

Certificate of Analysis

MEKK3, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-015, 16-015-K, 16-015M

Parent Lot # 225615

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-tagged, recombinant, human MEKK3 full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 52% by SDS-PAGE and Coomassie blue staining.
MW = 97.7 kDa.

Formulation: 0.75 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol, 20 mM β -Glycerophosphate, 10 mM Sodium Fluoride, 0.25 mM Na Vanadate. Frozen solution.

Specific Activity (Parent lot# 225615): 9 U/mg, where one unit of MEKK3 activity is defined as 1 nmol phosphate incorporated into 0.5 mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100 μ M.

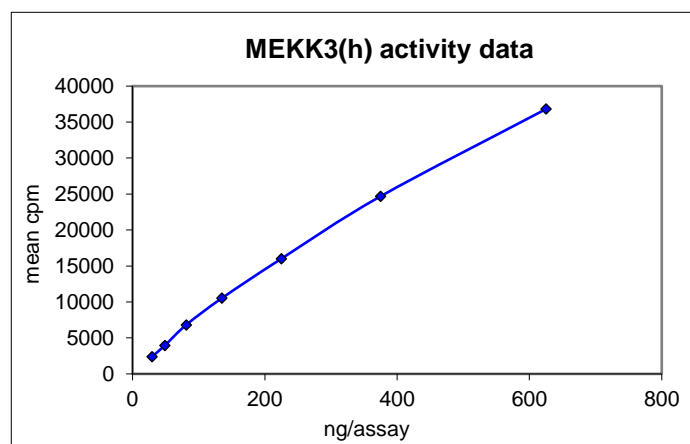
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

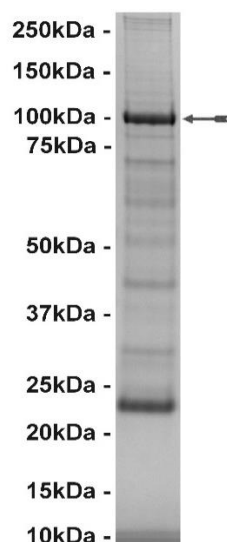
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 29.1–625 ng of this lot of enzyme phosphorylated 0.5 mg/ml myelin basic protein (MBP) 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 MEKK3 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of MEKK3, active..

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 50 mM HEPES pH 8.0, 1 mM EGTA, 0.01% Brij-35, 10 mM DTT.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.5 mg/ml. Make up a 3.3 mg/ml stock. Use 3.8 μ l of stock per assay point.
- 3. MEKK3, active:** 50 mM HEPES pH 8.0, 1 mM EGTA, 0.01% Brij-35, 10 mM DTT. Use 29.1–625 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 3.75 μ l of **MBP**.
3. Add **2.5 μ l (29.1–625 ng) MEKK3, active**.
4. Add 0.05 μ l DTT 1M.
5. Add 3.7 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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MEKK3, active Sequence Information

Protein	Human MEKK3
Tags	N-terminal GST
Native sequence	M231 of the recombinant protein is equivalent to M1 of human MEKK3
Accession number	GenBank NM_002401.3

Recombinant MEKK3 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMIA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLVK
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGQWQATF GGGDHPPKSD LEVLFQGPPEF MDEQEALNSI
241 MNDLVALQMN RRHRMPGYET MKNKDTGHSN RQSDVRIKFE HNGERRIIAF SRPVKYEDVE
301 HKVTTVFQGP LDLHYMNNEL SILKLNQDDL DKAIDILDRS SSMKSLRILL LSQDRNHNS
361 SPHSGVSRQV RIKASQSAGD INTIYQPPPEP RSRHLSVSSQ NPGRSSPPPG YVPERQQHIA
421 RQGSYTSINS EGEFIPETSE QCMLDPLSSA ENSLSGSCQS LDRSADSPSF RKSRMSRAQS
481 FPDNRQEYSD RETQLYDKGV KGGTYPRRYH VSVHKKDYS DGRRTFPRIR HQGNLFTLVP
541 SSRSLSTNGE NMGLAVQYLD PRGRLRSADS ENALSVQERN VPTKSPSAPI NWRRGKLLGQ
601 GAFGRVYLCY DVDTGRELAS KQVQFDPDSP ETSKEVSALE CEIQLLKNLQ HERIVQYYGC
661 LRDRAEKTLT IFMEYMPGGS VKDQLKAYGA LTESVTRKYT RQILEGMSYL HSNMIVHRDI
721 KGANILRDSA GNVKLGDFGA SKRLQITCMS GTGMRSVTGT PYWMSPEVIS GEGYGRKADV
781 WSLGCTVVEML LTEKPPWAEY EAMAAIFKIA TQPTNPQLPS HISEHGRDFL RRIFVEARQR
841 PSAEELLTHH FAQLMY
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Recombinant MEKK3 nucleotide sequence:

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1 atgtccccta tactagggta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
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1621 tccagccgct cctgagcac aatggcgag aacatgggtc tggctgtgca atacctggac
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2521 ccttcagctg aggagctgct cacacaccac tttgcacagc tcatgtactg a
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