

# Certificate of Analysis



## MYLK2

Myosin light chain kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: MYLK2

Synonyms: skMLCK; KMLC, MLCK

Product No.: 0445-0000-1

Lot: 001

**Description:** Human MYLK2, C/N/internal fragment, amino acids M<sub>1</sub>-V<sub>596</sub> (as in NCBI/Protein entry NP\_149109.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** MYLK2 Lot 001, was confirmed as MYLK2 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 95,209 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

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

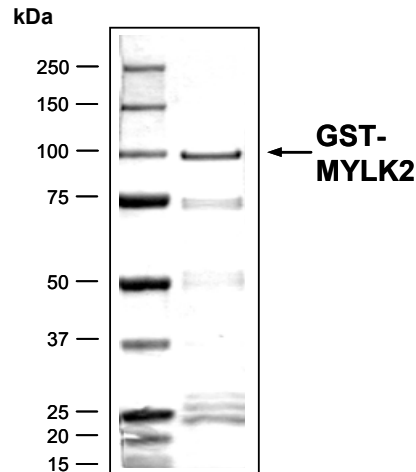
**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.154 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

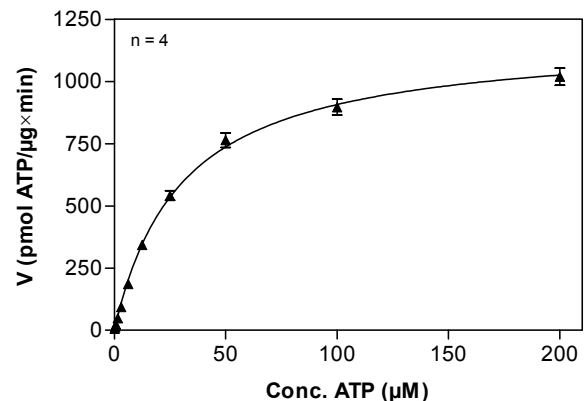
Specific kinase activity (P<sub>i</sub> transfer): 1180 pmol/µg×min  
ATP-K<sub>M</sub>: 30 µM

### MYLK2 Lot 001: Coomassie stain



2.0 µg GST-MYLK2

### MYLK2 Lot 001: 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: MLC-derived peptide (KKRPQRATSNVFS), 160 µg/ml
  - MYLK2: 1.0 µg / ml
- Filter binding assay
  - MSFC membrane (Millipore)

**Additional assay technology:** MYLK2 Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega

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## MYLK2

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MYLK2 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MGARGRMATE	NGAVELGIQN	PSTDKAPKGP	TGERPLAAGK	300
301	DPGPPDPKKA	PDPPTLKKDA	KAPASEKGDG	TLAQPSTSSQ	GPKGEGDRGG	GPAEGSAGPP	360
361	AALPQQTATP	ETSVKPKPAE	QGASGSQDPG	KPRVGKKAEE	GQAAARRGSP	AFLHSPSCPA	420
421	IISSEKLLA	KKPPSEASEL	TFEGVPMTHS	PTDPRPAKAE	EGKNILAESQ	KEVGEKTPGQ	480
481	AGQAKMQGDT	SRGIEFQAVP	SEKSEVGQAL	CLTAREEDCF	QILDCCPPPP	APFPHRMVEL	540
541	RTGNVSSEFS	MNSKEALGGG	KFGAVCTCME	KATGLKLAAK	VIKKQTPKDK	EMVLLIEVM	600
601	NQLNHRNLIQ	LYAAIETPHE	IVLFMEYIEG	GELFERIVDE	DYHLTEVDTM	VFVRQICDGI	660
661	LFMHKMRVLH	LDLKPENILC	VNTTGHVLKI	IDFGLARRYN	PNEKLVNFG	TPEFLSPEVV	720
721	NYDQISDKTD	MWSMGVITYM	LLSGLSPFLG	DDDTETLNNV	LSGNWYFDEE	TFEAVSDEAK	780
781	DFVSNLIVKD	QRARMNAAQC	LAHPWLNNLA	EKAKRCNRRL	KSQILLKKYL	MKRRWKKNFI	840
841	AVSAANRFKK	ISSSGALMAL	GV				900

1-218: GST **Red: HIS6-tag** **Pink:** Thrombin cleavage site **blue: MYLK2**

MYLK2 wt <sup>1</sup> amino acid sequence							
1	MATENGAVEL	GIQNPSTDKA	PKGPTGERPL	AAGKDPGPPD	PKKAPDPPTL	KDAKAPASE	60
61	KDGTTLAQPS	TSSQGPKEG	DRGGGPAEGS	AGPPAALPQQ	TATPETSCHK	PKAEQGASGS	120
121	QDPGKPRVGK	KAAEQAAAR	RGSPAFLHSP	SCPAAISSSE	KLLAKKPPSE	ASELTFEGVP	180
181	MTHSPTDPRP	AKAEEGKNIL	AESQKEVGEK	TPGQAGQAKM	QGDTSRGIEF	QAVPSEKSEV	240
241	GQALCLTARE	EDCFQILDCC	PPPPAPFPHR	MVELRTGNVS	SEFSMNSKEA	LGGGKFGAVC	300
301	TCMEKATGLK	LAAKVIKKQT	PKDKEMVLE	IEVMNQLNHR	NLIQLYAAIE	TPHEIVLFME	360
361	YIEGGELFER	IVDEDYHLTE	VDTMVFVRQI	CDGILFMHKM	RVLHLDLKPE	NILCVNTTGH	420
421	LVKIIDFGLA	RRYNPEKLE	VNFGTPEFLS	PEVVNYDQIS	DKTDMWSMGV	ITYMLLSGLS	480
481	PFLGDDDTET	LNNVLSGNWY	FDEETFEAVS	DEAKDFVSNL	IVKDQRARMN	AAQCLAHPLW	540
541	NNLAEKAKRC	NRRLKSQILL	KKYLMKRRWK	KNFIAVSAAN	RFKKISSSGA	LMALGV	600

**blue:** MYLK2 sequence expressed in fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_149109.1

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