Certificate of Analysis

REACTION BIOLOGY

ProQinase™ MLK3

mitogen-activated protein kinase kinase kinase 11

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP3K11

Synonyms: PTK1, SPRK, MEKK11

Product No.: 1999-0000-1

Lot: 001

Description: Human MLK3, N-terminal fragment, amino acids M_1-G_{488} (as in NCBI/Protein entry NP_002410.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: MLK3 Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 82377 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

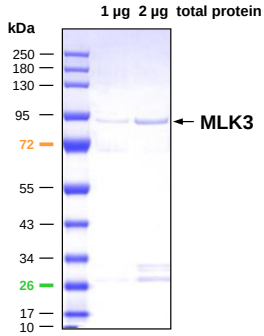
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

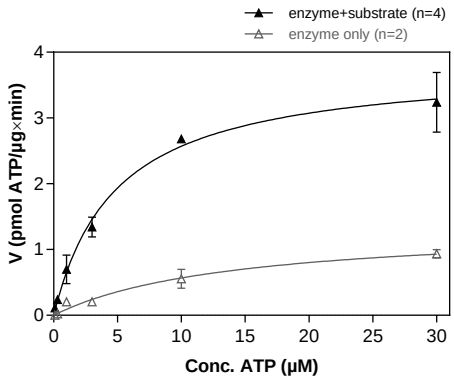
Protein concentration: 0.52 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 3.8 pmol/µg*min
ATP-K_M: 4.9 µM

MLK3 Lot 001:
Coomassie stain



MLK3 Lot 001:
Determination of V_{max} and K_M value for ATP



- 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: Myelin basic protein 20 µg/ml
  Kinase: 1 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-MLK3 Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID   60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
 121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG  240
 241  PMEPLKSLFL KSPLGSWNGS GSGGGGGGGG GRPEGSPKAA GYANPVWTAL FDYEPSGQDE  300
 301  LALRKGDRVE VLSRDAAISG DEGWWAGQVG GQVGIFPSNY VSRGGGPPPC EVASFQELRL  360
 361  EEVIGIGGFG KVYRGSWRGE LVAVKAARQD PDEDISVTAE SVRQEARLFA MLAHPNIIAL  420
 421  KAVCLEEPNL CLVMEYAAGG PLSRALAGRR VPPHVLVNWA VQIARGMHYL HCEALVPVIH  480
 481  RDLKSNNILL LQPIESDDME HKTLKITDFG LAREWHKTTQ MSAAGTYAWM APEVIKASTF  540
 541  SKGSDVWSFG VLLWELLTGE VPYRGIDCLA VAYGVAVNKL TLPIPSTCPE PFAQLMADCW  600
 601  AQDPHRRPDF ASILQQLEAL EAQVLREMPR DSFHSMQEGW KREIQGLFDE LRAKEKELLS  660
 661  REEELTRAAR EQRSQAEQLR RREHLLAQWE LEVFERELTL LLQQVDRERP HVRRRRGTFK  720
 721  RSKLRARDG                                                         780
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: MLK3 fragment

MLK3 wt[1] Amino Acid Sequence

```
1    MEPLKSLFLK SPLGSWNGSG SGGGGGGGGG RPEGSPKAAG YANPVWTALF DYEPSGQDEL   60
61   ALRKGDRVEV LSRDAAISGD EGWWAGQVGG QVGIFPSNYV SRGGGPPPCE VASFQELRLE  120
121  EVIGIGGFGK VYRGSWRGEL VAVKAARQDP DEDISVTAES VRQEARLFAM LAHPNIIALK  180
181  AVCLEEPNLC LVMEYAAGGP LSRALAGRRV PPHVLVNWAV QIARGMHYLH CEALVPVIHR  240
241  DLKSNNILLL QPIESDDMEH KTLKITDFGL AREWHKTTQM SAAGTYAWMA PEVIKASTFS  300
301  KGSDVWSFGV LLWELLTGEV PYRGIDCLAV AYGVAVNKLT LPIPSTCPEP FAQLMADCWA  360
361  QDPHRRPDFA SILQQLEALE AQVLREMPRD SFHSMQEGWK REIQGLFDEL RAKEKELLSR  420
421  EEELTRAARE QRSQAEQLRR REHLLAQWEL EVFERELTLL LQQVDRERPH VRRRRGTFKR  480
481  SKLRARDGGE RISMPLDFKH RITVQASPGL DRRRNVFEVG PGDSPTFPRF RAIQLEPAEP  540
541  GQAWGRQSPR RLEDSSNGER RACWAWGPSS PKPGEAQNGR RRSRMDEATW YLDSDDSSPL  600
601  GSPSTPPALN GNPPRPSLEP EEPKRPVPAE RGSSSGTPKL IQRALLRGTA LLASLGLGRD  660
661  LQPPGGPGRE RGESPTTPPT PTPAPCPTEP PPSPLICFSL KTPDSPPTPA PLLLDLGIPV  720
721  GQRSAKSPRR EEEPRGGTVS PPPGTSRSAP GTPGTPRSPP LGLISRPRPS PLRSRIDPWS  780
781  FVSAGPRPSP LPSPQPAPRR APWTLFPDSD PFWDSPPANP FQGGPQDCRA QTKDMGAQAP  840
841  WVPEAGP                                                           900
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blue: MLK3 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002410.1

Recombinant Proteins