

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SIK2 [2 - 926]

<u>Enzyme description:-</u>	SIK2 [2 – 926]
<u>Clone number:-</u>	DU 16624
<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	132,276.71 daltons
Average Mass	132,361.09 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.64

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

SIK2 [2 - 926]

Protein SIK2 [2 - 926]

Clone number DU 16624

Species Human

Accession number NM_015191.1

Tags N-terminal GST and HA

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPNSRVDYPYDVDPDY
AVMADGPRHLQRGPV RVGFYDIEGTLGKGNFAVVKLGRHRITKTEVAIK
IIDKSQLD AVNLEKIYREVQIMKMLDHPHI IKLYQVMETKSM LYLVT EY
AKNGE I FDYLANHGR LNESEARRKFWQILSAVDYCHGRKIVHRDLKAEN
LLLDNNMNIKIADFGFGNFFKSGELLATWCGSPPYAAPEVFEGQQYEGP
QLDIWSMGVVLYVLVCGALPFDGPTLPILRQRVLEGRFRIPYFMSEDCE
HLIRRMVLVDPSKRLTIAQIKEHKWMLIEVPVQRPVLYPQEQENEPSIG
EFNEQVLRLMHS LGIDQQKTIESLQNKSYNHFAAIYFLLVERLKSHRSS
FPVEQRLDGRQRRPSTIAEQTVAKAQTVGLPVTMHSPNMRLLSALLPQ
ASNVEAFSFPASGCQAEAAFMEEECV DTPK VNGCLLDPVPPVLVRKGCQ
SLPSNMMETSIDEGLETEGEAEEDPAHAFAEFQSTRSGQRRHTLSEVTN
QLVVM PGAGKIFSMNDSPSLDSVDSEYDMG SVQRDLN FLEDNPSLKDIM
LANQPSPRMTSPFISLRPTNPAMQALSSQKREVNHRSPVSFREGRRASD
TSLTQGI VAFRQHLQNLARTKGILELNKVQLLYEQIGPEADPNLAPAAP
QLQDLASSCPQEEVSQQQESVSTLPASVHPQLSPRQSLETQYLQHRLQK
PSLLSKAQNTCQLYCKEPPRSLEQQLOEHRLQQKRLFLQKQSQLOAYFN
QMQUIAESSYPQPSQQLPLPRQETPPPSQQAPPFSLTQPLSPVLEPSSEQ
MQYSPFLSQYQEMQLQPLPSTSGPRAAPPLPTQLQQQQPPPPPPPPPPR
QPGAAPAPLQFSYQTCELPSAASPAPDYPTPCQYPVDGAQQSDLTGPDC
PRSPGLQEAPSSYDPLALSELPGLFDC EMLDAVDPQHNGYVLVN

Native sequence Amino acids V2 – N926 (end) of human SIK2.
Residue V247 of the fusion protein is equivalent to V2 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag is located at residues 238 – 246.

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Protease cleavage

PreScission (LEVLFQGP) residues 221 - 229

Cloning sites

*Sal*I and *Not*I sites in pFastBAC GST

Nucleotide sequence of insert

gtcgactacccatacgaatgtgccagattacgccGTCATGGCGGATGGCC
CGAGGCACTTGCAGCGCGGGCCGGTCCGGGTGGGGTTCTACGACATCGA
GGGCACGCTGGGCAAGGGCAACTTCGCTGTGGTGAAGCTGGGGCGGCAC
CGGATCACCAAGACGGAGGTGGCAATAAAAAATAATCGATAAGTCTCAGC
TGGATGCAGTGAACCTTGAGAAAATCTACCGAGAAGTACAAATAATGAA
AATGTTAGACCACCCTCACATAATCAAACTTTATCAGGTAATGGAGACC
AAAAGTATGTTGTACCTTGTGACAGAATATGCCAAAAATGGAGAAATTT
TTGACTATCTTGCTAATCATGGCCGGTTAAATGAGTCTGAAGCCAGGCG
AAAATTCTGGCAAATCCTGTCTGCTGTTGATTATTGTCATGGTCGGAAG
ATTGTGCACCGTGACCTCAAAGCTGAAAATCTCCTGCTGGATAACAACA
TGAATATCAAAATAGCAGATTTTCGGTTTTGGAAATTTCTTTAAAAGTGG
TGAAGTCTGGCAACATGGTGTGGCAGCCCCCTTATGCAGCCCCAGAA
GTCTTTGAAGGGCAGCAGTATGAAGGACCACAGCTGGACATCTGGAGTA
TGGGAGTTGTTCTTTATGTCCTTGTCTGTGGAGCTCTGCCCTTTGATGG
ACCGACTCTTCCAATTTTGAGGCAGAGGGTTCTGGAAGGAAGATTCGGG
ATTCCGTATTTTCATGTCAGAAGATTGCGAGCACCTTATCCGAAGGATGT
TGGTCCTAGACCCATCCAAACGGCTAACCATAGCCCAAATCAAGGAGCA
TAAATGGATGCTCATAGAAGTTCCTGTCCAGAGACCTGTTCTCTATCCA
CAAGAGCAAGAAAATGAGCCATCCATCGGGGAGTTTAAATGAGCAGGTTT
TGCGACTGATGCACAGCCTTGAATAGATCAGCAGAAAACCATTTGAGTC
TTTGCAGAACAAAGAGCTATAACCACTTTGCTGCCATTTATTTCTTGTG
GTGGAGCGCCTGAAATCACATCGGAGCAGTTTCCCAGTGGAGCAGAGAC
TTGATGGCCGCCAGCGTCGGCCTAGCACCATTTGCTGAGCAAACAGTTGC
CAAGGCACAGACTGTGGGGCTCCCAGTGACCATGCATTCACCGAACATG
AGGCTGCTGCGATCTGCCCTCCTCCCCCAGGCATCCAACGTGGAGGCCT
TTTCATTTCCAGCATCTGGCTGTCAGGCGGAAGCTGCATTCATGGAAGA
AGAGTGTGTGGACACTCCAAAGGTCAATGGCTGTCTGCTTGACCCTGTG
CCTCCTGTCCTGGTGCAGGAAGGGATGCCAGTCACTGCCAGCAACATGA
TGGAGACCTCCATTGACGAAGGGCTGGAGACAGAAGGAGAGGCCGAGGA
AGACCCCGCTCATGCCTTTGAGGCATTTTCAGTCCACACGCAGCGGGCAG
AGACGGCACACTCTGTCAGAAGTGACCAATCAACTGGTCGTGATGCCTG
GGGCAGGGAAAATTTTCTCCATGAATGACAGCCCCTCCCTTGACAGTGT
GGACTCTGAGTATGATATGGGGTCTGTTTCAGAGGGACCTGAACTTTCTG
GAAGACAACCCTTCCCTTAAGGACATCATGTTAGCCAATCAGCCTTCAC
CCCGCATGACATCTCCCTTCATAAGCCTGAGACCTACCAACCCAGCCAT
GCAGGCTCTGAGCTCCCAGAAACGAGAGGTCCACAACAGGTCTCCAGTG
AGCTTCAGAGAGGGCCGCAGAGCATCAGATACCTCCCTCACCCAGGGAA
TTGTAGCATTTAGACAACATCTTCAGAATCTGGCTAGAACCAAAGGAAT
TCTAGAGTTGAACAAAGTGCAGTTGTTGTATGAACAAATAGGACCGGAG
GCAGACCCTAACCTGGCGCCGGCGGCTCCTCAGTCCAGGACCTTGCTA
GCAGCTGCCCTCAGGAAGAAGTTTCTCAGCAGCAGGAAAGCGTCTCCAC
TCTCCCTGCCAGCGTGCATCCCCAGCTGTCCCCACGGCAGAGCCTGGAG
ACCCAGTACCTGCAGCACAGACTCCAGAAGCCCAGCCTTCTGTCAAAGG

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CCCAGAACACCTGTCAGCTTTATTGCAAAGAACCACCGCGGAGCCTTGA
GCAGCAGCTGCAGGAACATAGGCTCCAGCAGAAGCGACTCTTTCTTCAG
AAGCAGTCTCAACTGCAGGCCTATTTTAATCAGATGCAGATAGCAGAGA
GCTCCTACCCACAGCCAAGTCAGCAGCTGCCCCCTCCCCGCCAGGAGAC
TCCACCGCCTTCTCAGCAGGCCCCACCGTTCAGCCTGACCCAGCCCCTG
AGCCCCGTCCTGGAGCCTTCCTCCGAGCAGATGCAATACAGCCCTTTCC
TCAGCCAGTACCAAGAGATGCAGCTTCAGCCCCCTGCCCTCCACTTCCGG
TCCCCGGGCTGCTCCTCCTCTGCCACGCAGCTACAGCAGCAGCAGCCG
CCACCGCCACCACCCCTCCACCACCACGACAGCCAGGAGCTGCCCCAG
CCCCCTTACAGTTCTCCTATCAGACTTGTGAGCTGCCAAGCGCTGCTTC
CCCTGCGCCAGACTATCCCACTCCCTGTCAGTATCCTGTGGATGGAGCC
CAGCAGAGCGACCTAACGGGGCCAGACTGTCCCAGAAGCCCAGGACTGC
AAGAGGCCCCCTCCAGCTACGACCCACTAGCCCTCTCTGAGCTACCTGG
ACTCTTTGATTGTGAAATGCTAGACGCTGTGGATCCACAACACAACGGG
TATGTCCTGGTGAATtagcgccgc