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Certificate of Analysis

MEK2, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-532, 14-532-K, 14-532M
Parent Lot # 1654882

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 6His-tagged recombinant, full-length human MEK2 expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 93.2% by SDS-PAGE and Coomassie blue staining. MW = 46kDa.

Specific Activity: As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with c-Raf (cat# 14-352).

Formulation: 1.778mg/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

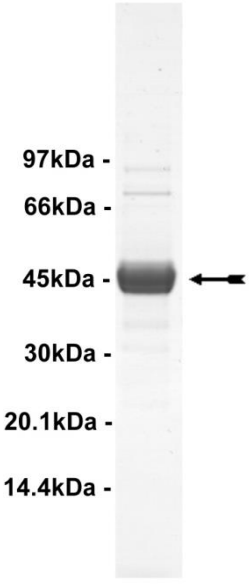
Quality Control Testing

Activation Assay: Unactive MEK2 was activated using c-Raf (cat# 14-352). 5.725µg of unactive MEK2 was activated using 183ng of c-Raf, then diluted 500-fold, used to activate MAP kinase 2 and the increased activity of the MAP kinase 2 against MBP determined. The activation of MEK2, subsequent activation of MAP kinase 2 and assay are described on page two. Results of this assay are shown below.

Active c-Raf	Unactive MEK2	MBP	Mean cpm	Comments
None	5.725µg	8.25µg	3128	Background
183ng	5.725µg	8.25µg	16719	Kinase activity

MS Tryptic Fingerprint: Confirmed identity as MEK2 with the translated sequence listed on page four.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MEK2, unactive.



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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. **MEK2, unactive (Catalogue# 14-532):** Add to a final assay concentration of 5µM (0.229mg/ml). Dilute with Dilution Buffer to 1.14mg/ml. Add 5µl of stock per assay point.
5. **c-Raf-1, active (Catalogue# 14-352):** Add to a final assay concentration of 0.1µM (7.3µg/ml). Dilute with Dilution Buffer to 73µg/ml. Use 2.5µl of stock per assay point.
6. **MAPK2 (Catalogue# 14-198):** Add to a final assay concentration of 1µM (67µg/ml). Dilute with Dilution Buffer to 0.67mg/ml. Use 2.5µl of stock 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):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock. Use 2.5µl of 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 MEK2*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 10µl dH_20.
3. Add 5µl of **MEK2, unactive**.
4. Add **2.5µl (183ng) 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 MEK2 500-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 (MAPK2).**
4. Add 2.5µl of diluted **MEK2** from **Stage One** above.
5. Add 5 µl 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.

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Stage Three: *Phosphorylation of MBP by Activated MAP kinase 2.*

1. Add 2.5µl 10 x assay buffer to a microcentrifuge tube.
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. Transfer a 20µl aliquot onto the centre of a 2cm x 2cm **P81** paper.
8. Wash the assay squares twice with 75mM phosphoric acid.
9. Wash the assay squares once for 2 minutes with acetone.
9. Transfer the assay squares to a scintillation vial and add 1ml 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 of 30% phosphoric acid and control samples with all assay components except c-Raf.

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MEK2 Sequence Information

Protein human MEK2

Tags *N*-terminal 6His

Native sequence M10 of the fusion protein is equivalent to M1 of human MEK2

Accession number GenBank NM_030662

Recombinant MEK2 amino acid sequence:

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  1 MHHHHHHEFM LARRKPVLPA LTINPTIAEG PSPTSEGASE ANLVDLQKKL EELELDEQQK
 61 KRLEAFLTQK AKVGELKDDD FERISELGAG NGGVVTKVQH RPSGLIMARK LIHLEIKPAI
121 RNQIIRELQV LHECNSPYIV GFYGAFYSDG EISICMEHMD GGSLDQVLKE AKRIPEEILG
181 KVSIAVLRGL AYLREKHQIM HRDVKPSNIL VNSRGEIKLC DFGVSGQLID SMANSFVGTR
241 SYMAPERLQG THYSVQSDIW SMGLSLVELA VGRYPIPPPD AKELEAIFGR PVVDGEEGEP
301 HSISPRPRPP GRPVSGHGMD SRPAMAIFEL LDYIVNEPPP KLPNGVFTPD FQEFVNKCLI
361 KNPAERADLK MLTNHTFIKR SEVEEVDFAG WLCKTLRLNQ PGTPTRTAV
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Recombinant MEK2 nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcatg ctggcccgga ggaagccggt gctgccggcg
  61 ctcaccatca accctaccat cgccgagggc ccatccccta ccagcgaggg cgcctccgag
 121 gcaaacctgg tggacctgca gaagaagctg gaggagctgg aacttgacga gcagcagaag
 181 aagcggctgg aagcctttct cacccagaaa gccaaggtcg gcgaactcaa agacgatgac
 241 ttcgaaagga tctcagagct gggcgcgggc aacggcgggg tggtcaccaa agtccagcac
 301 agaccctcgg gcctcatcat ggccaggaag ctgatccacc ttgagatcaa gccggccatc
 361 cggaaccaga tcatccgcga gctgcaggtc ctgcacgaat gcaactcgcc gtacatcgtg
 421 ggcttctacg gggccttcta cagtgacggg gagatcagca tttgcatgga acacatggac
 481 ggcggctccc tggaccaggt gctgaaagag gccaagagga ttcccgagga gatcctgggg
 541 aaagtcagca tcgcggttct ccggggcttg gcgtacctcc gagagaagca ccagatcatg
 601 caccgagatg tgaagccctc caacatcctc gtgaactcta gaggggagat caagctgtgt
 661 gacttcgggg tgagcggcca gctcatagac tccatggcca actccttcgt gggcacgcgc
 721 tcctacatgg ctccggagcg gttgcagggc acacattact cggtgcagtc ggacatctgg
 781 agcatgggcc tgtccctggt ggagctggcc gtcggaaggt accccatccc cccgcccgac
 841 gccaaagagc tggaggccat ctttggccgg cccgtggtcg acggggaaga aggagagcct
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 961 agccggcctg ccatggccat ctttgaactc ctggactata ttgtgaacga gccacctcct
1021 aagctgccca acggtgtgtt caccccgac ttccaggagt ttgtcaataa atgcctcatc
1081 aagaacccag cggagcgggc ggacctgaag atgctcacaa accacacctt catcaagcgg
1141 tccgaggtgg aagaagtgga ttttgccggc tggttgtgta aaaccctgcg gctgaaccag
1201 cccggcacac ccacgcgcac cgccgtgtga
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Reviewed and approved by site quality representative.