

# Certificate of Analysis

## SIK3

Salt-inducible kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: SIK3

Synonyms: L19, QSK, SIK-3

Product No.: 1340-0000-1

Lot: 003

**Description:** Human SIK3, N-terminal fragment, amino acids M<sub>1</sub>-L<sub>300</sub> (as in NCBI/Protein entry AAI28511.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** SIK3 Lot 003 was confirmed as SIK3 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 68,476 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, 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:** 0.090 µg/µl

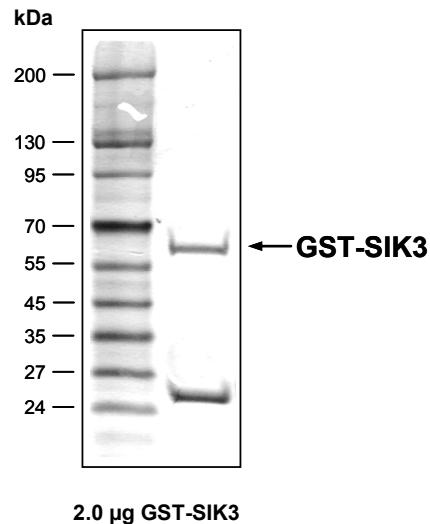
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

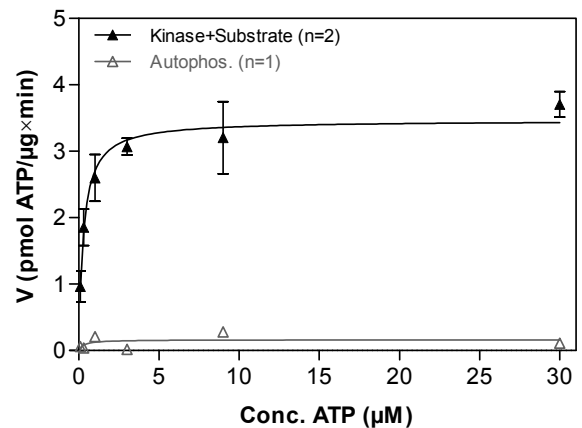
Specific kinase activity (P<sub>i</sub> transfer): 3.5pmol/µg×min

ATP-K<sub>M</sub>: 0.3 µM

**SIK3 Lot 003:  
Coomassie stain**



**SIK3 Lot 003:  
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, 80 µg/ml
  - SIK3: 1.0 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

**Additional assay technology:** SIK3 Lot 003

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

## SIK3

Product No.: 1340-0000-1

SIK3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLFGQ	240
241	PLAMLM PARI	GYEIDRTIG	KNFAVVKRA	THLVTKAKVA	IKIIDKTQLD	EENLKKIFRE	300
301	VQIMKMLCHP	HIIRLYQVME	TERMIYLVTE	YASGGEIFDH	LVAHGRMAEK	EARRKFQIV	360
361	TAVYFCHCRN	IVHRDLKAEN	LLLDANLNK	IADFGFSNLF	TPGQLLKTWC	GSPPYAAP EL	420
421	FEGKEYD GPK	VDIWSLGVVL	YVLVCGALPF	DGSTLQNLRA	RVLSGKFRIP	FFMSTECEHL	480
481	IRHMLVLDPN	KRLSMEQICK	HKWMKLG DAD	PNFDRLIAEC	QQLKEERQVD	PLNEDVLLAM	540
541	EDMGL						600

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:** SIK3 fragment

SIK3 wt <sup>1</sup> Amino Acid Sequence							
1	MPARIGYYEI	DRTIGKGNFA	VVKRATHLVT	KAKVAIKIID	KTQLDEENLK	KIFREVQIMK	1
61	MLCHPHIIRL	YQVMETERMI	YLVTEYASGG	EIFDHLVAHG	RMAEKEARRK	FKQIVTAVYF	61
121	CHCRNIVHRD	LKAENLLLLDA	NLNKIADFG	FSNLFTPGQL	LKTWCGSPPY	AAPELFEGKE	121
181	YDGPKVDIWS	LGVVLYVLVC	GALPFDGSTL	QNLRARVLSG	KFRIPFFMST	ECEHLIRHML	181
241	VLDPNKRLSM	EQICKHKWMK	LG DADPNFDR	LIAECQQLKE	ERQVDPLNED	VLLAMEDMGL	241
301	DKEQTLQAEQ	AGTAMNISVP	QVQLINPENQ	IVEPDGTLNL	DSDEGEESP	EALVRYLSMR	301
361	RHTVG VADPR	TEVMEDLQKL	LPGFPGVNPQ	APFLQVAPNV	NFMHNLLPMQ	NLQPTGQLEY	361
421	KEQSL LQPPT	LQLLNGMGPL	GRRASDGGAN	IQLHAQQLLK	RPRGSPPLVT	MTPAVPAVTP	421
481	VDEESSDGEP	DQEA VQRYLA	NRSKRHTLAM	TNPTAEIPPD	LQRQLGQQPF	RSRVWPPHLV	481
541	PDQHRSTYKD	SNTLHLPTER	FSPVRRFSDG	AASIQAFKAH	LEKMGNNSSI	KQLQQECEQL	541
601	QKMYGGQIDE	RTLEKTQQQH	MLYQQEQHHQ	ILQQQIQDSI	CPPQSPPLQ	AACENQPALL	601
661	THQLQRLRIQ	PSSPPP NHPN	NHLFRQPSNS	PPPMSSAMIQ	PHGAASSSQF	QGLPSRSAIF	661
721	QQQPENCSSP	PVALTCLGM	QQPAQSQQVT	IQVQEPVDML	SNMPGTAAGS	SGRGISISPS	721
781	AGQMOMQHRT	NLMATLSYGH	RPLSKQLSAD	SAEAHSAHQ	PPHYTT SALQ	QALLSPTPPD	781
841	YTRHQQVPHI	LQGLLSPRHS	LTGHSDIRLP	PTEFAQLIKR	QQQQRQQQQQ	QQQQQEYQEL	841
901	FRHMNQGDAG	SLAPSLGGQS	MTERQALS YQ	NADSYHHHTS	PQHLLQIRAQ	ECVSQASSPT	901
961	PPHGYAHQPA	LMHSESMEED	CSCEGAKDGF	QDSKSSSTLT	KGCHDSPLLL	STGGPGDPES	961
1021	LLGTVSHAQE	LGIHPYGHQP	TAAFSKNKVP	SREP VIGNCM	DRSSPGQAVE	LPDHNGLGYP	1021
1081	ARPSVHEHHR	PRALQRHHTI	QNSDDAYVQL	DNLPGMSLVA	GKALSSARMS	DAVLSQSSLM	1081
1141	GSQQFQDGEN	EECGASLGGH	EHPDLS DGSQ	HLNSSCYPST	CITDILLSYK	HPEVFSFMEQ	1141
1201	AGV						1201

**blue:** SIK3 sequence expressed in fusionprotein

<sup>1</sup>NCBI/Protein accession number AAI28511.1