

## ProQinase™ 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<sub>1</sub>-Y<sub>672</sub> (as in [NCBI/Protein](#) entry NP\_002392.2), N-terminal GST-HIS<sub>6</sub> 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**<sub>Fusion Protein</sub>: 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

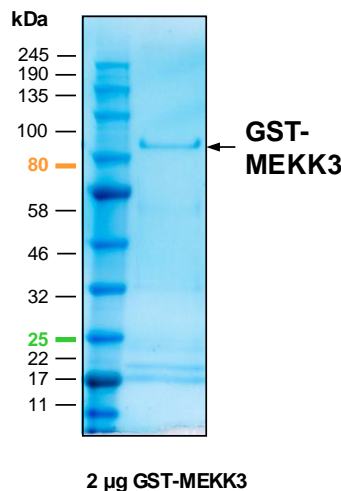
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

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

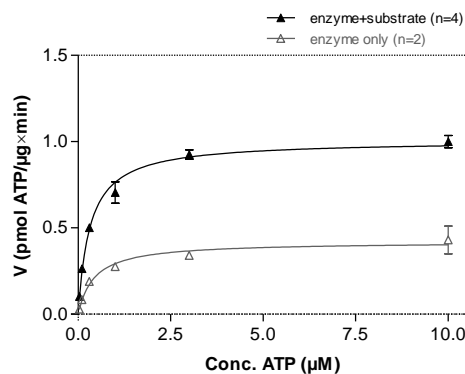
### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 1 pmol/µg × min  
ATP-K<sub>M</sub>: 0.32 µM

### MEKK3 Lot 005: Coomassie stain



### MEKK3 Lot 005: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: Casein, 20 µg/ml
  - Kinase: 4 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

## ProQinase™ MEKK3

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GST-MEKK3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGC	240
241	PLAMMDEQEA	LNSIMNDLVA	LQMNRRHRMP	GYETMKNKDT	GHSNRQSDVR	IKFEHNGERR	300
301	IIAFSRPVKY	EDVEHKVTV	FGQPLDLHYM	NNELSILLKN	QDDLKAIID	LDRSSSMKSL	360
361	RILLQSQRN	HNSSSPHSGV	SRQVRIKASQ	SAGDINTIYQ	PPEPRSRHLS	VSSQNPGRSS	420
421	PPPGYVPERQ	QHIAHQGSYT	SINSEGEFIP	ETSEQCMLDP	LSSAENSLSG	SCQSLDRSAD	480
481	SPSFRKSRMS	RAQSFDPNRQ	EYSDRETQLY	DKGVKGGTYP	RRYHVSVHHK	DYSDGRRTFP	540
541	RIRRHQGNLF	TLVPSSRSL	TNGENMGLAV	QYLDPRGRLR	SADSENALSV	QERNVPTKSP	600
601	SAPINWRRGK	LLQGGAFGRV	YLCYDVDTGR	ELASKQVQFD	PDSPETSKEV	SALECEIQLL	660
661	KNLQHERIVQ	YYGALDRAE	KTLTIFMEYM	PGSQVKDQLK	AYGALTESVT	RKYTRQILEG	720
721	MSYLHSNMIV	HRDIKGANIL	RDSAGNVKLG	DFGASKRLQT	ICMSGTMRS	VTGTPYWMSP	780
781	EVISGEGYGR	KADVWSLCT	VVEMLTEKPP	WAEYEAMAAI	FKIATQPTNP	QLPSHISEHG	840
841	RDFLRRIFFE	ARQRPAAEEL	LTHHFAQLMY				900

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: MEKK3

MEKK3 wt <sup>1</sup> Amino Acid Sequence							
1	MDEQEALNSI	MNDLVALQMN	RRHRMPGYET	MKNKDTGHSN	RQSDVRIKFE	HNGERRIAF	60
61	SRPVKYEDVE	HKVTTVFGQP	LDLHYMNNEL	SILLKNQDDL	DKAIDILDRS	SSMKSLRILL	120
121	LSQDRNHNS	SPHSGVSRQV	RIKASQSAGD	INTIYQPPPEP	RSRHLVSSQ	NPGRSSPPPG	180
181	YVPERQQHIA	RQGSYTSINS	EGEFIPETSE	QCMLDPLSSA	ENSLGSCQS	LDRSADSPSF	240
241	RKSRMSRAQS	FDPNRQEYSD	RETQLYDKGV	KGGTYPRIYH	VSVHHKDYSD	GRRTFPRIIR	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	SKRLQITCMS	GTGMRSVTGT	PYWMSPEVIS	540
541	GEGYGRKADV	WSLGCTVEM	LTEKPPWAEY	EAMAAIFKIA	TQPTNPQLPS	HISEHGRDFL	600
601	RRIFVEARQR	PSAEELLTHH	FAQLMY				660

blue: MEKK3 sequence expressed in recombinant protein

<sup>1</sup>NCBI/Protein accession number NP\_002392.2