eurofins | Discovery Services

Certificate of Analysis

MEK1, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-420, 14-420-K, 14-420M
Parent Lot # 2350988

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. Purity 76.8% using SDS-PAGE and Coomassie blue staining. MW = 71kDa.

Specific Activity (Parent lot# 2350988): Less than 1% of the value obtained with MEK1 that has been activated with c-Raf. MEK1 activity determined by activation of 1µM MAP kinase 2.

Formulation: 2.957mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 5% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. 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 microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

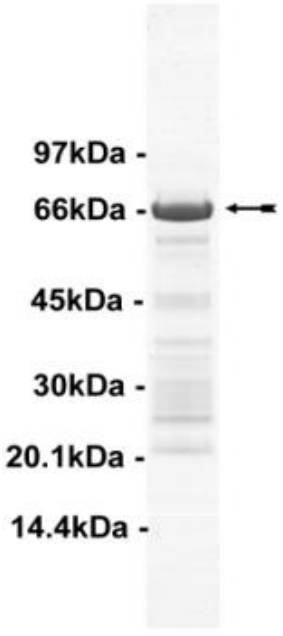
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 5µM unactive MEK1 was activated using 0.1µM cRaf, then diluted 100-fold, used to activate 1µM unactive MAP kinase 2, and the increased activity of the MAP kinase 2 against MBP determined. The activation of MEK1, subsequent activation of MAP kinase 2, and assay are described on page two. Results of this assay are shown below.

Active c-Raf	Unactive MEK1	Mean cpm	Comment
None	8.875µg	85	Inactive MEK1
110ng	8.875µg	13653	Activated MEK1

MS Tryptic Fingerprint: Confirmed product identity as MEK1 with the translated sequence listed on page four.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MEK1.

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. **10 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol, 0.3% Brij-35.
2. **Dilution Buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.1mM Na_3VO_4, 1mg/ml BSA.
3. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 1mM ATP.
4. **MEK1, unactive (Catalogue# 14-420):** Add to a final assay concentration of 5µM (0.355mg/ml). The volume is calculated by the formula, 0.355mg/ml divided by the concentration **2.957mg/ml** times 25µl.
5. **c-Raf-1, active (Catalogue# 14-352):** Add to a final assay concentration of 0.1µM (4.4µg/ml). Dilute with Dilution Buffer to 44µg/ml. Use 2.5µl per assay point.
6. **MAP kinase 2, unactive (Catalogue# 14-198):** Add to a final assay concentration of 1µM (67µg/ml). Dilute with Dilution Buffer to 0.67mg/ml. Use 2.5µl per assay point.
7. **Stage Two 5 x Mg/ATP:** 50mM magnesium acetate, 0.5mM ATP.
8. **10 x Assay Buffer:** 250mM Tris/HCl pH7.5, 2mM EGTA.
9. **MBP substrate (Catalogue# 13-104):** Dilute with dH_20 to prepare a 3.33mg/ml stock. Use 2.5µl stock per assay point.
10. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500cpm/pmol as required.)

Assay Procedure:

Stage One: *Activation of MEK1*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 12µl dH_20.
3. Add **3.0µl MEK1, unactive**.
4. Add 2.5µl **c-Raf, active**.
5. Add 5µl stage one 5 x Mg/ATP.
6. In appropriate controls, add dilution buffer to a final volume of 25µl.
7. Incubate for 30 minutes at 30°C.
8. Stop the reaction by diluting the MEK-1 250-fold in dilution buffer. Store on ice.

Stage Two: *Activation of MAPK2*

1. Add 2.5µl 10 x activation buffer to a microcentrifuge tube.
2. Add 12.5µl of dH_2O.
3. Add 2.5µl of **MAP kinase 2, unactive**.
4. Add 2.5µl of diluted **MEK1** from **Stage One**.
5. Add 5µl of stage two 5 x Mg/ATP.
6. Incubate for 15 minutes at 30°C.
7. Immediately transfer 1µl of the mixture to the stage three component mixture.

Certificate of Analysis

Stage Three: *Phosphorylation of MBP by Activated MAP kinase 2* (96 well plate format).

1. Add 2.5µl 10 x assay buffer per assay to wells.
2. Add 9µl dH_2O.
3. Add 2.5µl **MBP**.
4. Add 1µl of the **Stage Two** reaction product.
5. Add 10µl of the 2.5 x magnesium acetate/ [γ-^{33}P]ATP cocktail.
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.
9. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
10. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl 30% phosphoric acid and control samples with all assay components except c-Raf.

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 recombinant 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
1561 aggacccctg ggcggcccct cagctcgtat ggaatggata gccggcctcc catggcgatt
```

eurofins | Discovery Services

Certificate of Analysis

```
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.