Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ SGK1

serum/glucocorticoid regulated kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: SGK1

Synonyms: SGK

Product No.: 0199-0000-2

Lot: 005

Description: Human SGK1, full length, amino acids M_1-L_{431} (as in NCBI/Protein entry NP_005618.2), activated, N-terminal GST fusion protein, expressed in Sf9 insect cells

Product identity: SGK1 Lot 005 was confirmed as SGK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 74,950 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

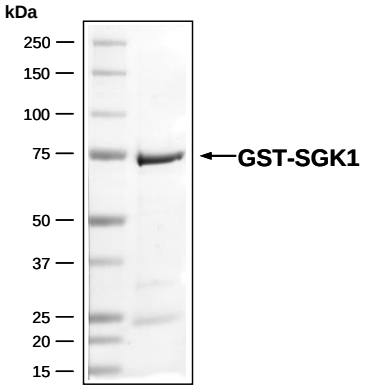
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

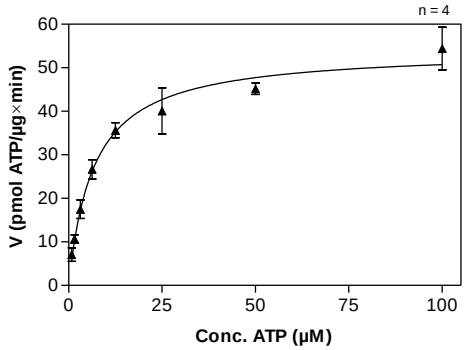
Biochemical Parameters:
Specific kinase activity (P_i transfer): 54 pmol/µg × min
ATP-K_M: 6.7 µM

SGK1 Lot 005:
Coomassie stain



500 ng GST-SGK1

SGK1 Lot 005:
Determination of V_{max} and K_M value for ATP



- 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: GSK3-derived peptide, 40 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
SGK1 Lot 005 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-SGK1 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 PGIQ**MTVKTE AAKGTLTYSR**	240
241	**MRGMVAILIA FMKQRRMGLN DFIQKIANNS YACKHPEVQS ILKISQPQEP ELMNANPSPP**	300
301	**PSPSQQINLG PSSNPHAKPS DFHFLKVIGK GSFGKVLLAR HKAEEVFYAV KVLQKKAILK**	360
361	**KKEEKHIMSE RNVLLKNVKH PFLVGLHFSF QTADKLYFVL DYINGGELFY HLQRERCFLE**	420
421	**PRARFYAAEI ASALGYLHSL NIVYRDLKPE NILLDSQGHI VLTDFGLCKE NIEHNSTTST**	480
481	**FCGTPEYLPE VLHKQPYDRT VDWWCLGAVL YEMLYGLPPF YSRNTAEMYD NILNKPLQLK**	540
541	**PNITNSARHL LEGLLQKDRT KRLGAKDDFM EIKSHVFFSL INWDDLINKK ITPPFNPNVS**	600
601	**GPNDLRHFDP EFTEEPVPNS IGKSPDSVLV TASVKEAAEA FLGFSYAPPT DSFL**	660

1-218: GST **blue**: SGK1

SGK1 wt[1] Amino Acid Sequence

1	**MTVKTEAAKG TLTYSRMRGM VAILIAFMKQ RRMGLNDFIQ KIANNSYACK HPEVQSILKI**	60
61	**SQPQEPELMN ANPSPPPSPS QQINLGPSSN PHAKPSDFHF LKVIGKGSFG KVLLARHKAE**	120
121	**EVFYAVKVLQ KKAILKKKEE KHIMSERNVL LKNVKHPFLV GLHFSFQTAD KLYFVLDYIN**	180
181	**GGELFYHLQR ERCFLEPRAR FYAAEIASAL GYLHSLNIVY RDLKPENILL DSQGHIVLTD**	240
241	**FGLCKENIEH NSTTSTFCGT PEYLAPEVLH KQPYDRTVDW WCLGAVLYEM LYGLPPFYSR**	300
301	**NTAEMYDNIL NKPLQLKPNI TNSARHLLEG LLQKDRTKRL GAKDDFMEIK SHVFFSLINW**	360
361	**DDLINKKITP PFNPNVSGPN DLRHFDPEFT EEPVPNSIGK SPDSVLVTAS VKEAAEAFLG**	420
421	**FSYAPPTDSF L**	480

blue: SGK1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005618.2

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