

Certificate of Analysis

Casein Kinase 2 α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-445, 14-445-K, 14-445M

Parent Lot # 2363220

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: N-terminal 6His-tagged, recombinant, human, full length Casein Kinase 2 alpha subunit. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 96.5% by SDS-PAGE and Coomassie blue staining. MW = 48.7kDa.

Formulation: 1.26mg/ml of enzyme in 20mM MOPS pH7.4, 1mM EDTA, 50% glycerol, 1mM benzamidine, 0.1% Triton X-100, 0.3% 2-mercaptoethanol. Liquid at -20°C.

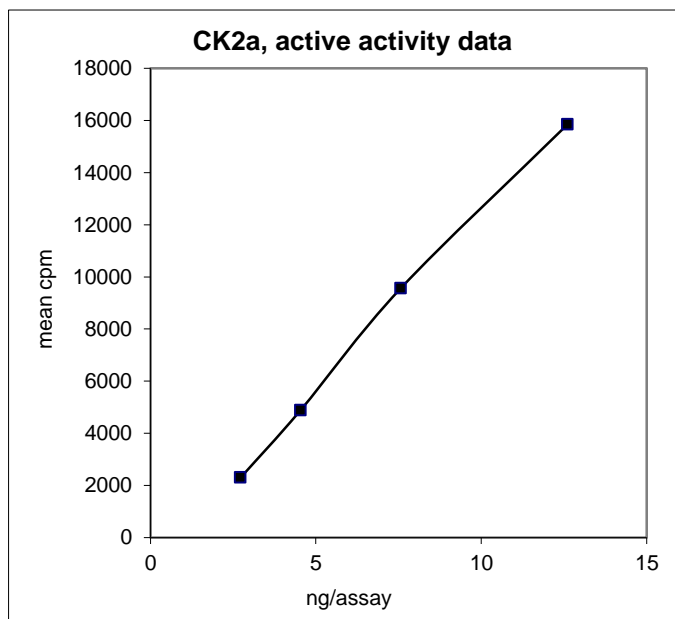
Specific Activity (Parent lot# 2363220): 1409U/mg, where one unit of Casein Kinase 2, active activity is defined as 1nmol phosphate incorporated into 165 μ M Casein Kinase 2 substrate peptide per minute at 30°C with a final ATP concentration of 100 μ M.

Storage and Stability: On receipt of material store at -70°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.

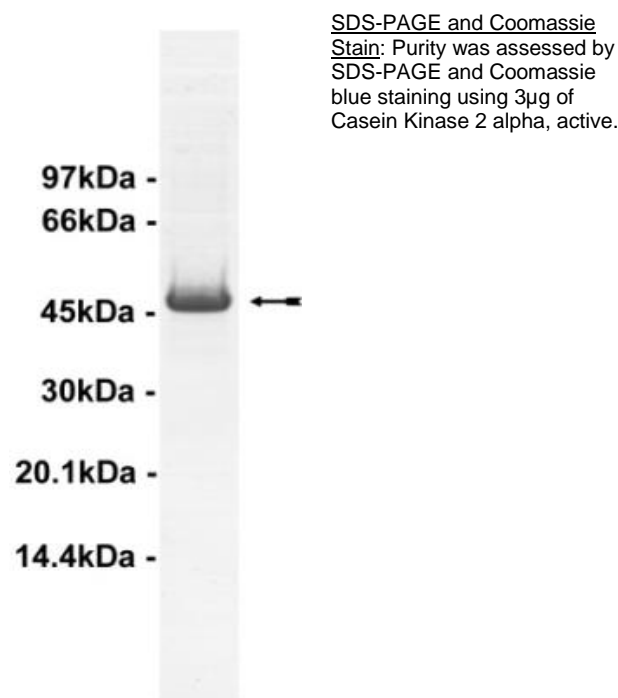
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3–13ng of this lot of enzyme phosphorylated 165 μ M Casein Kinase 2 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 identity as Casein Kinase 2 alpha with the translated sequence listed on page three.



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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. Casein Kinase 2 substrate peptide:** Use at a final concentration of 165 μ M. Prepare a 3.3mM stock and add 1.25 μ l of stock per assay point.
- 3. Casein Kinase 2 alpha, active:** Dilute with 20mM HEPES pH7.6, 0.15M NaCl, 0.1mM EGTA, 0.1% Triton X-100, 5mM DTT, 50% glycerol. Use 3–13ng 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 6.25 μ l of 4 x reaction buffer per assay to wells.
2. Add 1.25 μ l of **Casein Kinase 2 substrate peptide**.
3. Add **2.5 μ l (3–13ng) Casein Kinase 2 alpha, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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Casein Kinase 2 alpha Sequence Information

Protein	human Casein Kinase 2, alpha subunit
Tags	N-terminal 6His
Native sequence	M29 of the recombinant protein is equivalent to M1 of human Casein Kinase 2 alpha
Accession number	EMBL J02853

Recombinant Casein Kinase 2 alpha amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMPDMS GPVPSRARVY TDVNTHRPRE YWDYESHVVE
61 WGNQDDYQLV RKLGRGKYSE VFEAINITNN EKVVVKILKP VKKKKIKREI KILENLRGGP
121 NIITLADIVK DPVSRTPALV FEHVNNTDFK QLYQTLTDYD IRFMYEILK ALDYCHSMGI
181 MHRDVKPHNV MIDHEHRKLR LIDWGLAEFY HPGQEYNVRV ASRYFKGPEL 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 Casein Kinase 2 alpha nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcctatgga tcctatgtcg ggaccctgac caagcagggc cagagtttac
121 acagatgtta atacacacag acctcgagaa tactgggatt acgagtcaca tgtggtggaa
181 tggggaaatc aagatgacta ccagctgggt cgaaaattag gccgaggtaa atacagttaa
241 gtatttgaag ccatcaacat cacaataat gaaaaagttg ttgttaaaat tctcaagcca
301 gtaaaaaaga agaaaattaa gcgtgaaata aagatttttg agaatttgag aggaggtccc
361 aacatcatca cactggcaga cattgtaaaa gaccctgtgt cacgaacccc cgccttggtt
421 tttgaacacg taaacaacac agacttcaag caattgtacc agacgttaac agactatgat
481 attcgatatt acatgtatga gattctgaag gccctggatt attgtcacag catgggaatt
541 atgcacagag atgtcaagcc ccataatgtc atgattgatc atgagcacag aaagctacga
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1201 attgctgctg ccaacccctt tgggatgctt gttccagctg ccgctggcgc tcagcagtaa

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