Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CK1 epsilon [1 – 416]

Enzyme description:- CK1 epsilon [1 – 416]

Clone number:- DU 5127

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 75, 142.42 daltons
Average Mass 75, 190.42 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.10

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 epsilon [1 – 416]

Protein	CK1 epsilon [1 - 416]
Clone number	DU 5127
Species	Human
Accession number	NM_152221
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGSPEIPGSTRAAA**MELRVGNKYRLGRKIGSGSFGDIYLGAN** **IASGEEVAIKLECVKTKHPQLHIESKFYKMMQGGVGIPSIKWCGA** **EGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLADQMISRIEY** **IHSKNFIHRDVKPDNFLMGLGKKGNLVYIIDFGLAKKYRDARTHQ** **HIPYRENKNLTGTARYASINTHLGIEQSRRDDLESLGYVLMYFNL** **GSLPWQGLKAATKRQKYERISEKKMSTPIEVLCKGYPSEFSTYLN** **FCRSLRFDDKPDYSYLRQLFRNLFHRQGFSYDYVFDWNMLKFGAA** **RNPEDVDRERREHEREERMGQLRGSATRALPPGPPTGATANRLRS** **AAEPVASTPASRIQPAGNTSPRAISRVDRERKVSMRLHRGAPANV** **SSSDLTGRQEVSRIPASQTSVPFDHLGK**
Native sequence	Amino acids M1 – K416 of human CK1 epsilon. Residue M243 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	*Not*I sites of pGEX-6P-2

Nucleotide sequence of insert

gcggccgcgATGGAGCTACGTGTGGGGAACAAGTACCG
CCTGGGACGGAAGATCGGGAGCGGGTCCTTCGGAGATA
TCTACCTGGGTGCCAACATCGCCTCTGGTGAGGAAGTC
GCCATCAAGCTGGAGTGTGTGAAGACAAAGCACCCCCA
GCTGCACATCGAGAGCAAGTTCTACAAGATGATGCAGG
GTGGCGTGGGGATCCCGTCCATCAAGTGGTGCGGAGCT
GAGGGCGACTACAACGTGATGGTCATGGAGCTGCTGGG
GCCTAGCCTCGAGGACCTGTTCAACTTCTGTTCCCGCA
AATTCAGCCTCAAGACGGTGCTGCTCTTGGCCGACCAG
ATGATCAGCCGCATCGAGTATATCCACTCCAAGAACTT
CATCCACCGGGACGTCAAGCCCGACAACTTCCTCATGG
GGCTGGGGAAGAAGGGCAACCTGGTCTACATCATCGAC
TTCGGCCTGGCCAAGAAGTACCGGGACGCCCGCACCCA
CCAGCACATTCCCTACCGGGAAAACAAGAACCTGACCG
GCACGGCCCGCTACGCTTCCATCAACACGCACCTGGGC
ATTGAGCAAAGCCGTCGAGATGACCTGGAGAGCCTGGG
CTACGTGCTCATGTACTTCAACCTGGGCTCCCTGCCCT
GGCAGGGGCTCAAAGCAGCCACCAAGCGCCAGAAGTAT
GAACGGATCAGCGAGAAGAAGATGTCAACGCCCATCGA
GGTCCTCTGCAAAGGCTATCCCTCCGAATTCTCAACAT
ACCTCAACTTCTGCCGCTCCCTGCGGTTTGACGACAAG
CCCGACTACTCTTACCTACGTCAGCTCTTCCGCAACCT
CTTCCACCGGCAGGGCTTCTCCTATGACTACGTCTTTG
ACTGGAACATGCTGAAATTCGGTGCAGCCCGGAATCCC
GAGGATGTGGACCGGGAGCGGCGAGAACACGAACGCGA
GGAGAGGATGGGGCAGCTACGGGGGTCCGCGACCCGAG
CCCTGCCCCCTGGCCCACCCACGGGGGCCACTGCCAAC
CGGCTCCGCAGTGCCGCCGAGCCCGTGGCTTCCACGCC
AGCCTCCCGCATCCAGCCGGCTGGCAATACTTCTCCCA
GAGCGATCTCGCGGGTCGACCGGGAGAGGAAGGTGAGT
ATGAGGCTGCACAGGGGTGCGCCCGCCAACGTCTCCTC
CTCAGACCTCACTGGGCGGCAAGAGGTCTCCCGGATCC
CAGCCTCACAGACAAGTGTGCCATTTGACCATCTCGGG
AAGTGAgcggccgc