Certificate of Analysis

ProQinase
TARGETING CANCER

SGK3

Serum/glucocorticoid regulated kinase-like

Recombinant Human Active Protein Kinase

HGNC Symbol: SGK3

Synonyms: CISK, SGKL, SGK2

Product No.: 0198-0000-2

Lot: 004

Description: Human SGK3, full length, amino acids M_1-L_{496} (as in NCBI/Protein entry NP_037389.4), activated, N-terminal GST-fusion protein, expressed in Sf9 insect cells

Product identity: SGK3 Lot 004, was confirmed as SGK3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 83,186 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: With PDK1 (GenBank entry NM_002613)

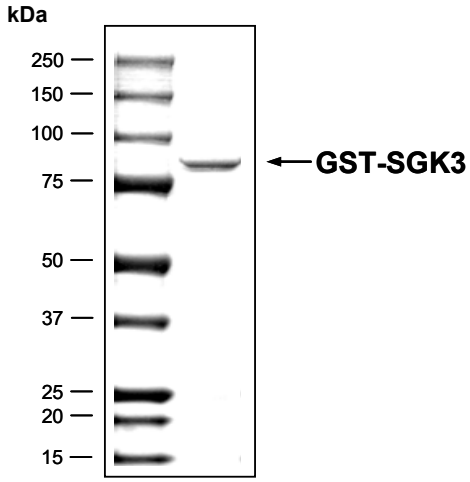
Storage buffer: 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

Protein concentration: 0.201 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

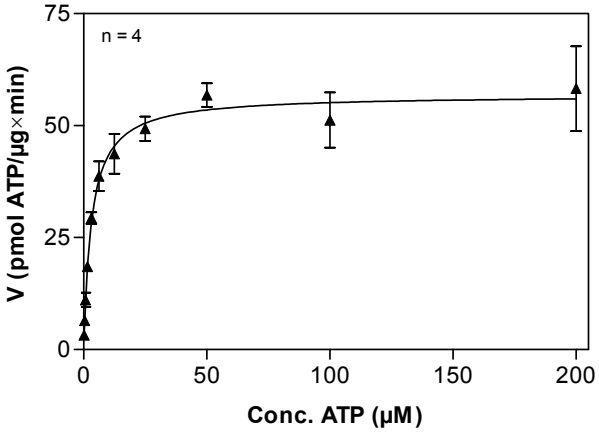
Biochemical Parameters:
Specific kinase activity (P_i transfer): 57 pmol/µg×min
ATP-K_M: 3.1 µM

SGK3 Lot 004:
Coomassie stain



2.0 µg GST-SGK3

SGK3 Lot 004:
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: GSK3-derived peptide (R11-SGRARTSSFAEPGGK), 100 µg / ml
  SGK3: 4.0 µg / ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology: SGK3 Lot 004 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega

ProQinase GmbH • Breisacher Str. 117 • D-79106 Freiburg • Germany • www.proqinase.com • info@proqinase.com

SGK3

Product No.: 0198-0000-2

SGK3 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 PGIQMQRDHT MDYKESCPSV	240
241	SIPSSDEHRE KKKRFTVYKV LVSVGRSEWF VFRRYAEFDK LYNTLKKQFP AMALKIPAKR	300
301	IFGDNFDPDF IKQRRAGLNE FIQNLVRYPE LYNHPDVRAF LQMDSPKHQS DPSEDEDERS	360
361	SQKLHSTSQN INLGPSGNPH AKPTDFDFLK VIGKGSFGKV LLAKRKLDGK FYAVKVLQKK	420
421	IVLNRKEQKH IMAERNVLLK NVKHPFLVGL HYSFQTTEKL YFVLDFVNGG ELFFHLQRER	480
481	SFPEHRARFY AAEIASALGY LHSIKIVYRD LKPENILLDS VGHVVLTDFG LCKEGIAISD	540
541	TTTTFCGTPE YLAPEVIRKQ PYDNTVDWWC LGAVLYEMLY GLPPFYCRDV AEMYDNILHK	600
601	PLSLRPGVSL TAWSILEELL EKDRQNRLGA KEDFLEIQNH PFFESLSWAD LVQKKIPPPF	660
661	NPNVAGPDDI RNFDTAFTEE TVPYSVCVSS DYSIVNASVL EADDAFVGFS YAPPSEDLFL	720

1-218: GST **blue**: **SGK3** **boxed**:variation from RefSeq

SGK3 wt[1] amino acid sequence

1	MQRDHTMDYK ESCPSVSIPS SDEHREKKKR FTVYKVLVSV GRSEWFVFRR YAEVDKLYNT	60
61	LKKQFPAMAL KIPAKRIFGD NFDPDFIKQR RAGLNEFIQN LVRYPELYNH PDVRAFLQMD	120
121	SPKHQSGPSE DEDERSSQKL HSTSQNINLG PSGNPHAKPT DFDFLKVIGK GSFGKVLLAK	180
181	RKLDGKVYAV KVLQKKIVLN RKEQKHIMAE RNVLLKNVKH PFLVGLHYSF QTTEKLYFVL	240
241	DFVNGGELFF HLQRERSFPE HRARFYAAEI ASALGYLHSI KIVYRDLKPE NILVDSVGHV	300
301	VLTDFGLCKE GIAISDTTTT FCGTPEYLAP EVIRKQPYDN TVDWWCLGAV LYEMLYGLPP	360
361	FYCRDVAEMY DNILHKPLSL RPGVSLRAWS ILEELLEKDR QNRLGAKEDF LEIQNHPFFE	420
421	SLSWADLVQK KIPPPFNPNV AGPDDIRNFD TAFTEETVPY SVCVSSDYSI VNASVLEADD	480
481	AFVGFSYAPP SEDLFL	540

blue: SGK3 sequence expressed in fusionprotein **Red**: variant in fusionprotein

[1]NCBI/Protein accession number NP_037389.4
G127D; V187F: documented sequence conflict, see UniProt Q96BR1/ENA entry AAF27051