Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CSNK1G2 [1 – 415]

Enzyme description:- CSNK1G2 [1 - 415]

Clone number:- DU 35306

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 74, 233.82 daltons
Average Mass 74, 281.43 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.56

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM 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 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
KRRRALS*VASLPGL (where S* is phospho Ser) Final concentration: 300 μM

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Clone Data Sheet

CSNK1G2 [1 - 415]

Protein	CSNK1G2 [1 - 415]
Clone number	DU 35306
Species	Human
Accession number	BC018693.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDFDKKGGKGETEE** **GRRMSKAGGGRSSHGIRSSGTSSGVLMVGPNFRVGKKIGCGNFGELRLG** **KNLYTNEYVAIKLEPIKSRAPQLHLEYRFYKQLSATEGVPQVYYFGPCG** **KYNAMVLELLGPSLEDLFDLCDRTFTLKTVLMIAIQLITRMEYVHTKSL** **IYRDVKPENFLVGRPGTKRQHAIHIIDFGLAKEYIDPETKKHIPYREHK** **SLTGTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQGLKADT** **LKERYQKIGDTKRATPIEVLCENFPEEMATYLRYVRRLDFFEKPDYDYL** **RKLFTDLFDRSGFVFDYEYDWAGKPLPTPIGTVHTDLPSQPQLRDKTQP** **HSKNQALNSTNGELNADDPTAGHSNAPITAPAEVEVADETKCCCFFKRR** **KRKSLQRHK**
Native sequence	Amino acids M1 – K415 of human CSNK1G2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGGATTTTGACAAGAAAGGAGGGAAAGGGGAGACGGAGGAGG
GCCGGAGAATGTCCAAGGCCGGCGGGGGCCGGAGCAGCCACGGCATCCG
GAGCTCGGGGACCAGCTCGGGGGTCCTGATGGTGGGCCCCAACTTCCGC
GTCGGCAAGAAGATCGGCTGCGGCAACTTCGGGGAGCTCCGCCTAGGAA
AGAATCTCTATACAAATGAATACGTGGCTATCAAATTGGAGCCGATCAA
GTCCCGGGCCCCGCAGCTGCACCTGGAGTACCGGTTCTACAAGCAGCTC
AGCGCCACAGAGGGCGTCCCTCAGGTCTACTACTTCGGTCCGTGCGGGA
AGTACAACGCCATGGTGCTGGAGCTGCTGGGGCCCAGCCTGGAGGACCT
GTTCGACCTGTGCGACCGGACCTTCACGCTCAAGACGGTGCTGATGATC
GCCATCCAGCTGATCACGCGCATGGAGTATGTGCACACCAAGAGCCTAA
TCTACCGGGACGTGAAGCCCGAGAACTTCCTGGTGGGCCGCCCGGGGAC
CAAGCGGCAGCATGCCATCCACATCATCGACTTCGGGCTGGCCAAGGAG
TACATCGACCCCGAGACCAAGAAGCACATCCCGTACCGCGAGCACAAGA
GCCTGACGGGCACGGCGCGCTACATGAGCATCAACACGCACCTGGGCAA
GGAGCAGAGCCGCCGCGACGACCTGGAGGCGCTGGGCCACATGTTCATG
TACTTCCTGCGCGGCAGCCTCCCCTGGCAGGGGCTCAAGGCCGACACGC
TCAAGGAGCGGTACCAGAAGATCGGGGACACCAAACGCGCCACGCCCAT
CGAGGTGCTCTGCGAGAACTTCCCAGAGGAGATGGCCACGTACCTGCGC
TATGTGCGGCGCCTGGACTTCTTCGAGAAGCCCGACTATGACTACCTGC
GGAAGCTCTTCACCGACCTCTTCGACCGCAGTGGCTTCGTGTTCGACTA
TGAGTACGACTGGGCCGGGAAGCCCCTGCCGACCCCCATCGGCACCGTC
CACACCGACCTGCCCTCCCAGCCTCAGCTCCGGGACAAAACCCAGCCGC
ACAGCAAAAACCAGGCGTTGAACTCCACCAACGGGGAGCTGAATGCGGA
CGACCCCACGGCCGGCCACTCCAACGCCCCGATCACAGCGCCTGCAGAG
GTGGAGGTGGCCGATGAAACCAAATGCTGCTGTTTCTTCAAGAGGAGAA
AGAGAAAATCGCTGCAGCGACACAAGtgagcggccgc