

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SIK3 [2 - 1369]

Enzyme description:- SIK3 [2 – 1369]
Clone number:- DU 16623
Source:- Recombinant
Expression system:- Baculovirus expression vector system
Tag:- N-terminal GST and HA
Purification method:- 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:-
KKKVSRSGLYRSPSPENLNRPR Final concentration: 300 µM

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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
LEFPNLPHYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAELSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSYPYDVPDYAAAAAA
SGAGGAAGAGTGGAGPAGRLPPPAPGSPAAPAAVSPAAGQPRPPAPAS
RGMPARIGYYEIDRTIGKGNFAVVKRATHLVTKAKVAIKIIDKTQLDE
ENLKKIFREVQIMKMLCHPHIIRLYQVMETERMIYLVTEYASGGEIFDH
LVAHGRMAEKEARRKFKQIVTAVYFCHCRNIVHRDLKAENLLLDANLNI
KIADFGFSNLFTPGQLLKTWCGSPPYAAPELFEGKEYDGPKVDIWSLGV
VLYVLCGALPFDGSTLQNLRARVLSGKFRIPFFMSTECEHLIRHMLVL
DPNKRLSMEQICKHKWMLGDADPNFDRLIAECQOLKEERQVDPLNEDV
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SMPRALAFQAPVNIQAEQAGTAMNISVPQVQLINPENQIVEPDGTLNLD
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QSMTERQALSYQNADSYHHHTSPQHLLQIRAQECVSQASSPTPPHGYAH
QPALMHSESMEEDCCEGAKDGFQDSKSSSTLTGCHDSPLLLSTGGPG
DPESLLGTVSHAQELGIHPYGHQPTAAF SKNKVPSREPVI GNCMDRSSP
GQAVELPDHNLGYPARPSVHEHHRPRALQRHHTIQNSSDDAYVQLDNLP
GMSLVAGKALSSARMSDAVLSQSSLMGSQQFQDGENEECGASLGGHEHP
DLSDGSQHLNSSCYPSTCITDILLSYKHPEVSFSMEQAGV

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Native sequence 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.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 229

Cloning sites *Bam*H1 sites in pFastBAC GST

Nucleotide sequence of insert

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ggatcctaccatacgaatgtgccagattacgccGCGGCGGGCGGCGGCGA
GCGGAGCTGGCGGGGCTGCCGGGGCCGGGACTGGGGGAGCCGGGCCCCG
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GCCGTGTCCCCTGCGGCCGGCCAGCCGCGTCCCCAGCCCCGGCCTCCC
GCGGACCCATGCCCGCCGTATCGGCTACTACGAGATCGACCCGACCAT
CGGCAAGGGCAACTTCGCGGTGGTCAAGCGGGCCACGCACCTCGTCACC
AAGGCAAGGTTGCTATCAAGATCATAGATAAGACCCAGCTGGATGAAG
AAAACCTGAAGAAGATTTTCCGGGAAGTTCAAATTATGAAGATGCTTTG
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ATTTATCTGGTGACAGAATATGCTAGTGGAGGGGAAATATTTGACCACC
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