

# Certificate of Analysis

## MELK

maternal embryonic leucine zipper kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: MELK

Synonyms: HPK38

Product No.: 0662-0000-1

Lot: 001

**Description:** Human MELK, N-terminal fragment, amino acids M<sub>1</sub>-A<sub>342</sub> (as in NCBI/Protein entry NP\_055606.1), untagged, expressed in Sf9 insect cells

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

**Theoretical MW**<sub>Fusion Protein</sub>: 38,852 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** By GST-Affinity Chromatography and proteolytic removal of the tag sequence

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

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 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:** 0.191 µg/µl

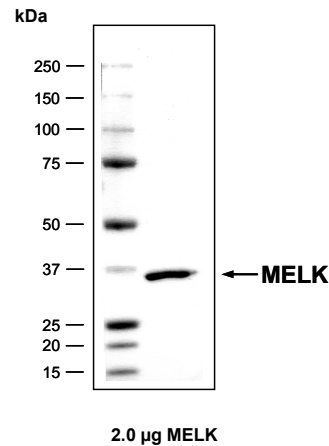
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

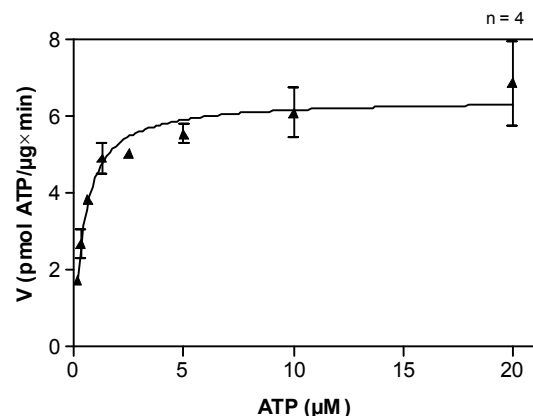
Specific kinase activity (P<sub>i</sub> transfer): 6.4 pmol/µg × min

ATP-K<sub>M</sub>: 0.44 µM

### MELK Lot 001: Coomassie stain



### MELK 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: RBER-CHKtide 100 µg/ml
  - Kinase: 2.0 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

### Additional assay technology: MELK Lot 001

was also successfully tested by ProQinase 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

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MELK Recombinant Fusion Protein Amino Acid Sequence							
1	GPLA	MKDYDE	LLKYELHET	IGTGGFAKVK	LACHILTGEM	VAIKIMDKNT LGSDLPRIKT	60
61	EIEALKNLRH	QHICQLYHVL	ETANKIFMVL	EYCPGGELFD	YIISQDRLSE	EETRNVFRQI	120
121	VSAVAVVHSQ	GYAHRDLKPE	NLLFDEYHKL	KLIDFGLCAK	PKGKNDYHLQ	TCCGSLAYAA	180
181	PELIQKSYL	GSEADVWSMG	ILLYVLMCGF	LPFDDDNVMA	LYKKIMRGKY	DVPKWLSPSS	240
241	ILLLQOMLQV	DPKKRISMKN	LLNHPWIMQD	YNYPVEWQSK	NPFIHLDDDC	VTLSVHHRN	300
301	NRQTMEDLIS	LWQYDHLTAT	YLLLLAKKAR	GKPVRLRLSS	FSCGQA		360

1-4: legacy from tag cleavage **blue**: MELK fragment **boxed**: variation from RefSeq

MELK wt <sup>1</sup> Amino Acid Sequence							
1	MKDYDELLKY	YELHETIGTG	GFAKVKLACH	ILTGEMVAIK	IMDKNTLGSD	LPRIKTEIEA	60
61	LKNLRHQHIC	QLYHVLETAN	KIFMVLEYCP	GGELFDYIIS	QDRLSEEETR	VVFRQIVSAV	120
121	AYVHSQGYAH	RDLKPENLLF	DEYHKLKLID	FGLCAKPKGN	KDYHLQTCGG	SLAYAAPELI	180
181	QGKSYLGSEA	DVWSMGILLY	VLMCGFLPFD	DDNVMALYKK	IMRGKYDVPK	WLSRSSILLL	240
241	QOMLQVDPKK	RISMKNLLNH	PWIMQDYNYP	VEWQSKNFFI	HLDDDCVTEL	SVHHRNNRQT	300
301	MEDLISLWQY	DHLTATYLLL	LAKKARGKPV	RLRLSSFSCG	QASATPFTDI	KSNNWSLEDV	360
361	TASDKNYVAG	LIDYDWCEDD	LSTGAATPRT	SQFTKYWTES	NGVESKSLTP	ALCRTPANKL	420
421	KNKENVYTPK	SAVKNEEYFM	FPEPKTPVNK	NQHKREILTT	PNRYTTPSKA	RNQLKETPI	480
481	KIPVNSTGTD	KLMTGVISPE	RRCRSVELDL	NQAHMEETPK	RKGAKVFGSL	ERGLDKVITV	540
541	LTRSKRKGSA	RDGPRRLKLH	YNVTTTRLVN	PDQLLNEIMS	ILPKKHVDFV	QKGYTLKCQT	600
600	QSDFGKVTMQ	FELEVCQLQK	PDVVGIRRQR	LKGDAWVYKR	LVEDILSSCK	V	660

**blue**: MELK sequence expressed in fusion protein

<sup>1</sup>NCBI/Protein accession number NP\_055606.1