

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Casein Kinase 2, alpha 1 [2 - 391]

<u>Enzyme description:-</u>	Casein Kinase 2 alpha 1 [2 - 391]
<u>Clone number:-</u>	DU 813
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 48, 353.17 daltons
Average Mass 48, 384.01 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.75

Purity:- >80 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay buffer:-

20 mM Hepes pH 7.5, 5 mM DTT, 0.1 mM EDTA, 150 mM NaCl, 0.1 % Triton X-100,
10 mM MgAc

Substrate:-

CK2 substrate peptide [RRRDDDSDDD] Final concentration: 300 µM

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Clone Data Sheet

Casein Kinase 2, alpha 1 [2 - 391]

<u>Protein</u>	CK2 alpha 1 [2 - 391]
<u>Clone number</u>	DU 813
<u>Species</u>	Human
<u>Accession number</u>	NM_001895
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQ<u>GAMGSS</u>SGPVPSRARVYTDVNTHRPRE YWDYESHVVEWGNQDDYQLVRKLGRGKYSEVFEAINITNNEKVVKILK PVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNTD FKQLYQTLTDYDIRFYMEILKALDYCHSMGIMHRDVKPHNVMIDHEHR KLRLIDWGLAEFYHPGQYINVRVASRYFKGPELLVDYQMYDYSLDMWSL GCMLASMIFRKEPFFHGHNDYDQLVRIAKVLGTEDLYDYIDKYNIELDP RFNDILGRHSRKRWERFVHSENQHLVSPEALDFDKLLRYDHQSRLTAR EAMEHPYFYTVVKDQARMGSSSMPPGGSTPVSSANMMSGISSVPTPSPLG PLAGSPVIAAANPLGMPVPAAGAQQ
<u>Native sequence</u>	Amino acids S2 – Q391 (end) of human CK2 alpha 1. Residue S29 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pFastBAC HTb

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**Nucleotide
sequence of insert**

ggatcccTCGGGACCCGTGCCAAGCAGGGCCAGAGTTTACACAGATGTTA
ATACACACAGACCTCGAGAATACTGGGATTACGAGTCACATGTGGTGGA
ATGGGGAAATCAAGATGACTACCAGCTGGTTTCGAAAATTAGGCCGAGGT
AAATACAGTGAAGTATTTGAAGCCATCAACATCACAAATAATGAAAAAG
TTGTTGTTAAAAATTCTCAAGCCAGTAAAAAAGAAGAAAATTAAGCGTGA
AATAAAGATTTTGGAGAATTTGAGAGGAGGTCCCAACATCATCACACTG
GCAGACATTGTAAGACCCTGTGTACGAACCCCGCCTTGGTTTTTTG
AACACGTAAACAACACAGACTTCAAGCAATTGTACCAGACGTTAACAGA
CTATGATATTCGATTTTACATGTATGAGATTCTGAAGGCCCTGGATTAT
TGTCACAGCATGGGAATTATGCACAGAGATGTCAAGCCCCATAATGTCA
TGATTGATCATGAGCACAGAAAGCTACGACTAATAGACTGGGGTTTTGGC
TGAGTTTTATCATCCTGGCCAAGAATATAATGTCCGAGTTGCTTCCCGA
TACTTCAAAGGTCCTGAGCTACTTGTAGACTATCAGATGTACGATTATA
GTTTGGATATGTGGAGTTTGGGTGTATGCTGGCAAGTATGATCTTTCG
GAAGGAGCCATTTTTCCATGGACATGACAATTATGATCAGTTGGTGAGG
ATAGCCAAGGTTCTGGGGACAGAAGATTTATATGACTATATTGACAAAT
ACAACATTGAATTAGATCCACGTTTCAATGATATCTTGGGCAGACACTC
TCGAAAGCGATGGGAACGCTTTGTCCACAGTGAAAATCAGCACCTTGTC
AGCCCTGAGGCCTTGGATTTCCCTGGACAAACTGCTGCGATATGACCACC
AGTCACGGCTTACTGCAAGAGAGGCAATGGAGCACCCCTATTTCTACAC
TGTTGTGAAGGACCAGGCTCGAATGGGTTCATCTAGCATGCCAGGGGGC
AGTACGCCCGTCAGCAGCGCCAATATGATGTGAGGGATTTCTTCAGTGC
CAACCCCTTCACCCCTTGGACCTCTGGCAGGCTCACCAGTGATTGCTGC
TGCCAACCCCTTGGGATGCCTGTTCCAGCTGCCGCTGGCGCTCAGCAG
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