

Certificate of Analysis

Casein Kinase 2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-197, 14-197-K, 14-197M

Parent Lot # 2363294

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: An *N*-terminal 6His-tagged full-length human CK α subunit reconstituted with *N*-terminal GST-tagged full-length human CK β subunit. These are expressed individually by baculovirus in Sf21 insect cells. The CK α subunit was purified using Ni²⁺/NTA-agarose, the β subunit using glutathione-agarose, and the purified subunits reconstituted *in vitro*. Purity of alpha/beta mix = 86.9% by SDS-PAGE and Coomassie blue staining. α subunit MW = 48.7kDa, β subunit MW = 52.6kDa.

Formulation: 1.668mg/ml of 20mM MOPS pH7.0, 1mM EDTA, 50% glycerol, 0.1mM Triton X-100, 0.3% 2-mercaptoethanol, 1mM benzamidine. Liquid at -20°C.

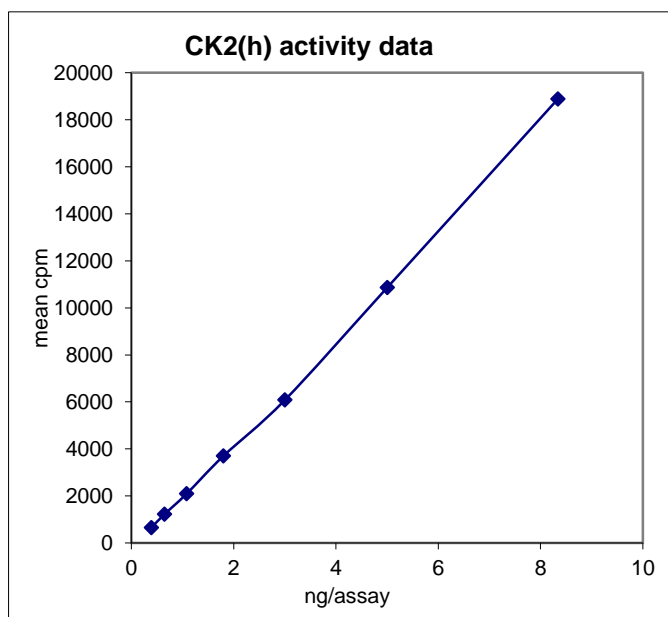
Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Specific Activity (Parent lot# 2363294): 2296U/mg, where one unit of Casein Kinase 2 $\alpha\beta$ activity is defined as 1nmol phosphate incorporated into 165 μ M casein kinase 2 substrate peptide (RRRDDSDDD) per minute at 30°C with a final ATP concentration of 100 μ M.

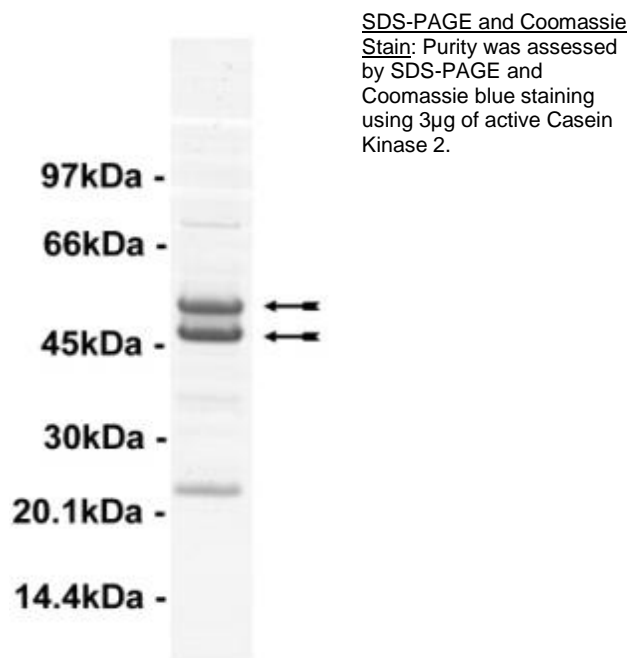
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.4–8.3ng of this lot of enzyme phosphorylated 165 μ M CK2 substrate peptide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as Casein Kinase 2 with the translated sequence listed on pages three and four.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 1. 4 x Reaction Buffer:** 80mM HEPES pH7.6, 0.6M NaCl, 0.4mM EDTA, 20mM DTT, 0.4% Triton X-100.
- 2. CK2 substrate peptide (RRRDDDSDDD):** Use at a final assay concentration of 165µM. Prepare a 3.3mM stock. Add 1.25µl of stock per assay point.
- 3. Casein Kinase 2, active:** Dilute with 20mM HEPES pH7.6, 0.15M NaCl, 0.1mM EGTA, 5mM DTT, 0.1% Triton X-100, 50% glycerol. Use 0.4–8.3ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 6.25µl of 4 x reaction buffer per assay to wells
2. Add 1.25µl of **CK2 substrate peptide**.
3. Add **2.5µl (0.4–8.3ng) Casein Kinase 2, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Casein Kinase 2 alpha subunit Sequence Information

<u>Protein</u>	Human Casein Kinase 2 alpha
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M29 of the fusion protein is equivalent to M1 of human casein kinase 2 alpha
<u>Accession number</u>	EMBL J02853

Recombinant CK2α amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPM S GPVPSRARVY TDVNTHRPRE YWDYESHVVE
61 WGNQDDYQLV RKLGRGKYSE VFEAINITNN EKVVVKILKP VKKKKIKREI KILENLRGGP
121 NIITLADIVK DPVSRTPALV FEHVNNTDFK QLYQTLTDYD IRFYMYEILK ALDYCHSMGI
181 MHRDVKPHNV MIDHEHRKLR LIDWGLAEFY HPGQEYNVRV ASRYFKGPPEL LVDYQMYDYS
241 LDMWSLGCML ASMIFRKEPF FHGHDNYDQL VRIAKVLGTE DLYDYIDKYN IELDPRFNDI
301 LGRHSRKRWE RFVHSENQHL VSPEALDFLD KLLRYDHQSR LTAREAMEHP YFYTVVKDQA
361 RMGSSSMPGG STPVSSANMM SGISSVPTPS PLGPLAGSPV IAAANPLGMP VPAAAGAQQ
  
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Recombinant CK2α nucleotide sequence:

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1 atgtcg tact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tcctatgtcg ggacccgtgc caagcagggc cagagtttac
121 acagatgtta atacacacag acctcgagaa tactgggatt acgagtcaca tgtgggtgaa
181 tggggaaatc aagatgacta ccagctgggt cgaaaattag gccgaggtaa atacagtga
241 gtatttgaag ccatcaacat cacaaataat gaaaaagtgt ttgttaaaat tctcaagcca
301 gtaaaaaaga agaaaattaa gcgtgaaata aagattttgg agaatttgag aggaggtccc
361 aacatcatca cactggcaga cattgtaaaa gaccctgtgt cacgaacccc cgccttggtt
421 tttgaacacg taaacaacac agacttcaag caattgtacc agacgttaac agactatgat
481 attcgat tttt acatgtatga gattctgaag gccctggatt attgtcacag catgggaatt
541 atgcacagag atgtcaagcc ccataatgtc atgattgatc atgagcacag aaagctacga
601 ctaatagact ggggtttggc tgagttttat catcctggcc aagaatataa tgtccgagtt
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721 ttggatatgt ggagtttggg ttgtatgctg gcaagtatga tctttcggaa ggagccattt
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1021 cttactgcaa gagaggcaat ggagcacccc tatttctaca ctggttgtaa ggaccaggct
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1141 tcagggattt cttcagtgcc aacccttca ccccttggac ctctggcagg ctcaccagtg
1201 attgctgctg ccaacccct tgggatgcct gttccagctg ccgctggcgc tcagcagtaa
  
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Certificate of Analysis

Casein Kinase 2 beta subunit Sequence Information

<u>Protein</u>	Human Casein Kinase 2 beta
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M237 of the fusion protein is equivalent to M1 of human Casein Kinase 2 beta
<u>Accession number</u>	GenBank NM_001320

Recombinant CK2 β amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGP EF KGLRRPMSSS
241 EEVSWISWFC GLRGNEFFCE VDEDYIQDKF NLTGLNEQVP HYRQALDMIL DLEPDEELED
301 NPNQSDLIEQ AAEMLYGLIH ARYILTNRGI AQMLEKYQQG DFGYCPRVYC ENQPMLPIGL
361 SDIPGEAMVK LYCPKCMDVY TPKSSRHHHT DGAYFGTGFP HMLFMVHPEY RPKR PANQFV
421 PRLYGFKIHP MAYQLQLQAA SNFKSPVKTI R

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Recombinant CK2 β nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttcca atcttcctta ttatattgat
181 ggtgatgta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagtcc tgtccaggg gcccaattc aaaggcctac gtcgacctat gaggcagctca
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781 gtggatgaag actacatcca ggacaaattt aatcttactg gactcaatga gcaggctcct
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1141 acaccaagt catcaagaca ccatcacacg gatggcgcct acttcggcac tggtttcctt
1201 cacatgctct tcatggtgca tcccagatc cggcccaaga gacctgcaa ccagtttgtg
1261 cccaggctct acggtttcaa gatccatccg atggcctacc agctgcagct ccaagccgcc
1321 agcaacttca agagcccagt caagacgatt cgctga

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Reviewed and approved by site quality representative.

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