

## *Division of Signal Transduction 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  $\mu$ M

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**Clone Data Sheet**

**CSNK1G2 [1 - 415]**

<b><u>Protein</u></b>	CSNK1G2 [1 - 415]
<b><u>Clone number</u></b>	DU 35306
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	BC018693.2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMDFDKKGGKGETEE <b>GRRMSKAGGGRSSHGIRSSGTSSGVLVMPNFRVGGKIGCGNFGELRLG</b> <b>KNLYTNEYVAIKLEPIKSRAPQLHLEYRFYKQLSATEGVPQVYFGPCG</b> <b>KYNAMVLELLGPSLEDLFDLCDRTFTLKTVLMIAIQLITRMEYVHTKSL</b> <b>IYRDVKPENFLVGRPGTKRQHAIHIIDFGLAKEYIDPETKKHIPYREHK</b> <b>SLTGTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSPLPWQGLKADT</b> <b>LKERYQKIGDTKRATPIEVL CENFPEEMATYLRYVRRLDFFEKPDYDYL</b> <b>RKLF TDLFDRSGFVFDYEYDWAGKPLPTPIGTVHTDLPSQPQLRDKTQP</b> <b>HSKNQALNSTNGELNADDPTAGHSNAPI TAPAEVEVADETKCCCFKRR</b> <b>KRKS LQRHK</b></p>
<b><u>Native sequence</u></b>	<p>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.</p>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 229
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX 6P-1

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**Nucleotide**  
**Sequence of insert**

ggatccATGGATTTTGACAAGAAAGGAGGGAAAGGGGAGACGGAGGAGG  
GCCGGAGAATGTCCAAGGCCGGGGCCGGAGCAGCCACGGCATCCG  
GAGCTCGGGGACCAGCTCGGGGGTCCTGATGGTGGGCCCAACTTCCGC  
GTCGGCAAGAAGATCGGCTGCGGCAACTTCGGGGAGCTCCGCCTAGGAA  
AGAATCTCTATACAAATGAATACGTGGCTATCAAATTGGAGCCGATCAA  
GTCCCAGGGCCCCGAGCTGCACCTGGAGTACCGGTTCTACAAGCAGCTC  
AGCGCCACAGAGGGCGTCCCTCAGGTCTACTACTTCGGTCCGTGCGGGA  
AGTACAACGCCATGGTGCTGGAGCTGCTGGGGCCAGCCTGGAGGACCT  
GTTGACCTGTGCGACCGGACCTTCACGCTCAAGACGGTGCTGATGATC  
GCCATCCAGCTGATCACGCGCATGGAGTATGTGCACACCAAGAGCCTAA  
TCTACCGGGACGTGAAGCCCGAGAACTTCCTGGTGGGCCCGCCGGGGAC  
CAAGCGGCAGCATGCCATCCACATCATCGACTTCGGGCTGGCCAAGGAG  
TACATCGACCCCGAGACCAAGAAGCACATCCCGTACCGCGAGCACAAGA  
GCCTGACGGGCACGGCGCGCTACATGAGCATCAACACGCACCTGGGCAA  
GGAGCAGAGCCCGCCGCGACGACCTGGAGGCGCTGGGCCACATGTTTCATG  
TACTTCCTGCGCGGCAGCCTCCCTGGCAGGGCTCAAGGCCGACACGC  
TCAAGGAGCGGTACCAGAAGATCGGGGACACCAAACGCGCCACGCCCAT  
CGAGGTGCTCTGCGAGAACTTCCAGAGGAGATGGCCACGTACCTGCGC  
TATGTGCGGCGCCTGGACTTCTTCGAGAAGCCCGACTATGACTACCTGC  
GGAAGCTCTTCACCGACCTCTTCGACCGCAGTGGCTTCGTGTTGACTA  
TGAGTACGACTGGGCCGGGAAGCCCCTGCCGACCCCATCGGCACCGTC  
CACACCGACCTGCCCTCCAGCCTCAGCTCCGGGACAAAACCCAGCCGC  
ACAGCAAAAACAGGCGTTGAACTCCACCAACGGGGAGCTGAATGCGGA  
CGACCCACGGCCGGCCACTCCAACGCCCCGATCACAGCGCCTGCAGAG  
GTGGAGGTGGCCGATGAAACCAAATGCTGCTGTTTCTTCAAGAGGAGAA  
AGAGAAAATCGCTGCAGCGACACAAGtgagcggccgc