

## ProQinase™ MLK4 mitogen-activated protein kinase kinase kinase 21

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP3K21

Synonyms: KIAA1804

Product No.: 1078-0000-1

Lot: 002

**Description:** Human MLK4, internal fragment, amino acids A<sub>98</sub>-L<sub>451</sub> (as in [NCBI/Protein](#) entry NP\_115811.2), N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** MLK4 Lot 002, was confirmed as MLK4 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 68,091 Da

**Expression host:** Sf9 insect cells/E.coli

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

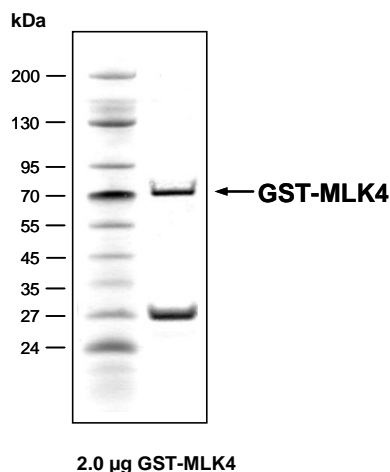
### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 28 pmol/µg × min  
ATP-K<sub>M</sub>: 2.5 µM

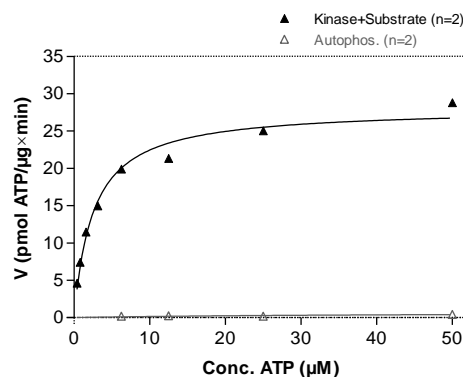
### Additional assay technology:

MLK4 Lot 002 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

### MLK4 Lot 002: Coomassie stain



### MLK4 Lot 002: 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: bio-lkBa, 8 µg/ml
  - MLK4: 1 µg/ml
- Filter binding assay  
MSPH membrane (Millipore)

# ProQinase™ MLK4

Product No.: 1078-0000-1

GST-MLK4 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	WPLQGQWQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RDS <b>LEVLFCG</b>	240
241	PLAMVAPCRP	<b>AASPAPPPSR</b>	<b>PSSPVHVAFE</b>	<b>RLELKEBIGA</b>	<b>GGFGQVYRAT</b>	<b>WQGQEVAVKA</b>	300
301	<b>ARQDPEQDAA</b>	<b>AAAESVREA</b>	<b>RLFAMLRHPN</b>	<b>I IELRGVCLQ</b>	<b>QPHLCVLVLEF</b>	<b>ARGGALNRAL</b>	360
361	<b>AAANAAPDPR</b>	<b>APGPRRRARI</b>	<b>PPHVLVNWAV</b>	<b>QIARGMLYLH</b>	<b>EEAFVPIILHR</b>	<b>DLKSSNILL</b>	420
421	<b>EKIEHDDICN</b>	<b>KTLKITDFGL</b>	<b>AREWHRTTKM</b>	<b>STAGTYAWMA</b>	<b>PEVIKSSLFS</b>	<b>KGSDIWSYGV</b>	480
481	<b>LLWELLTGEV</b>	<b>PYRGIDGLAV</b>	<b>AYGVAVNKLT</b>	<b>LPIPSTCPEP</b>	<b>FAKLMKECWQ</b>	<b>QDPHIRPSFA</b>	540
541	<b>LILEQLTAIE</b>	<b>GAVMTEMPQE</b>	<b>SFHSMQDDWK</b>	<b>LEIQQMFDL</b>	<b>RTKEKELRSR</b>	<b>EEELTRAA</b>	600

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: MLK4 fragment

MLK4 wt <sup>1</sup> Amino Acid Sequence							
1	MALRGAAGAT	DTPVSSAGGA	PGGSASSSST	SSGGSASAGA	GLWAALYDYE	ARGEDELSLR	60
61	RGQLVEVLSQ	DAAVSGDEGW	WAGQVQRRIG	IFPANYV <b>APC</b>	<b>RPAASPAPP</b>	<b>SRPSSPVHVA</b>	120
121	<b>FERLELKELEI</b>	<b>GAGGFGQVYR</b>	<b>ATWQGQEVAV</b>	<b>KAARQDPEQD</b>	<b>AAAAAESVRR</b>	<b>EARLFAMLRH</b>	180
181	<b>PNIIELRGVC</b>	<b>LQQPHLCVLV</b>	<b>EFARGGALNR</b>	<b>ALAAANAAPD</b>	<b>PRAPGPRRAR</b>	<b>RIPPHVLVNW</b>	240
241	<b>AVQIARGMLY</b>	<b>LHEEAFVPII</b>	<b>HRDLKSSNIL</b>	<b>LLEKIEHDDI</b>	<b>CNKTLKITDF</b>	<b>GLAREWHRTT</b>	300
301	<b>KMSTAGTYAW</b>	<b>MAPEVIKSSL</b>	<b>FSKGSDIWSY</b>	<b>GVLLWELLTG</b>	<b>EVPIRGIDGL</b>	<b>AVAYGVAVNK</b>	360
361	<b>LTLPIPISTCP</b>	<b>EPFAKLMKEC</b>	<b>WQDQPHIRPS</b>	<b>FALILEQLTA</b>	<b>IEGAVMTEMP</b>	<b>QESFHSMQDD</b>	420
421	<b>WKLEIQQMFD</b>	<b>ELRTKEKELR</b>	<b>SREEELTRAA</b>	<b>LQQKSQEELL</b>	<b>KRREQQLAER</b>	<b>EIDVLERELN</b>	480
481	ILIFQLNQEK	PKVKKRKGKF	KRSRLKLDKG	HRISLPSDFQ	HKITVQASPN	LDKRRSLNSS	540
541	SSSPSSPTM	MPRLRAIQLT	SDESNTWGR	NTVFRQEEFE	DVKNRNFKKK	CTWGPNSIQM	600
600	KDRTDCKERI	RPLSDGNSPW	STILIKNQKT	MPLASLFDVQ	PGSCEEPKLS	PDGLEHRKPK	660
661	QIKLPSQAYI	DLPLGKDAQR	ENPAEAESE	EAASANAATV	SIEMTPTNSL	SRSPQRKKT	720
721	SALYGCTVLL	ASVALGLDLR	ELHKAQAEE	PLPKKEKKK	EGIFQRASKS	RRSASPPTSL	780
781	PSTCGEASSP	PSLPLSSALG	ILSTPSFSTK	CLLQMDSEDP	LVDSAPVTC	SEMLTPDFCP	840
841	TAPGSGREPA	LMPRLDTDCS	VSRNLPSFL	QOTCGNVPYC	ASSKHRPSSH	RRTMSDGNPT	900
901	PTGATIIISAT	GASALPLCPS	PAPSHLPRE	VSPKKHSTVH	IVPQRRPASL	RSRSDLQAY	960
961	PQTAVSQAQ	TACVVGRPGP	HPTQFLAAKE	RTKSHVPSLL	DADVEGQSRD	YTVPLCRMRS	1020
1021	KTSRPSIYEL	EKEFLS					1080

**blue**: MLK4 sequence expressed in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_115811.2