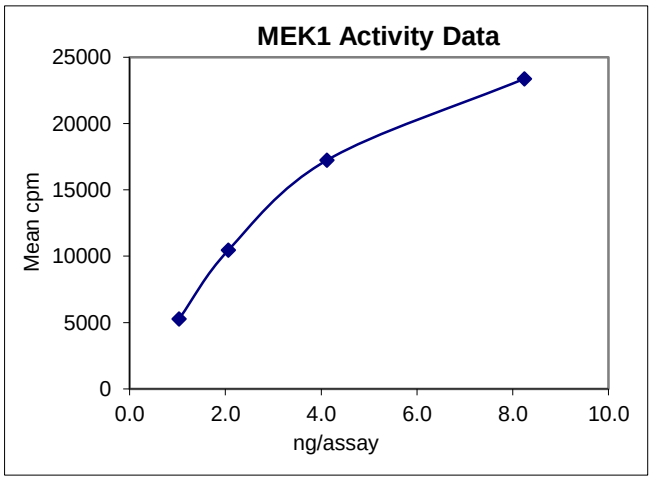
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

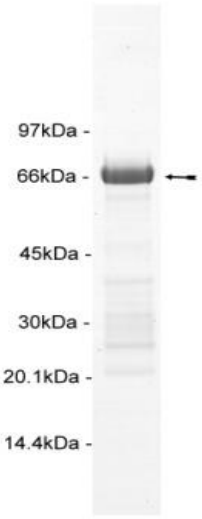
Quality Control Testing

Kinase Assay: 1.0–8.2ng of this lot of enzyme phosphorylated 1µM unactive MAPK2 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 product identity as MEK1 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 MEK1, active.



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

eurofins | Discovery Services

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x MAPK2 Activation Buffer:** 250mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol, 0.05% Brij-35.
2. **10 x Reaction Buffer:** 250mM Tris/HCl pH7.5, 0.2mM EGTA.
3. **MAPK2 unactive:** Use at a final assay concentration of 1µM (0.067mg/ml). Prepare a 0.67mg/ml stock and add 2.5µl of stock per assay point. Prepare using MEK1 dilution buffer.
4. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
5. **MEK1, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.0–8.2ng per assay point.
6. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 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.)

Assay Procedure:

Stage One*: Activation of MAPK2.*

1. Add 5µl of MAPK2 activation buffer to a microcentrifuge tube.
2. Add 2µl of **MAPK2 unactive**.
3. Add **2.5µl (1.0–8.2ng) MEK1, active**.
4. Add 10µl of dH_2O.
5. Add 5µl of stage one Mg/ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Immediately transfer 1µl of the mixture to the second stage component mixture.

Stage Two*: Phosphorylation of MBP by activated MAP kinase* (96 well plate format)

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **MBP**.
3. Add 9µl of dH_2O.
4. Add 10µl of diluted [γ-^{33}P]ATP mixture.
5. Add **1µl** of **Stage One** reaction product.
6. Incubate for 15 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.

eurofins | Discovery Services

Certificate of Analysis

MEK1 Sequence Information

Protein Human MEK1

Tags *N*-terminal GST and *C*-terminal 6His tags

Native sequence M231 of the fusion protein is equivalent to M1 of human MEK1

Accession number EMBL Z30163, possessing amino acid substitution A327G. Please note, introduction of the A327G mutation to the rabbit sequence (Z30163) results in expression of recombinant MEK1 protein that is 100% identical to wild-type human MEK1 described in SwissProt Q02750, note this SwissProt entry fails to record M1 and starts at P2.

Recombinant MEK1 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 IEGRGIPRSA MPKKKPTPIQ
 241 LNPAPDGSAV NGTSSAETNL EALQKKLEEL ELDEQQRKRL EAFLTQKQKV GELKDDDFEK
 301 ISELGAGNGG VVFKVSHKPS GLVMARKLIH LEIKPAIRNQ IIRELQVLHE CNSPYIVGFY
 361 GAFYSDGEIS ICMEHMDGGS LDQVLKKAGR IPEQILGKVS IAVIKGLTYL REKHKIMHRD
 421 VKPSNILVNS RGEIKLCDFG VSGQLIDSMA NSFVGTRSYM SPERLQGTHY SVQSDIWSMG
 481 LSLVEMAVGR YPIPPPDAKE LELMFGCQVE GDAAETPPRP RTPGRPLSSY GMDSRPPMAI
 541 FELLDYIVNE PPPKLPSGVF SLEFQDFVNK CLIKNPAERA DLKQLMVHAF IKRSDAEEVD
 601 FAGWLCSTIG LNQPSTPTHA AGVHHHHHH
```

Recombinant MEK1 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 atcgaaggtc gtgggatccc ccgatccgcc atgccaaaga agaagcccac ccccatccag
 721 ctgaatcctg cccctgacgg ctcggcggtg aacggcacca gctcggcgga gaccaacctg
 781 gaggccttgc agaagaagct ggaggagctg gagcttgacg agcagcagcg gaagcgcctg
 841 gaggccttcc tcacccagaa gcagaaagtg ggagagctga aggacgatga cttcgagaag
 901 atcagtgagc tgggagccgg caacggcggc gtggtgttca aggtctccca caagccgtcc
 961 ggcctggtca tggccagaaa gcttattcac ctggagatca aacctgctat ccggaaccag
1021 atcataaggg agctgcaggt tctgcacgag tgcaactccc cgtacatcgt gggcttctac
1081 ggggcattct acagcgatgg cgagatcagc atctgcatgg agcacatgga cgggggttcc
1141 ttggatcaag tcctgaagaa agctggacgg attcccgagc aaattttggg gaaagttagc
1201 attgctgtga tcaagggcct gacgtatctg agggagaagc acaagatcat gcacagagac
1261 gtcaagccct ccaacatcct ggtcaactcc cgcggggaga tcaagctctg tgacttcggg
1321 gtcagtgggc agctcatcga ctccatggcc aactccttcg tgggcaccag gtcttatatg
1381 tcgcccgaga gactccaggg gacacactac tctgtgcagt cggacatctg gagcatgggg
1441 ctgtccctgg tggagatggc ggtggggcgg taccccatcc cgccccccga cgccaaggag
1501 ctggagctga tgtttgggtg ccaggtggag ggcgatgcgg ccgagactcc gcccaggccc
```

eurofins | Discovery Services

Certificate of Analysis

```
1561 aggacccctg ggcggcccct cagctcgtat ggaatggata gccggcctcc catggcgatt
1621 tttgagctgc tggattacat cgtcaatgag cctcctccga aactccccag cggagtcttc
1681 agcctggagt ttcaagattt tgtgaataaa tgcttaataa aaaaccccgc cgagagagca
1741 gacttgaagc agctcatggt tcatgctttt atcaagaggt ctgatgccga ggaggtggat
1801 tttgctggtt ggctgtgctc caccatcggc cttaaccagc ccagcacgcc gacgcacgcg
1861 gccggtgtgc atcatcacca ccatcactaa
```

Reviewed and approved by site quality representative.