

ProQinase™ MEK1 S218E/S222E

MAPK / ERK activating kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K1

Synonyms: MAP2K1, MKK1

Product No.: 0385-0000-1

Lot: 001

Description: Human MEK1, full length, amino acids M₁-V₃₉₃ (as in [NCBI/Protein](#) entry NP_002746.1), with constitutive activating mutations S218E and S222E, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MEK1 S218E/S222E, Lot 001, was confirmed as MEK1 by MEK1 specific Western blot

Theoretical MW_{Fusion Protein}: 72,918 Da

Expression host: Sf9 insect cells/E.coli

Purification: GST-Affinity Chromatography

Activation: constitutively active due to S218E/S222E point mutations, no further procedures were applied to activate this kinase

Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C

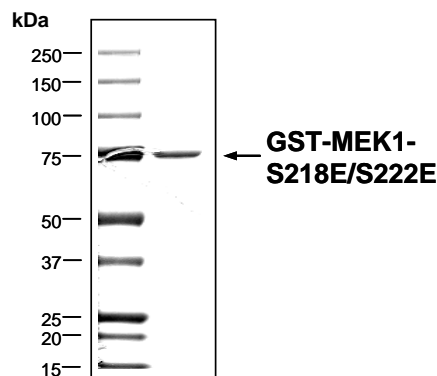
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.379 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

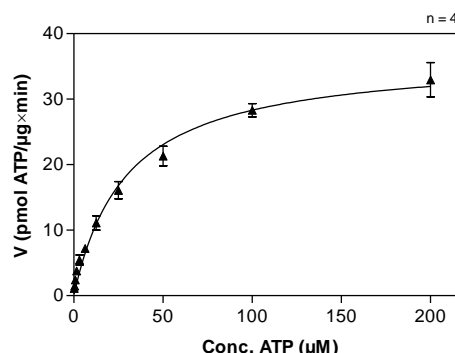
Specific kinase activity (P_i transfer): 37 pmol/µg × min
ATP-K_M: 30 µM

**MEK1 S218E/S222E Lot 001:
Coomassie stain**



2.0 µg GST-MEK1 S218E/S222E

**MEK1 S218E/S222E Lot 001:
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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg/ml PEG_{20,000}
 - ATP (variable)
 - Substrate: ERK2 K54R 100 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
MSFC membrane (Millipore)

ProQinase™ MEK1 S218E/S222E

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GST-MEK1 S218E/S222E Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRMPKKK	PTPIQLNPAP	DGSAVNGTSS	AETNLEALQK	KLEELELDEQ	300
301	QRKRLEAFLT	QKQKVGELKD	DDFEKISELG	AGNGGVVFKV	SHKPSGLVMA	RKLIHLEIKP	360
361	AIRNQIIREL	QVLHECNQSPY	IVGFYGAFFYS	DGEISICMEH	MDGGSLDQVL	KKAGRIPEQI	420
421	LGKVSIAVIK	GLTYLREKHK	IMHRDVKPSN	ILVNSRGEIK	LCDFGVSGQL	IDE ^{MANE} FVFG	480
481	TRSYMSPERL	QGTHYSVQSD	IWSMGLSLVE	MAVGRYPIPP	PDAKELELMF	GCQVEGDAAE	540
541	TPPRPRTQGR	PLSSYGMSDR	PPMAIFELLD	YIVNEPPPKL	PSGVFSLEFQ	DFVNKCLIKN	600
601	PAERADLKQL	MVHAFIKRSD	AAEVDVFAWGL	CSTIGLNQPS	TPTHAAGV		660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: MEK1 **boxed**: variation from RefSeq

MEK1 wt ¹ Amino Acid Sequence							
1	MPKKKPTPIQ	LNPAPDGSVA	NGTSSAETNL	EALQKKLEEL	ELDEQQRKRL	EAFLTQKQKV	60
61	GELKDDDFEK	ISELGAGNGG	VVFKVSHKPS	GLVMARKLIH	LEIKPAIRNQ	IIRELQVLHE	120
121	CNSPYIVGFY	GAFYSDGEIS	ICMEHMDGGS	LDQVLKKAGR	IPEQILGKVS	IAVIKGLTYL	180
181	REKHKIMHRD	VKPSNILVNS	RGEIKLDFG	VSGQLID ^{SMA}	NSFVGTRSYM	SPERLQTHY	240
241	SVQSDIWSMG	LSLVEMAVGR	YPIPPPDAAKE	LELMFGCQVE	GDAAE ^T PPRP	RTPGRPLSSY	300
301	GMSRPPMAI	FELLDYIVNE	PPPKLPSGVF	SLEFQDFVNK	CLIKNPAERA	DLKQLMVHAF	360
361	IKRSDAEVD	FAGWLCSTIG	LNQSTP ^{THA}	AGV			420

blue: MEK1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

¹[NCBI/Protein](#) accession number NP_002746.1