*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of active CK1 gamma 1 [1 – 422]

**Enzyme description:-** CK1 gamma 1 [1 - 422]

**Clone number:-** DU 31197

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method**:- GSH Sepharose

**Calculated molecular mass:-**
Monoisotopic 75, 287.24 daltons
Average Mass 75, 335.49 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 8.54

**Purity:-** >80 %

**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 Serine) Final concentration: 300 $\mu$M

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# Clone Data Sheet

## CK1 gamma 1 [1 – 422]

| | |
|---|---|
| **Protein** | CK1 gamma 1 [1 - 422] |
| **Clone number** | DU 31197 |
| **Species** | Human |
| **Accession number** | NM_022048.3 |
| **Tags** | N-terminal GST |
| **Bacterially expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br>LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA<br>VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH<br>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS<br>KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDHPSREKDERQRT**<br>**TKPMAQRSAHCSRPSGSSSSSGVLMVGPNFRVGKKIGCGNFGELRLGKN**<br>**LYTNEYVAIKLEPIKSRAPQLHLEYRFYKQLGSAGEGLPQVYYFGPCGK**<br>**YNAMVLELLGPSLEDLFDLCDRTFTLKTVLMIAIQLLSRMEYVHSKNLI**<br>**YRDVKPENFLIGRQGNKKEHVIHIIDFGLAKEYIDPETKKHIPYREHKS**<br>**LTGTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQGLKADTL**<br>**KERYQKIGDTKRNTPIEALCENFPEEMATYLRYVRRLDFFEKPDYEYLR**<br>**TLFTDLFEKKGYTFDYAYDWVGRPIPTPVGSVHVDSGASAITRESHTHR**<br>**DRPSQQQPLRNQVVSSTNGELNVDDPTGAHSNAPITAHAEVEVVEEAKC**<br>**CCFFKRKRKKTAQRHK** |
| **Native sequence** | Amino acids M1 – K422 (end) of human CK1 gamma 1.<br>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 sites of pGEX 6P-1 |

**Nucleotide Sequence of insert**

ggatccATGGACCATCCTAGTAGGGAAAAGGATGAAAGACAA
CGGACAACTAAACCCATGGCACAAAGGAGTGCACACTGCTCT
CGACCATCTGGCTCCTCATCGTCCTCTGGGGTTCTTATGGTG
GGACCCAACTTCAGGGTTGGCAAGAAGATAGGATGTGGGAAC
TTCGGAGAGCTCAGATTAGGTAAAAATCTCTACACCAATGAA
TATGTAGCAATCAAACTGGAACCAATAAAATCACGTGCTCCA
CAGCTTCATTTAGAGTACAGATTTTATAAACAGCTTGGCAGT
GCAGGTGAAGGTCTCCCACAGGTGTATTACTTTGGACCATGT
GGGAAATATAATGCCATGGTGCTGGAGCTCCTTGGCCCTAGC
TTGGAGGACTTGTTTGACCTCTGTGACCGAACATTTACTTTG
AAGACGGTGTTAATGATAGCCATCCAGCTGCTTTCTCGAATG
GAATACGTGCACTCAAAGAACCTCATTTACCGAGATGTCAAG
CCAGAGAACTTCCTGATTGGTCGACAAGGCAATAAGAAAGAG
CATGTTATACACATTATAGACTTTGGACTGGCCAAGGAATAC
ATTGACCCCGAAACCAAAAAACACATACCTTATAGGGAACAC
AAAAGTTTAACTGGAACTGCAAGATATATGTCTATCAACACG
CATCTTGGCAAAGAGCAAAGCCGGAGAGATGATTTGGAAGCC
CTAGGCCACATGTTCATGTATTTCCTTCGAGGCAGCCTCCCC
TGGCAAGGACTCAAGGCTGACACATTAAAAGAGAGATATCAA
AAAATTGGTGACACCAAAAGGAATACTCCCATTGAAGCTCTC
TGTGAGAACTTTCCAGAGGAGATGGCAACCTACCTTCGATAT
GTCAGGCGACTGGACTTCTTTGAAAAACCTGATTATGAGTAT
TTACGGACCCTCTTCACAGACCTCTTTGAAAAGAAAGGCTAC
ACCTTTGACTATGCCTATGATTGGGTTGGGAGACCTATTCCT
ACTCCAGTAGGGTCAGTTCACGTAGATTCTGGTGCATCTGCA
ATAACTCGAGAAAGCCACACACATAGGGATCGGCCATCACAA
CAGCAGCCTCTTCGAAATCAGGTGGTTAGCTCAACCAATGGA
GAGCTGAATGTTGATGATCCCACGGGAGCCCACTCCAATGCA
CCAATCACAGCTCATGCCGAGGTGGAGGTAGTGGAGGAAGCT
AAGTGCTGCTGTTTCTTTAAGAGGAAAAGGAAGAAGACTGCT
CAGCGCCACAAGtgaggatcc