Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ TSK2

testis specific serine kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: TSSK2

Synonyms: STK22B; SPOGA2

Product No.: 0220-0000-1

Lot: 002

Description: Human TSK2, full length, amino acids M_1-T_{358} (as in NCBI/Protein entry NP_443732.3), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: TSK2 Lot 002, was confirmed as TSK2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 73,916 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

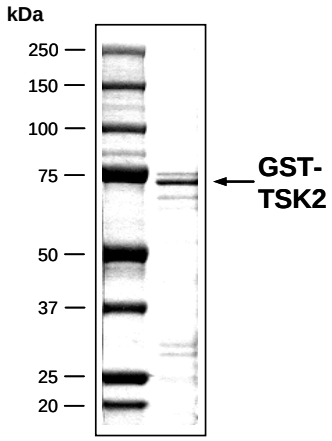
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

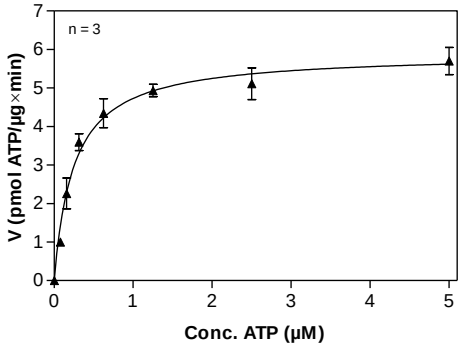
Biochemical Parameters:
Specific kinase activity (P_i transfer): 5.9 pmol/µg × min
ATP-K_M: 0.2 µM

TSK2 Lot 002:
Coomassie stain



SP002, 2.0 µg

TSK2 Lot 002:
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: Histone H2A, 50 µg/ml
  TSK2: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
TSK2 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

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GST-TSK2 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 GIYARGIQAS MGARGRQCDG YLQNSPLMDD ATVLRKKGYI VGINLGKGSY	300
301	AKVKSAYSER LKFNVAVKII DRKKTPTDFV ERFLPREMDI LATVNHGSII KTYEIFETSD	360
361	GRIYIIMELG AQGDLLEFIK CQGALHEDVA RKMFRQLSSA VKYCHDLDIV HRDLKCENLL	420
421	LDKDFNIKLS DFGFSKRCLR DSNGRIILSK TFCGSAAYAA PEVLQSIPYQ PKVYDIWSLG	480
481	VILYIMVCGS MPYDDSDIRK MLRIQKEHRV DFPRSKNLTC ECKDLIYRML QPDVSQRLHI	540
541	DEILSHSWLQ PPKPKATSSA SFKREGEGKY RAECKLDTKT GLRPDHRPDH KLGAKTQHRL	600
601	LVVPENENRM EDRLAETSRA KDHHISGAEV GKASTKGEFQ HTGGRY	660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: TSK2 **boxed**: variation from RefSeq

TSK2 wt[1] Amino Acid Sequence

1	MDDATVLRKK GYIVGINLGK GSYAKVKSAY SERLKFNVAV KIIDRKKTPT DFVERFLPRE	60
61	MDILATVNHG SIIKTYEIFE TSDGRIYIIM ELGVQGDLLE FIKCQGALHE DVARKMFRQL	120
121	SSAVKYCHDL DIVHRDLKCE NLLLDKDFNI KLSDFGFSKR CLRDSNGRII LSKTFCGSAA	180
181	YAAPEVLQSI PYQPKVYDIW SLGVILYIMV CGSMPYDDSD IRKMLRIQKE HRVDFPRSKN	240
241	LTCECKDLIY RMLQPDVSQR LHIDEILSHS WLQPPKPKAT SSASFKREGE GKYRAECKLD	300
301	TKTGLRPDHR PDHKLGAKTQ HRLLVVPENE NRMEDRLAET SRAKDHHISG AEVGKAST	360

blue: TSK2 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_443732.3

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