

Certificate of Analysis

MEKK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-963, 14-963-K, 14-963M

Parent Lot # D15EP011N

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, human MEKK2, full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 74kDa.

Specific Activity (Parent lot# D15EP011N): 118U/mg, where one unit of MEKK2, active activity is defined as 1nmol phosphate incorporated into 1mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.46mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

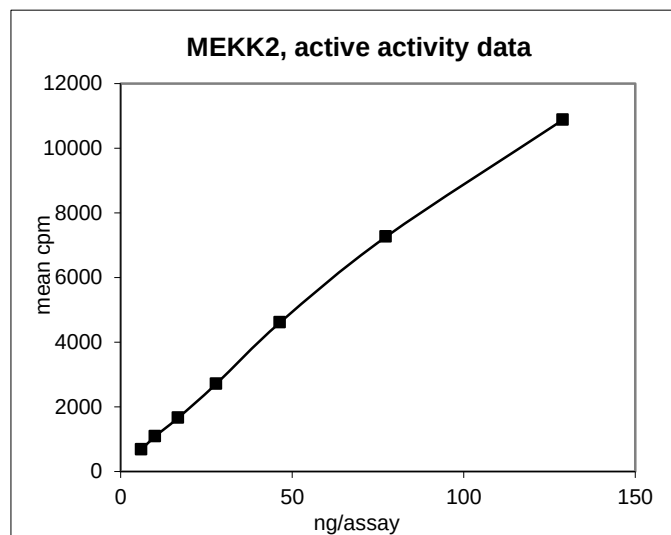
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

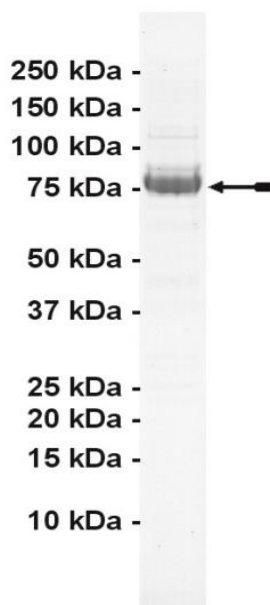
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6–129ng of this lot of enzyme phosphorylated 1mg/ml myelin basic protein 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 MEKK2 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 MEKK2, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 1mg/ml. Prepare a 10mg/ml stock and add 2.5µl of stock per assay point.
- 3. MEKK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–129ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of MBP.
3. Add 5µl of dH₂O.
4. Add **2.5µl (6–129ng) MEKK2, active.**
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 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.

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MEKK2 Sequence Information

Protein	Human MEKK2
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human MEKK2
Accession number	GenBank NM_006609.3

Recombinant MEKK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDDQQALNSI MQDLAVLHKA SRPALSLOET
61 RKAKSSSPKK QNDVRVKFEH RGEKRILQFP RPKVLEDLRS KAKIAFGQSM DLHYTNNELV
121 IPLTTQDDLD KAVELLDRSI HMKSLKILLV INGSTQATNL EPLPSLEDLD NTVFGAERKK
181 RLSIIGPTSR DRSSPPPGYI PDELHQVARN GSFTSINSEG EFIPESMDQM LDPLSLSSPE
241 NSGSGSCPSL DSPLDGESYP KSRMPRAQSY PDNHQEFSDY DNPIFEKFGK GGTYPRRYHV
301 SYHHQEYNDG RKTFFPRARRT QGTSLRSPVS FSPTDHSLSST SSGSSIFTPE YDDSRIRRRG
361 SDIDNPTLTV MDISPPSRSP RAPTNWRLGK LLGQGAFGRV YLCYDVDVDTGR ELAVKQVQFD
421 PDSPETSKEV NALECEIQLL KNLLHERIVQ YYGCLRDPQE KTLSIFMEYM PGGSIKDQLK
481 AYGALTENVY RKYTRQILEG VHYLHSNMIV HRDIKGANIL RDSTGNVKLG DFGASKRLQT
541 ICLSGTGMKS VTGTPYWMSP EVISGEGYGR KADIWSVACT VVEMLTEKPP WAEFEAMAAI
601 FKIAATQPTNP KLPPHVSDYT RDFLKRIFVE AKLRPSADEL LRHMFVHYH

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Recombinant MEKK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tattttcagg ggcgcattgga tccggaattc atggatgata agcaagcttt gaactcaatc
121 atgcaagatt tggctgtcct tcataaggcc agtcgaccag cattatcctt gcaggaaacc
181 agaaaagcaa aatcttcata accaaaaaaa cagaatgatg tccgagtcaa atttgaacat
241 agaggagaaa aaagaatcct tcagttcccc agaccagtta aactggaaga tctgagatct
301 aaagctaaaa ttgccttttg acagtctatg gatctacatt ataccaataa cgagttggta
361 attccattaa ctactcaaga tgacttggaac aaagctgtgg aactgctgga tcgtagtatt
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1441 gcatatggcg ctcttactga gaatgtgact aggaaataca cccgtcagat tctggagggt
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1561 cgagattcaa caggcaacgt caaactagga gattttgggg ccagcaaacg gcttcagacc
1621 atctgtctct cagggaacag aatgaagtct gtcacgggca caccatactg gatgagccct

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1681 gaagtcatca gtggagaagg ctatggaaga aaagcagaca tctggagtgt tgcatgtact
1741 gtggtagaaa tgctaactga aaagccgcct tgggctgaat ttgaagcaat ggctgccatc
1801 tttaaaatcg ccactcagcc aacaaaccca aagctgccac ctcatgtctc agactatact
1861 cgagatttcc tcaaacggat ttttgtagag gccaaactga gaccttcagc tgatgaactc
1921 ttaaggcaca tgtttgtgca ttatcactag
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