Division of Signal Tranduction 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

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 74, 106.86 daltons
Average Mass 74, 154.24daltons
[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

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Clone Data Sheet

CK1 delta [1 – 415]

Protein	CK1 delta [1 - 415]
Clone number	DU 19064
Species	Human
Accession number	NM_001893
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIA DKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQ IDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQ GPLGS**MELRVGNRYRLGRKIGSGSFGDIYLGTDIAAGE** **EVAIKLECVKTKHPQLHIESKIYKMMQGGVGIPTIRWC** **GAEGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLA** **DQMISRIEYIHSKNFIHRDVKPDNFLMGLGKKGNLVYI** **IDFGLAKKYRDARTHQHIPYRENKNLTGTARYASINTH** **LGIEQSRRDDLESLGYVLMYFNLGSLPWQGLKAATKRQ** **KYERISEKKMSTPIEVLCKGYPSEFATYLNFCRSLRFD** **DKPDYSYLRQLFRNLFHRQGFSYDYVFDWNMLKFGASR** **AADDAERERRDREERLRHSRNPATRGLPSTASGRLRGT** **QEVAPPTPLTPTSHTANTSPRPVSGMERERKVSMRLHR** **GAPVNISSSDLTGRQDTSRMSTSQIPGRVASSGLQSVV** **HR**
Native sequence	Amino acids M1 – R415 of human CK1 delta. Residue M232 of the fusion protein is equilivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*HI and *Not*I sites of pGEX-6PB-1

Nucleotide sequence of insert

ggatccATGGAGCTGAGAGTCGGGAACAGGTACCGGCTGGGCCGG
AAGATCGGCAGCGGCTCCTTCGGAGACATCTATCTCGGTACGGAC
ATTGCTGCAGGAGAAGAGGTTGCCATCAAGCTTGAATGTGTCAAA
ACCAAACACCCTCAGCTCCACATTGAGAGCAAAATCTACAAGATG
ATGCAGGGAGGAGTGGGCATCCCCACCATCAGATGGTGCGGGGCA
GAGGGGGACTACAACGTCATGGTGATGGAGCTGCTGGGGCCAAGC
CTGGAGGACCTCTTCAACTTCTGCTCCAGGAAATTCAGCCTCAAA
ACCGTCCTGCTGCTTGCTGACCAAATGATCAGTCGCATCGAATAC
ATTCATTCAAAGAACTTCATCCACCGGGATGTGAAGCCAGACAAC
TTCCTCATGGGCCTGGGGAAGAAGGGCAACCTGGTGTACATCATC
GACTTCGGGCTGGCCAAGAAGTACCGGGATGCACGCACCCACCAG
CACATCCCCTATCGTGAGAACAAGAACCTCACGGGGACGGCGCGG
TACGCCTCCATCAACACGCACCTTGGAATTGAACAATCCCGAAGA
GATGACTTGGAGTCTCTGGGCTACGTGCTAATGTACTTCAACCTG
GGCTCTCTCCCCTGGCAGGGGCTGAAGGCTGCCACCAAGAGACAG
AAATACGAAAGGATTAGCGAGAAGAAAATGTCCACCCCCATCGAA
GTGTTGTGTAAAGGCTACCCTTCCGAATTTGCCACATACCTGAAT
TTCTGCCGTTCCTTGCGTTTTGACGACAAGCCTGACTACTCGTAC
CTGCGGCAGCTTTTCCGGAATCTGTTCCATCGCCAGGGCTTCTCC
TATGACTACGTGTTCGACTGGAACATGCTCAAATTTGGTGCCAGC
CGGGCCGCCGATGACGCCGAGCGGGAGCGCAGGGACCGAGAGGAG
CGGCTGAGACACTCGCGGAACCCGGCTACCCGCGGCCTCCCTTCC
ACAGCCTCCGGCCGCCTGCGGGGGACGCAGGAAGTGGCTCCCCCC
ACACCCCTCACCCCTACCTCACACACGGCTAACACCTCCCCCCGG
CCCGTCTCCGGCATGGAGAGAGAGCGGAAAGTGAGTATGCGGCTG
CACCGCGGGGCCCCCGTCAACATCTCCTCGTCCGACCTCACAGGC
CGACAAGATACCTCTCGCATGTCCACCTCACAGATTCCTGGTCGG
GTGGCTTCCAGTGGTCTTCAGTCTGTCGTGCACCGAtgagcggcc
gc