Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ MARK3

MAP/microtubule affinity-regulating kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: MARK3

Synonyms: cTAK1, EMK2, KP78, PAR1A

Product No.: 0433-0000-1

Lot: 001

Description: MARK3, full length, amino acids M_1-L_{713} (as in NCBI/Protein entry NP_001122392.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MARK1 Lot 001, was confirmed as MARK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 109,443 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

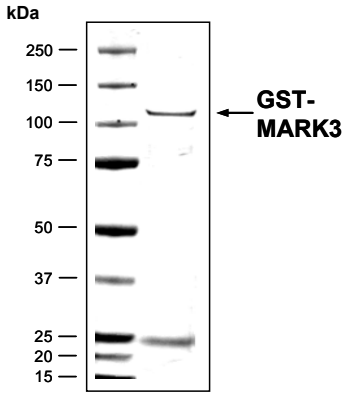
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

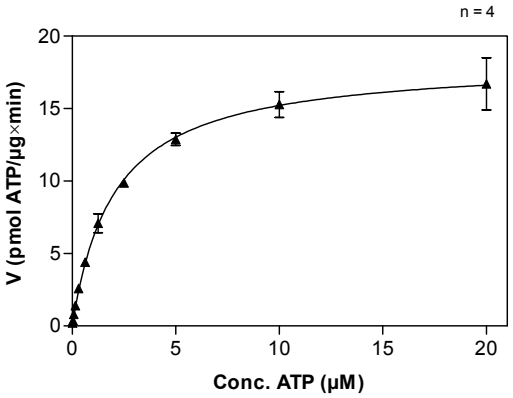
Biochemical Parameters:
Specific kinase activity (P_i transfer): 18 pmol/µg × min
ATP-K_M: 2 µM

MARK3 Lot 001:
Coomassie stain



2.0 µg GST-MARK3

MARK3 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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: Example#1 0.0 µg/ml
  Kinase: 1.0 µg/ml
- Filter binding assay
  MSFC/PH membrane (Millipore)

Additional assay technology: MARK3 Lot 001 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

REACTION BIOLOGY

ProQinase™ MARK3

Product No.: 0433-0000-1

MARK3 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI	240
241	LVPRGSPGLD GIYARQMSTR TPLPTVNERD TENHTSHGDG RQEVTSRTSR SGARCRNSIA	300
301	SCADEQPHIG NYRLLKTIGK GNFAKVKLAR HILTGREVAI KIIDKTQLNP TSLQKLFREV	360
361	RIMKILNHPN IVKLFEVIET EKTLYLIMEY ASGGEVFDYL VAHGRMKEKE ARSKFRQIVS	420
421	AVQYCHQKRI VHRDLKAENL LLDADMNIKI ADFGFSNEFT VGGKLDTFCG SPPYAAPELF	480
481	QGKKYDGPEV DVWSLGVILY TLVSGSLPFD GQNLKELRER VLRGKYRIPF YMSTDCENLL	540
541	KRFLVLNPIK RGTLEQIMKD RWINAGHEED ELKPFVEPEL DISDQKRIDI MVGMGYSQEE	600
601	IQESLSKMKY DEITATYLLL GRKSSEVRPS SDLNNSTGQS PHHKVQRSVS SSQKQRRYSD	660
661	HAGPAIPSVV AYPKRSQTST ADSDLKEDGI SSRKSSGSAV GGKGIAPASP MLGNASNPNK	720
721	ADIPERKKSS TVPSSNTASG GMTRRNTYVC SERTTADRHS VIQNGKENST IPDQRTPVAS	780
781	THSISSAATP DRIRFPRGTA SRSTFHGQPR ERRTATYNGP PASPSLSHEA TPLSQTRSRG	840
841	STNLFSKLTS KLTRSRNVSA EQKDENKEAK PRSLRFTWSM KTTSSMDPGD MMREIRKVLD	900
901	ANNCDYEQRE RFLLFCVHGD GHAENLVQWE MEVCKLPRLS LNGVRFKRIS GTSIAFKNIA	960
961	SKIANELKL	1020

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue**:MARK3

MARK3 wt[1] Amino Acid Sequence

1	MSTRTPLPTV NERDTENHTS HGDGRQEVTS RTSRSGARCR NSIASCADEQ PHIGNYRLLK	60
61	TIGKGNFAKV KLARHILTGR EVAIKIIDKT QLNPTSLQKL FREVRIMKIL NHPNIVKLFE	120
121	VIETEKTLYL IMEYASGGEV FDYLVAHGRM KEKEARSKFR QIVSAVQYCH QKRIVHRDLK	180
181	AENLLLDADM NIKIADFGFS NEFTVGGKLD TFCGSPPYAA PELFQGKKYD GPEVDVWSLG	240
241	VILYTLVSGS LPFDGQNLKE LRERVLRGKY RIPFYMSTDC ENLLKRFLVL NPIKRGTLEQ	300
301	IMKDRWINAG HEEDELKPFV EPELDISDQK RIDIMVGMGY SQEEIQESLS KMKYDEITAT	360
361	YLLLGRKSSE VRPSSDLNNS TGQSPHHKVQ RSVSSSQKQR RYSDHAGPAI PSVVAYPKRS	420
421	QTSTADSDLK EDGISSRKSS GSAVGGKGIA PASPMLGNAS NPNKADIPER KKSSTVPSSN	480
481	TASGGMTRRN TYVCSERTTA DRHSVIQNGK ENSTIPDQRT PVASTHSISS AATPDRIRFP	540
541	RGTASRSTFH GQPRERRTAT YNGPPASPSL SHEATPLSQT RSRGSTNLFS KLTSKLTRSR	600
601	NVSAEQKDEN KEAKPRSLRF TWSMKTTSSM DPGDMMREIR KVLDANNCDY EQRERFLLFC	660
661	VHGDGHAENL VQWEMEVCKL PRLSLNGVRF KRISGTSIAF KNIASKIANE LKL	720

blue: MARK3 sequence expressed in fusion protein

[1]NCBI/Protein accession number NP_001122392.2

Recombinant Proteins