Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SIK2 [2 - 926]

Enzyme description:- SIK2 [2 - 926]

Clone number:- DU 16624

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST and HA

Purification method:- 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:-
KKKVSRSGLYRSPSMPENLNRPR Final concentration: 300 μM

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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	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD*YPYDVPDYA***VMAD** **GPRHLQRGPVRVGFYDIEGTLGKGNFAVVKLGRHRITKTEVAIKIIDKSQ** **LDAVNLEKIYREVQIMKMLDHPHIIKLYQVMETKSMLYLVTEYAKNGEIF** **DYLANHGRLNESEARRKFWQILSAVDYCHGRKIVHRDLKAENLLLDNNMN** **IKIADFGFGNFFKSGELLATWCGSPPYAAPEVFEGQQYEGPQLDIWSMGV** **VLYVLVCGALPFDGPTLPILRQRVLEGRFRIPYFMSEDCEHLIRRMLVLD** **PSKRLTIAQIKEHKWMLIEVPVQRPVLYPQEQENEPSIGEFNEQVLRLMH** **SLGIDQQKTIESLQNKSYNHFAAIYFLLVERLKSHRSSFPVEQRLDGRQR** **RPSTIAEQTVAKAQTVGLPVTMHSPNMRLLRSALLPQASNVEAFSFPASG** **CQAEAAFMEEECVDTPKVNGCLLDPVPPVLVRKGCQSLPSNMMETSIDEG** **LETEGEAEEDPAHAFEAFQSTRSGQRRHTLSEVTNQLVVMPGAGKIFSMN** **DSPSLDSVDSEYDMGSVQRDLNFLEDNPSLKDIMLANQPSPRMTSPFISL** **RPTNPAMQALSSQKREVHNRSPVSFREGRRASDTSLTQGIVAFRQHLQNL** **ARTKGILELNKVQLLYEQIGPEADPNLAPAAPQLQDLASSCPQEEVSQQQ** **ESVSTLPASVHPQLSPRQSLETQYLQHRLQKPSLLSKAQNTCQLYCKEPP** **RSLEQQLQEHRLQQKRLFLQKQSQLQAYFNQMQIAESSYPQPSQQLPLPR** **QETPPPSQQAPPFSLTQPLSPVLEPSSEQMQYSPFLSQYQEMQLQPLPST** **SGPRAAPPLPTQLQQQPPPPPPPPPPPRQPGAAPAPLQFSYQTCELPSAA** **SPAPDYPTPCQYPVDGAQQSDLTGPDCPRSPGLQEAPSSYDPLALSELPG** **LFDCEMLDAVDPQHNGYVLVN**
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.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 229

Cloning sites *Sal*1 and *Not*1 sites in pFastBAC GST

Nucleotide sequence of insert

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gtcgactacccatacgatgtgccagattacgccGTCATGGCGGATGGCCC
GAGGCACTTGCAGCGCGGGCCGGTCCGGGTGGGGTTCTACGACATCGAGG
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