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Certificate of Analysis

MEK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-528, 14-528-K, 14-528M
Parent Lot # WAD0049

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, activated using c-Raf (cat# 14-352) and then re-purified using Ni^{2+}/NTA agarose. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 46kDa.

Specific Activity (Parent lot# WAD0049): 3954U/mg, where one unit of MEK2, active activity is defined as the amount of MEK2 which activates 1µM MAPK2 (cat# 14-198) 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 myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.40mg/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.

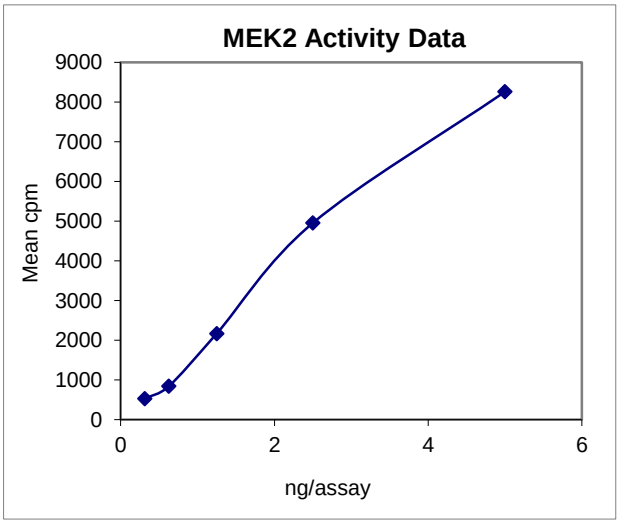
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

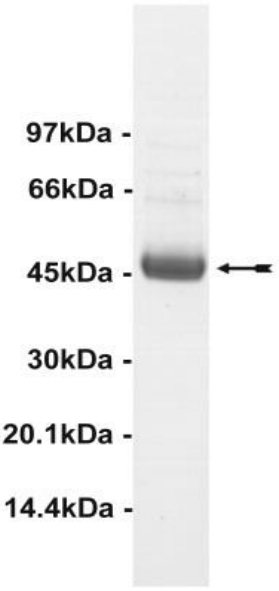
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.31–5ng 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 identity as MEK2 with the translated sequence listed on page three.



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

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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 MEK2 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. **MEK2, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.31–5ng 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 2.5µl of **MAPK2 unactive**.
3. Add **2.5µl (0.31–5ng) MEK2, active**.
4. Add 10 µl 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 **Stage Two** component mixture.

***Stage Two**: Phosphorylation of MBP by activated MAP kinase 2* (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 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
8. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
9. Wash the filtermat once for 2 minutes with methanol.
10. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
11. 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.

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MEK2 Sequence Information

Protein Human MEK2

Tags *N*-terminal 6His

Native sequence M10 of the recombinant 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
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 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.