

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CK1 delta [1 – 415]

Enzyme description:- CK1 delta [1 – 415]

Clone number:- DU 19064

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 74, 106.86 daltons

Average Mass 74, 154.24 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.16

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, 0.2 mM PMSF, 1 mM Benzamidine

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:-

KRRRALS*VASLPGL (where S* is phospho Serine) Final concentration: 300 μM

Division of Signal Transduction Therapy

Clone Data Sheet

CK1 delta [1 – 415]

<u>Protein</u>	CK1 delta [1 - 415]
<u>Clone number</u>	DU 19064
<u>Species</u>	Human
<u>Accession number</u>	NM_001893
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEG DKWRNKKFELGLEFPNLPYYIDGDVKLTSMAIIRYIA DKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPO IDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFO GPLGSMELRVGNRYRLGRKIGSGSFGDIYLGTDIAAGE EVAIKLECVKTKHPQLHIESKIYKMMQGGVGIP TIRWC GAEGDYNVMVME LLGPSLEDLFNFCSRKFS LKTVLLLA DQMISRIEYIHSKNFIHRDVKPDNFLMGLGKKGNLVYI IDFGLAKKYRDARTHQHIPYRENKNLTGTARYASINTH LGIEQSRRDDLES LGYVLMYFNLGSLPWQGLKAATKRQ KYERISEKKMSTPIEVLCKGYPSEFATYLNFCRSLRFD DKPDYSYL RQLFRNLFHRQGSYDYVFDWNMLKFGASR AADDAERERRDREERLRHSRNPATRGLPSTASGRLRGT QEVAPPTPLTPTSH TANTSPRPVSGMERERKVSMLHR GAPVNISSSDLTGRQDTSRMSTSQIPGRVASSGLQSVV HR</p>
<u>Native sequence</u>	Amino acids M1 – R415 of human CK1 delta. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> HI and <i>Not</i> I sites of pGEX-6PB-1

Division of Signal Transduction Therapy

**Nucleotide
sequence of insert**

ggatccATGGAGCTGAGAGTCGGGAACAGGTACCGGCTGGGCCGG
AAGATCGGCAGCGGCTCCTTCGGAGACATCTATCTCGGTACGGAC
ATTGCTGCAGGAGAAGAGGTTGCCATCAAGCTTGAATGTGTCAAA
ACCAAACACCCTCAGCTCCACATTGAGAGCAAAATCTACAAGATG
ATGCAGGGAGGAGTGGGCATCCCCACCATCAGATGGTGCGGGGCA
GAGGGGGACTACAACGTCATGGTGATGGAGCTGCTGGGGCCAAGC
CTGGAGGACCTCTTCAACTTCTGCTCCAGGAAATTCAGCCTCAAA
ACCGTCCTGCTGCTTGCTGACCAAATGATCAGTCGCATCGAATAC
ATTCATTCAAAGAACTTCATCCACCGGGATGTGAAGCCAGACAAC
TTCCTCATGGGCCTGGGGAAGAAGGGCAACCTGGTGTACATCATC
GACTTCGGGCTGGCCAAGAAGTACCGGGATGCACGCACCCACCAG
CACATCCCCTATCGTGAGAACAAGAACCACGGGGACGGCGCGG
TACGCCTCCATCAACACGCACCTTGAATTGAACAATCCCGAAGA
GATGACTTGGAGTCTCTGGGCTACGTGCTAATGTACTTCAACCTG
GGCTCTCTCCCCTGGCAGGGGCTGAAGGCTGCCACCAAGAGACAG
AAATACGAAAGGATTAGCGAGAAGAAAATGTCCACCCCATCGAA
GTGTTGTGTAAAGGCTACCCTTCCGAATTTGCCACATACCTGAAT
TTCTGCCGTTCCCTTGCGTTTTGACGACAAGCCTGACTACTCGTAC
CTGCGGCAGCTTTTCCGGAATCTGTTCCATCGCCAGGGCTTCTCC
TATGACTACGTGTTGACTGGAACATGCTCAAATTTGGTGCCAGC
CGGGCCGCGATGACGCCGAGCGGGAGCGCAGGGACCGAGAGGAG
CGGCTGAGACACTCGCGGAACCCGGCTACCCGCGGCCTCCCTTCC
ACAGCCTCCGGCCGCCTGCGGGGACGCAGGAAGTGGCTCCCCC
ACACCCCTCACCCCTACCTCACACACGGCTAACACCTCCCCCGG
CCCGTCTCCGGCATGGAGAGAGAGCGGAAAGTGAGTATGCGGCTG
CACCGCGGGGCCCGTCAACATCTCCTCGTCCGACCTCACAGGC
CGACAAGATACCTCTCGCATGTCCACCTCACAGATTCTGGTCCG
GTGGCTTCCAGTGGTCTTCAGTCTGTGTCGTGCACCGAtgagcggcc
gc