

Certificate of Analysis

Casein Kinase 2 α 2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-689, 14-689-K, 14-689M

Parent Lot # 28467U

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, full length, human Casein Kinase 2 α 2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 93% by SDS-PAGE and Coomassie blue staining. MW = 45.8kDa.

Specific Activity (Parent lot# 28467U): 3079U/mg, where one unit of Casein Kinase 2 α 2, active activity is defined as 1nmol phosphate incorporated into 165 μ M casein Kinase 2 substrate peptide (RRRDDSDDDD) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.719mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

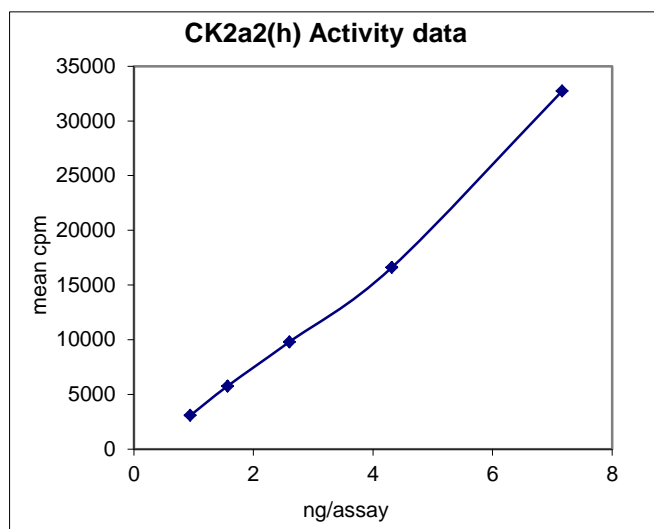
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

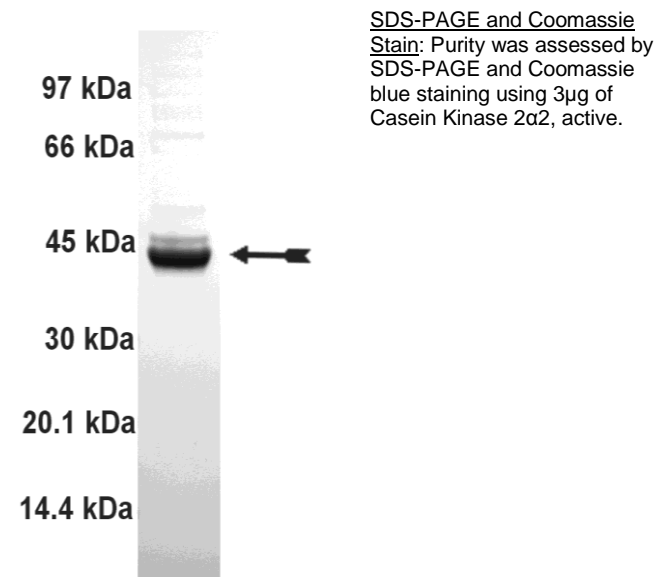
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.9–7.2ng of this lot of enzyme phosphorylated 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 α 2, with the translated native sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
- 2. Casein Kinase 2 substrate peptide (RRRDDDSDDD):** Use at a final assay concentration of 330 μ M. Prepare a 3.3mM stock. Add 2.5 μ l of stock per assay point.
- 3. Casein Kinase 2 α 2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.9–7.2ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **casein Kinase 2** substrate peptide.
3. Add **2.5 μ l (0.9–7.2ng) Casein Kinase 2 α 2, 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.

Casein Kinase 2α2 Sequence Information

Protein	Human Casein Kinase 2α2
Tags	N-terminal 6His
Native sequence	M37 of recombinant protein is equivalent to M1 of human Casein Kinase 2α2
Accession number	GenBank NM_001896

Recombinant Casein Kinase 2α2 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPGP AAGSRARVYA EVNSLRSREY
61 WDYEAHVPSW GNQDDYQLVR KLGRGKYSEV FEAINITNNE RVVVKILKPV KKKKIKREVK
121 ILENLRGGTN IIKLIDTVKD PVSKTALVF EYINNTDFKQ LYQILTDFDI RFYMYELLKA
181 LDYCHSKGIM HRDVKPHNVM IDHQQKLRRL IDWGLAEFYH PAQEYNRVA SRYFKGPELL
241 VDYQMYDYSL DMWSLGCMLA SMIFRREPEF HGQDNYDQLV RIAKVLGTEE LYGYLKKYHI
301 DLDPHFNDIL GQHSRKRWEN FIHSENRHLV SPEALDLLDK LLRYDHQQRLL TAKEAMEHPY
361 FYPVWKEQSQ PCADNAVLSS GLTAAR
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Recombinant Casein Kinase 2α2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcccgccccg
121 gccgcgggca gcaggcccg ggtctacgcc gaggtgaaca gtctgaggag ccgcgagtac
181 tgggactacg aggctcacgt cccgagctgg ggtaatcaag atgattacca actggttcga
241 aaacttggtc ggggaaaata tagtgaagta tttgaggcca ttaatatac caacaatgag
301 agagtggttg taaaaatcct gaagccagtg aagaaaaaga agataaaacg agaggttaag
361 attctggaga accttcgtgg tgaacaaat atcattaagc tgattgacac tgtaaaggac
421 cccgtgtcaa agacaccagc tttggtattt gaatatatca ataatacaga ttttaagcaa
481 ctctaccaga tcctgacaga ctttgatatc cggttttata tgtatgaact acttaaagct
541 ctggattact gccacagcaa gggaatcatg cacagggatg tgaaacctca caatgtcatg
601 atagatcacc aacagaaaaa gctgcgactg atagattggg gtctggcaga attctatcat
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1141 ggtctcacgg cagcacgata a
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