*Division of Signal Transduction Therapy*

# Standard Operating Procedure

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

**Enzyme description:-** Casein Kinase 2 alpha 1 [2 - 391]

**Clone number:-** DU 813

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal His(6)

**Purification method**:- $Ni^{2+}$-NTA agarose

**Expression level:-** 2 mg/L

**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 [Long term stability to be determined]

**Assay:-** Standard filter binding assay

**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 $\mu$M

**Specific activity range:-** To be determined

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# Clone Data Sheet

## Casein Kinase 2, alpha 1 [2 - 391]

| | |
|---|---|
| **Protein** | CK2 alpha 1 [2 - 391] |
| **Clone number** | DU 813 |
| **Species** | Human |
| **Accession number** | NM_001895 |
| **Tags** | N-terminal His(6) |
| **Baculovirus expressed protein** | MSYYHHHHHHDYDIPTTENLYFQGAMGS**SGPVPSRARVYTDVNTHRPRE YWDYESHVVEWGNQDDYQLVRKLGRGKYSEVFEAINITNNEKVVVKILK PVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNTD FKQLYQTLTDYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHR KLRLIDWGLAEFYHPGQEYNVRVASRYFKGPELLVDYQMYDYSLDMWSL GCMLASMIFRKEPFFHGHDNYDQLVRIAKVLGTEDLYDYIDKYNIELDP RFNDILGRHSRKRWERFVHSENQHLVSPEALDFLDKLLRYDHQSRLTAR EAMEHPYFYTVVKDQARMGSSSMPGGSTPVSSANMMSGISSVPTPSPLG PLAGSPVIAAANPLGMPVPAAAGAQQ** |
| **Native sequence** | Amino acids S2 – Q391 (end) of human CK2 alpha 1.<br>Residue S29 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10. |
| **Protease cleavage** | rTEV (ENLYFQG) residues 18 - 24 |
| **Cloning sites** | *Bam*H1 and *Eco*R1 sites of pFastBAC HTb |

**Nucleotide sequence of insert**

ggatccTCGGGACCCGTGCCAAGCAGGGCCAGAGTTTACACAGATGTTA
ATACACACAGACCTCGAGAATACTGGGATTACGAGTCACATGTGGTGGA
ATGGGGAAATCAAGATGACTACCAGCTGGTTCGAAAATTAGGCCGAGGT
AAATACAGTGAAGTATTTGAAGCCATCAACATCACAAATAATGAAAAAG
TTGTTGTTAAAATTCTCAAGCCAGTAAAAAAGAAGAAAATTAAGCGTGA
AATAAAGATTTTGGAGAATTTGAGAGGAGGTCCCAACATCATCACACTG
GCAGACATTGTAAAAGACCCTGTGTCACGAACCCCCGCCTTGGTTTTTG
AACACGTAAACAACACAGACTTCAAGCAATTGTACCAGACGTTAACAGA
CTATGATATTCGATTTTACATGTATGAGATTCTGAAGGCCCTGGATTAT
TGTCACAGCATGGGAATTATGCACAGAGATGTCAAGCCCCATAATGTCA
TGATTGATCATGAGCACAGAAAGCTACGACTAATAGACTGGGGTTTGGC
TGAGTTTTATCATCCTGGCCAAGAATATAATGTCCGAGTTGCTTCCCGA
TACTTCAAAGGTCCTGAGCTACTTGTAGACTATCAGATGTACGATTATA
GTTTGGATATGTGGAGTTTGGGTTGTATGCTGGCAAGTATGATCTTTCG
GAAGGAGCCATTTTTCCATGGACATGACAATTATGATCAGTTGGTGAGG
ATAGCCAAGGTTCTGGGGACAGAAGATTTATATGACTATATTGACAAAT
ACAACATTGAATTAGATCCACGTTTCAATGATATCTTGGGCAGACACTC
TCGAAAGCGATGGGAACGCTTTGTCCACAGTGAAAATCAGCACCTTGTC
AGCCCTGAGGCCTTGGATTTCCTGGACAAACTGCTGCGATATGACCACC
AGTCACGGCTTACTGCAAGAGAGGCAATGGAGCACCCCTATTTCTACAC
TGTTGTGAAGGACCAGGCTCGAATGGGTTCATCTAGCATGCCAGGGGGC
AGTACGCCCGTCAGCAGCGCCAATATGATGTCAGGGATTTCTTCAGTGC
CAACCCCTTCACCCCTTGGACCTCTGGCAGGCTCACCAGTGATTGCTGC
TGCCAACCCCCTTGGGATGCCTGTTCCAGCTGCCGCTGGCGCTCAGCAG
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