

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SIK3 [2 - 1369]

<u>Enzyme description:-</u>	SIK3 [2 – 1369]
<u>Clone number:-</u>	DU 16623
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST and HA
<u>Purification method:-</u>	Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic	178, 221.87 daltons
Average Mass	178, 334.97 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 6.45

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF.

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

KKKVSRLGLYRSPSPENLNRPR Final concentration: 300 μ M

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Clone Data Sheet

SIK3 [2 - 1369]

Protein SIK3 [2 - 1369]

Clone number DU 16623

Species Human

Accession number Sugen Kinase Database 15721

Tags N-terminal GST and HA

Baculovirus expressed protein MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSYPYDVPDYAAAAAA
SGAGGAAGAGTGGAGPAGRLLPPPAPGSPAAPAAVSPAAGQPRPPAPAS
RGMPARIGYYEIDRTIGKGNFAVVKRATHLVTKAKVAIKIIDKTQLDE
ENLKKIFREVQIMKMLCHPHIIRLYQVMETERMIYLVTEYASGGEIFDH
LVAHGRMAEKEARRKFKQIVTAVYFCHCRNIVHRDLKAENLLLDANLNI
KIADFGFSNLFTPGQLLKTWCGSPPYAAPELFEGKEYDGPKVDIWSLGV
VLYVLVCGALPFDGSTLQNLRARVLSGKFRIFFFMSTECEHLIRHMLVL
DPNKRLSMEQICKHKWMKLGADPNFDRLIAECQQLKEERQVDPLNEDV
LLAMEDMGLDKEQTLQSLRSDAYDHYS AIYSLLCDRHKRHKT LRLGALP
SMPRALAFQAPVNIQAEQAGTAMNISVPQVQLINPENQIVEPDGTLNLD
SDEGEEPSPEALVRYLSMRRTVGVADPRTEVMEDLQKLLPGFPGVNPQ
APFLQVAPNVNFMHNLLPMQNLQPTGQLEYKEQSLLQPPTLQLLNGMGP
LGRRASDGGANIQLHAQQLKRPRGPSPLVTMTPAVPAVTPVDEESSDG
EPDQEAVQRYLANRSKRHTLAMTNPTAEIPPD LQRQLGQQPFRSRVWPP
HLVPDQHRSTYKDSNTLHLPTERFSPVRRFSDGAASIQAFKAHLEKMGN
NSSIKQLQQECEQLQKMYGGQIDERTLEKTQQQHMLYQQEQHHQILOQQ
IQDSICPPQPSPPLQAACENQPALLTHQLQRLRIQPSSPPPNHPNNHLF
RQPSNSPPPMSSAMIQPHGAASSSQFQGLPSRSAIFQQQPENCSSPPNV
ALTCLGMQQPAQSQQVTIQVQEPVDMLSNMPGTAAGSSGRGISISPSAG
QMOMQHRTNLMATLSYGHRPLSKQLSADSAEAHSLNVNRFSPAN YDQAH
LHPHLFSDQSRGSPSSYSPSTGVGFSPTQALKVPPLDQFPTFPSSAHQQ
PPHYTT SALQQALLSPTPPDYTRHQQVPHILOGLLSPRHSLTGHS D IRL
PPTEFAQLIKRQQQQRQQQQQQQQQQQEQELFRHMNQGDAGSLAPSLGG
QSMTERQALSYQNADSYHHHTSPQHLLQIRAQECVSQASSPTPPHGYAH
QPALMHSESMEEDCCEGAKDGFQDSKSSSTLTGCHDSPLLLSTGGPG
DPESLLGTVSHAQELGIHPYGHQPTAAFSKNKVPSREPVIGNCMDRSSP
GQAVELPDHNLGYPARPSVHEHHRPRALQRHHTIQNSSDDAYVQLDNLP
GMSLVAGKALSSARMSDAVLSSQSSLMGSSQQFQDGENEECGASLGGHEHP
DLSDGSQHLNSSCYPSTCITDILLSYKHPEVSFSMEQAGV

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<u>Native sequence</u>	Amino acids A2 – V1369 (end) of human SIK3. Residue A241 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag is located at residues 232 – 240.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 sites in pFastBAC GST
<u>Nucleotide sequence of insert</u>	<pre>ggatcctaccatacgaatgtgccagattacgccGCGGCGGCGGCGGCGA GCGGAGCTGGCGGGGCTGCCGGGGCCGGGACTGGGGGAGCCGGGCCCCG GGCCCGCCTGCTGCCTCCGCCCCGCGCCGGGGTCCCCAGCCGCCCCCGCT GCCGTGTCCCCTGCGGCCGGCCAGCCGCGTCCCCCAGCCCCGGCCTCCC GCGGACCCATGCCCCGCCGTATCGGCTACTACGAGATCGACCGCACCAT CGGCAAGGGCAACTTCGCGGTGGTCAAGCGGGCCACGCACCTCGTCACC AAGGCCAAGGTTGCTATCAAGATCATAGATAAGACCCAGCTGGATGAAG AAAACCTGAAGAAGATTTTCCGGGAAGTTCAAATTATGAAGATGCTTTG CCACCCCATATCATCAGGCTCTACCAGGTTATGGAGACAGAACGGATG ATTTATCTGGTGACAGAATATGCTAGTGAGGGGAAATATTTGACCACC TGGTGGCCCATGGTAGAATGGCAGAAAAGGAGGCACGTCGGAAGTTCAA ACAGATCGTCACAGCTGTCTATTTTTGTCACTGTTCGGAACATTGTTTCAT CGTGATTTAAAAGCTGAAAATTTACTTCTGGATGCCAATCTGAATATCA AAATAGCAGATTTTGGTTTTCAGTAACCTCTTCACTCCTGGGCAGCTGCT GAAGACCTGGTGTGGCAGCCCTCCCTATGCTGCACCTGAACTCTTTGAA GGAAAAGAATATGATGGGCCCAAAGTGGACATCTGGAGCCTTGGAGTTG TCCTCTACGTGCTTGTGTGCGGTGCCCTGCCATTTGATGGAAGCACACT GCAGAATCTGCGGGCCCGCGTGCTGAGTGGAAGTTCCGCATCCCATTT TTTATGTCCACAGAATGTGAGCATTTGATCCGCCATATGTTGGTGTAG ATCCCAATAAGCGCCTCTCCATGGAGCAGATCTGCAAGCACAAAGTGGAT GAAGCTAGGGGACGCCGATCCCAACTTTGACAGGTTAATAGCTGAATGC CAACAACTAAAGGAAGAAAGACAGGTGGACCCCTGAATGAGGATGTCC TCTTGGCCATGGAGGACATGGGACTGGACAAAGAACAGACACTGCAGTC ATTAAGATCAGATGCCTATGATCACTATAGTGCAATCTACAGCCTGCTG TGTGATCGACATAAGAGACATAAAACCCTGCGTCTCGGAGCACTTCCTA GCATGCCCCGAGCCCTGGCCTTTCAAGCACCAGTCAATATCCAGGCGGA GCAGGCAGGTACTGCTATGAACATCAGCGTTCCCCAGGTGCAGCTGATC AAGCCAGAGAACCAAATTGTGGAGCCGGATGGGACACTGAATTTGGACA GTGATGAGGGTGAAGAGCCTTCCCCTGAAGCATTGGTGCCTATTTGTC AATGAGGAGGCACACAGTGGGTGTGGCTGACCCACGCACGGAAGTTATG GAAGATCTGCAGAAGCTCCTACCTGGCTTTCCTGGAGTCAACCCCGAGG CTCCATTCTTGCAGGTGGCCCCAATGTGAACTTCATGCACAACCTGTT GCCTATGCAAACTTGCAACCAACCGGGCAACTTGAGTACAAGGAGCAG TCTCTCCTACAGCCGCCACGCTACAGCTGTTGAATGGAATGGGCCCCC TTGGCCGGAGGGCATCAGATGGAGGAGCCAACATCCAACCTGCATGCCCA GCAGCTGCTGAAGCGCCACGGGGACCTCTCCGCTTGTACCATGACA</pre>

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CCAGCAGTGCCAGCAGTTACCCCTGTGGACGAGGAGAGCTCAGACGGGG
AGCCAGACCAGGAAGCTGTGCAGAGGTACTTGGCAAATAGGTCCAAAAG
ACATACACTGGCCATGACCAACCCTACAGCTGAGATCCCACCGGACCTA
CAACGGCAGCTAGGACAGCAGCCTTTCCGTTCCCGGGTCTGGCCTCCTC
ACCTGGTACCTGATCAGCATCGCTCTACCTACAAGGACTCCAACACTCT
GCACCTCCCTACGGAGCGTTTCTCCCCTGTGCGCCGGTCTCAGATGGG
GCTGCGAGCATCCAGGCCTTCAAAGCTCACCTGGAAAAAATGGGCAACA
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GGCAGCCCAGTAATAGTCCTCCCCCATGAGCAGTGCCATGATCCAGCC
TCACGGGGCTGCATCTTCTTCCAGTTTCAAGGCTTACCTTCCCGCAGT
GCAATCTTTCAGCAGCAACCTGAGAACTGTTCTCTCTCCCAACGTGG
CACTAACCTGCTTGGGTATGCAGCAGCCTGCTCAGTCACAGCAGGTCAC
CATCCAAGTCCAAGAGCCTGTTGACATGCTCAGCAACATGCCAGGCACA
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AGAGCATGACAGAGCGCCAGGCTTTATCTTATCAAAATGCTGACTCTTA
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ACCCTGAATCTTTGCTAGGAACTGTGAGTCATGCCCAAGAATTGGGGAT
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TGCAGTTCTCAGTCAGTCTTCGCTCATGGGCAGCCAGCAGTTTCAGGAT
GGGGAAAATGAGGAATGTGGGGCAAGCCTGGGAGGTCATGAGCACCCAG
ACCTGAGTGATGGCAGCCAGCATTTAACTCCTCTTGCTATCCATCTAC
GTGTATTACAGACATTCTGCTCAGCTACAAGCACCCCGAAGTCTCCTTC

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AGCATGGAGCAGGCAGGCGTGtaaaagccgaattccagcacactggcgg
ccgttactagtggatcc