

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Casein Kinase 1 alpha 1 [2 - 337]

Enzyme description:- CK1 alpha 1 [2 - 337]

Clone number:- DU 329

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 65, 565.48 daltons

Average Mass 65, 607.83 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.02

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

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 Ser)

Final concentration: 300 μ M

Division of Signal Transduction Therapy

Clone Data Sheet

Casein Kinase 1 alpha 1 [2 - 337]

<u>Protein</u>	CK1 alpha [2 - 337]
<u>Clone number</u>	DU 329
<u>Species</u>	Human
<u>Accession number</u>	X80693
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLOGWQATFGGGDHPKSDLEVLFOGPLGSASSSGSKAE FIVGGKYKLVKIGSGSFGDIYLAINITNGEEVALKLESQKARHPOLL YESKLYKILQGGVGIPHIRWYGQEKDYNVLMVDLLGPSLEDLNFCSR RFTMKTVLMADQMSRIEYVHTKNFIHRDIKPDNFLMGIGRHCNKLF LIDFGLAKKYRDNRTROHIPYREDKNLTGTARYASINAHLGIEQSRRD DMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCK GFPAEFAMYLNYCRGLRFEEAPDYMRLRQLFRILFRTLNHQYDYTFDW TMLKQKAAQQAASSSGOGQAQTPTGKQTDKSKSNMKGF</p>
<u>Native sequence</u>	<p>Amino acids A2 – F337 (end) of human CK1 alpha 1.</p> <p>Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	Prescission site (<u>LEVLFOGP</u>) at residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX-6P-1

Division of Signal Transduction Therapy

Nucleotide
sequence of insert

ggatccGCGAGTAGCAGCGGCTCCAAGGCTGAATTCATTGTCCGGTGGG
AAATATAAACTGGTACGGAAGATCGGGTCTGGCTCCTTCGGGGACATC
TATTTGGCGATCAACATCACCAACGGCGAGGAAGTGGCACTGAAGCTA
GAATCTCAGAAGGCCAGGCATCCCCAGTTGCTGTACGAGAGCAAGCTC
TATAAGATTCTTCAAGGTGGGGTTGGCATCCCCACATACGGTGGTAT
GGTCAGGAAAAAGACTACAATGTACTAGTCATGGATCTTCTGGGACCT
AGCCTCGAAGACCTCTTCAATTTCTGTTCAAGAAGGTTTACAATGAAA
ACTGTACTTATGTTAGCTGACCAGATGATCAGTAGAATTGAATATGTG
CATAAAAGAATTTTATACACAGAGACATTA AACAGATAACTTCCTA
ATGGGTATTGGGCGTCACTGTAATAAGTTATTCCTTATTGATTTTGGT
TTGGCCAAAAGTACAGAGACAACAGGACAAGGCAACACATACCATA
AGAGAAGATAAAAACCTCACTGGCACTGCCGATATGCTAGCATCAAT
GCACATCTTGGTATTGAGCAGAGTCGCCGAGATGACATGGAATCATT
GGATATGTTTTGATGTATTTTAATAGAACCAGCCTGCCATGGCAAGGG
CTAAAGGCTGCAACAAAGAAACAAAATATGAAAAGATTAGTGAAAAG
AAGATGTCCACGCCTGTTGAAGTTTTATGTAAGGGGTTTCCTGCAGAA
TTTGCGATGTACTTAAACTATTGTCGTGGGCTACGCTTTGAGGAAGCC
CCAGATTACATGTATCTGAGGCAGCTATTCCGCATTCTTTTCAGGACC
CTGAACCATCAATATGACTACACATTTGATTGGACAATGTTAAAGCAG
AAAGCAGCACAGCAGGCAGCCTCTTCAAGTGGGCAGGGTCAGCAGGCC
CAAACCCACAGGCAAGCAA ACTGACAAATCCAAGAGTAACATGAAA
GGTTTct aagcggccgc