Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CSNK1G3 [1 – 423]

Enzyme description:- CSNK1G3 [1 - 423]

Clone number:- DU 35305

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 75, 674.27 daltons
Average Mass 75, 723.09 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.61

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

CSNK1G3 [1 - 423]

Protein	CSNK1G3 [1 - 423]
Clone number	DU 35305
Species	Human
Accession number	AAH47567.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MENKKKDKDKSDDR** **MARPSGRSGHNTRGTGSSSSGVLMVGPNFRVGKKIGCGNFGELRLGKNL** **YTNEYVAIKLEPMKSRAPQLHLEYRFYEQLGSGDGIPQVYYFGPCGKYN** **AMVLELLGPSLEDLFDLCDRTFSLKTVLMIAIQLISRMEYVHSKNLIYR** **DVKPENFLIGRPGNKTQQVIHIIDFGLAKEYIDPETKKHIPYREHKSLT** **GTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQGLKADTLKE** **RYQKIGDTKRATPIEVLCENFPEMATYLRYVRRLDFFEKPDYDYLRKLF** **TDLFDRKGYMFDYEYDWIGKQLPTPVGAVQQDPALSSNREAHQHRDKMQ** **QSKNQVVSSTNGELNTDDPTAGRSNAPITAPTEVEVMDETNCQKVLNMW** **CCCFFKRRKRKTIQRHK**
Native sequence	Amino acids M1 – K423 (end) of human CSNK1G3. 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

ggatccATGGAAAATAAAAAGAAAGACAAGGACAAATCAGATGATAGAATGGCACGACC
TAGTGGTCGATCGGGACACAACACTCGAGGAACTGGGTCTTCATCGTCTGGAGTTTTAA
TGGTTGGACCTAACTTTAGAGTTGGAAAAAAAATTGGATGTGGCAATTTTGGAGAATTA
CGATTAGGGAAAAATTTATACACAAATGAATATGTGGCAATTAAGTTGGAGCCCATGAA
ATCAAGAGCACCACAGCTACATTTGGAATACAGATTCTATGAGCAGTTAGGATCTGGAG
ATGGTATACCTCAAGTTTACTATTTCGGCCCTTGTGGTAAATACAATGCTATGGTGCTG
GAACTGCTGGGACCTAGTTTGGAAGACTTGTTTGACTTGTGTGACAGAACATTTTCTCT
TAAAACAGTTCTCATGATAGCTATACAACTGATTTCTCGCATGGAATATGTCCATTCAA
AGAACTTGATATACAGAGATGTAAAACCTGAGAACTTCTTAATAGGACGACCAGGAAAC
AAAACCCAGCAAGTTATTCACATTATAGATTTTGGTTTGGCAAAGGAATATATTGATCC
GGAGACAAAGAAACACATACCATACAGAGAACACAAGAGCCTTACAGGAACAGCTAGAT
ATATGAGCATAAACACACATTTAGGAAAAGAACAAAGTAGAAGAGACGATTTAGAAGCT
TTAGGTCATATGTTCATGTATTTTCTGAGAGGCAGTCTTCCTTGGCAAGGCTTAAAGGC
TGACACATTAAAGGAGAGGTATCAGAAAATTGGAGATACAAAACGGGCTACACCAATAG
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AAACGAAGGAAAAGGAAAACCATACAGCGCCACAAAtgagcggccgc