Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

MEKK3

mitogen-activated protein kinase kinase kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP3K3

Synonyms: MAPKKK3, MEK kinase3

Product No.: 0859-0000-1

Lot: 005

Description: Human MEKK3, full length, amino acids M_1-Y_{672} (as in NCBI/Protein entry NP_002392.2), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: MEKK3 Lot 005, was confirmed as MEKK3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 99,304 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

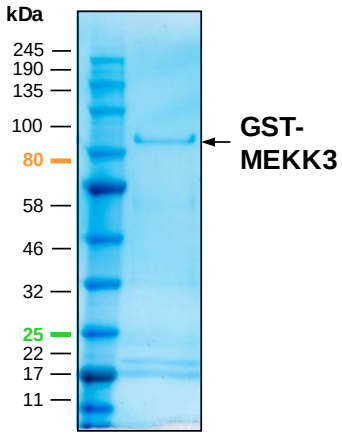
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.264 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

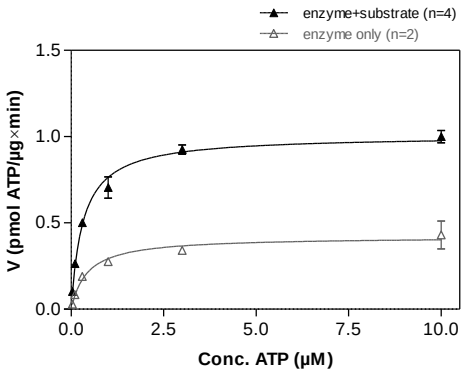
Biochemical Parameters:
Specific kinase activity (P_i transfer): 1 pmol/µg × min
ATP-K_M: 0.32 µM

MEKK3 Lot 005:
Coomassie stain



2 µg GST-MEKK3

MEKK3 Lot 005:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate:Casein, 20 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

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GST-MEKK3 Recombinant Fusion Protein Amino Acid Sequence

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241 PLAMMDEQEA LNSIMNDLVA LQMNRRHRMP GYETMKNKDT GHSNRQSDVR IKFEHNGERR 300
301 IIAFSRPVKY EDVEHKVTTV FGQPLDLHYM NNELSILLKN QDDLDKAIDI LDRSSSMKSL 360
361 RILLLSQDRN HNSSSPHSGV SRQVRIKASQ SAGDINTIYQ PPEPRSRHLS VSSQNPGRSS 420
421 PPPGYVPERQ QHIARQGSYT SINSEGEFIP ETSEQCMLDP LSSAENSLSG SCQSLDRSAD 480
481 SPSFRKSRMS RAQSFPDNRQ EYSDRETQLY DKGVKGGTYP RRYHVSVHHK DYSDGRRTFP 540
541 RIRRHQGNLF TLVPSSRSLS TNGENMGLAV QYLDPRGRLR SADSENALSV QERNVPTKSP 600
601 SAPINWRRGK LLGQGAFGRV YLCYDVDTGR ELASKQVQFD PDSPETSKEV SALECEIQLL 660
661 KNLQHERIVQ YYGCLRDRAE KTLTIFMEYM PGGSVKDQLK AYGALTESVT RKYTRQILEG 720
721 MSYLHSNMIV HRDIKGANIL RDSAGNVKLG DFGASKRLQT ICMSGTGMRS VTGTPYWMSP 780
781 EVISGEGYGR KADVWSLGCT VVEMLTEKPP WAEYEAMAAI FKIATQPTNP QLPSHISEHG 840
841 RDFLRRIFVE ARQRPSAEEL LTHHFAQLMY                                  900
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: MEKK3

MEKK3 wt[1] Amino Acid Sequence

```
  1 MDEQEALNSI MNDLVALQMN RRHRMPGYET MKNKDTGHSN RQSDVRIKFE HNGERRIIAF  60
 61 SRPVKYEDVE HKVTTVFGQP LDLHYMNNEL SILLKNQDDL DKAIDILDRS SSMKSLRILL 120
121 LSQDRNHNSS SPHSGVSRQV RIKASQSAGD INTIYQPPEP RSRHLSVSSQ NPGRSSPPPG 180
181 YVPERQQHIA RQGSYTSINS EGEFIPETSE QCMLDPLSSA ENSLSGSCQS LDRSADSPSF 240
241 RKSRMSRAQS FPDNRQEYSD RETQLYDKGV KGGTYPRRYH VSVHHKDYSD GRRTFPRIRR 300
301 HQGNLFTLVP SSRSLSTNGE NMGLAVQYLD PRGRLRSADS ENALSVQERN VPTKSPSAPI 360
361 NWRRGKLLGQ GAFGRVYLCY DVDTGRELAS KQVQFDPDSP ETSKEVSALE CEIQLLKNLQ 420
421 HERIVQYYGC LRDRAEKTLT IFMEYMPGGS VKDQLKAYGA LTESVTRKYT RQILEGMSYL 480
481 HSNMIVHRDI KGANILRDSA GNVKLGDFGA SKRLQTICMS GTGMRSVTGT PYWMSPEVIS 540
541 GEGYGRKADV WSLGCTVVEM LTEKPPWAEY EAMAAIFKIA TQPTNPQLPS HISEHGRDFL 600
601 RRIFVEARQR PSAEELLTHH FAQLMY                                      660
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blue: MEKK3 sequence expressed in recombinant protein

[1] NCBI/Protein accession number NP_002392.2

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