Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CK1 delta [1 – 294]

Enzyme description:- CK1 delta [1 – 294]

Clone number:- DU 1632

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:- 60, 909 daltons

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
RRKDLHDDEEDEAMSITA Final concentration: 250 µM

Specific activity range:- 400 – 800 U/mg

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Clone Data Sheet

CK1 delta [1 – 294]

Protein	CK1 delta [1 - 294]
Clone number	DU 1632
Species	Rat
Accession number	AB063114
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG DKWRNKKFELGLEFPNPLYYIDGDVKLTQSMAIIRYIA DKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPEMLHMFEDRLCHKTYLNGDHVTHPD FMLYDALAVVLYMDPMCLDAFPKLVCFKKRIEAIPQID KYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPG IRL**MELRVGNRYRLGRKIGSGSFGDIYLGTDIAAGEEV** **AIKLECVKTKHPQLHIESKIYKMMQGGVGIPTIRWCGA** **EGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLADQ** **MISRIEYIHSKNFIHRDVKPDNFLMGLGKKGNLVYIID** **FGLAKKYRDARTHQHIPYRENKNLTGTARYASINTHLG** **IEQSRRDDLESLGYVLMYFNLGSLPWQGLKAATKRQKY** **ERISEKKMSTPIEVLCKGYPSEFATYLNFCRSLRFDDK** **PDYSYLRQLFRNLFHRQGFSYDYVFDWNMLK**
Native sequence	Amino acids M1 – K294 of rat CK1 delta. [Full length protein ends at residue R415] 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	Thrombin (LVPRGS) residues 221 - 226
Cloning sites	*Eco*RI and *Sma*I sites of pGEX-2T

Nucleotide sequence of insert

ATGGAGCTGAGGGTCGGGAACAGGTACCGACTGGGCCGCAAGATC
GGCAGCGGCTCCTTCGGAGACATCTATCTCGGTACGGACATTGCT
GCAGGAGAAGAAGTTGCCATCAAGCTTGAATGTGTCAAAACCAAA
CATCCTCAGCTCCACATTGAGAGCAAGATCTACAAAATGATGCAG
GGAGGAGTGGGCATCCCTACCATCAGATGGTGTGGGGCTGAGGGG
GACTACAATGTCATGGTGATGGAGCTACTGGGACCCAGCCTGGAA
GACCTATTCAACTTCTGTTCAAGGAAGTTTAGTCTCAAAACTGTT
CTGTTGCTTGCTGACCAAATGATAAGTCGTATTGAGTACATTCAT
TCGAAGAATTTTATCCACCGAGACGTGAAACCAGATAACTTCCTC
ATGGGGCTGGGAAAGAAAGGCAACCTGGTCTACATCATTGACTTT
GGGCTGGCCAAGAAGTATCGGGATGCCCGCACCCACCAGCATATC
CCCTATCGAGAGAACAAGAACCTCACAGGGACAGCACGCTATGCC
TCCATCAACACACACCTTGGCATTGAACAATCTCGAAGGGATGAC
TTGGAGTCTCTGGGGTACGTGCTGATGTACTTCAACCTGGGCTCT
CTCCCCTGGCAGGGGCTGAAGGCCGCCACCAAGAGGCAGAAGTAT
GAGAGGATCAGTGAGAAGAAGATGTCCACTCCAATTGAAGTGCTG
TGCAAGGGCTATCCTTCTGAATTTGCCACATACCTGAATTTCTGC
CGTTCCTTACGTTTTGATGACAAACCTGACTACTCCTACCTGAGA
CAGCTCTTCAGAAATCTGTTCCATCGCCAGGGCTTCTCCTACGAC
TATGTGTTCGACTGGAACATGCTCAAAtag