

Certificate of Analysis

CaM Kinase II α , active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-962, 14-962-K, 14-962M

Parent Lot # D15CP011N

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 CaM kinase II α full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography

Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 55 kDa.

Specific Activity (Parent lot# D15CP011N): 9003U/mg, where one unit of CaM kinase II α , active activity is defined as 1nmol phosphate incorporated into 250 μ M KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.54mg/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.

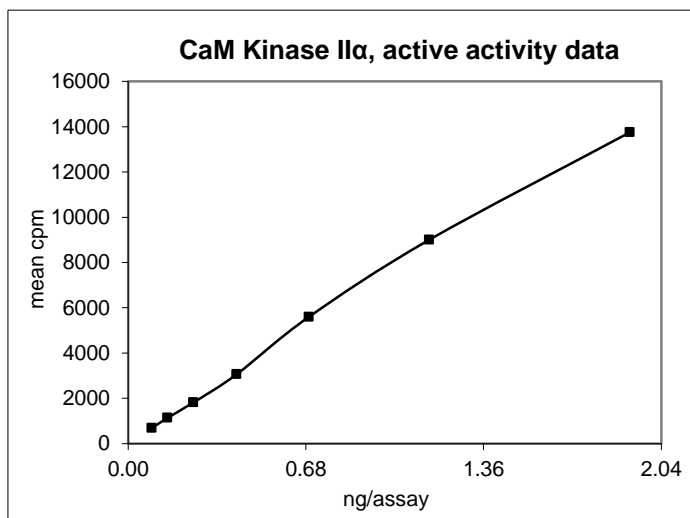
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

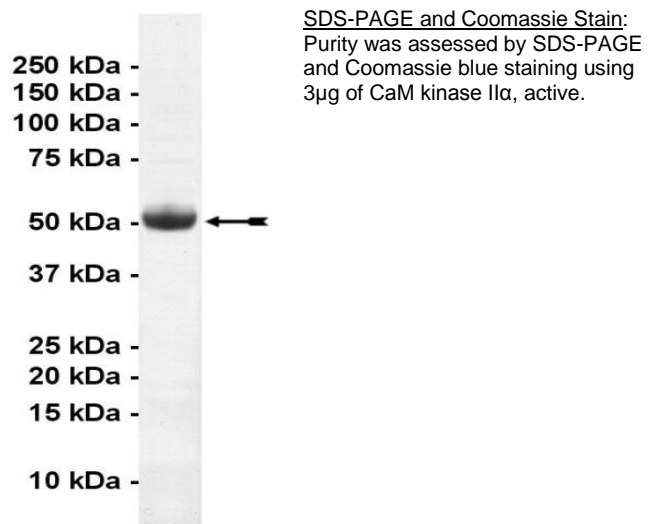
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.9–1.9ng of this lot of enzyme phosphorylated 250 μ M KKLNRTLSFAEPG 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 CaM kinase II α with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- KKLNRTLSFAEPG:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
- Calcium Chloride:** Use at a final assay concentration of 0.5mM. Prepare a 50mM stock and use 0.25 μ l per assay point.
- Calmodulin:** Use at a final assay concentration of 0.016mg/ml. Prepare a 0.3mg/ml stock and use 1.325 μ l per assay point.
- CaM kinase II α , active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.9–1.9ng per assay point.
- [γ -³³P]ATP:** 2.5 x MgAc/[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):

- Add 5 μ l of 5 x reaction buffer per assay to wells.
- Add 3.425 μ l of dH₂O.
- Add 0.25 μ l of 50mM calcium chloride.
- Add 1.325 μ l of 0.3mg/ml calmodulin.
- Add 2.5 μ l of KKLNRTLSFAEPG.
- Add **2.5 μ l (0.09–1.9ng) CaM kinase II α , active.**
- Add 10 μ l of diluted [γ -³³P]ATP mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5 μ l of 3% phosphoric acid.
- Transfer a 10 μ l aliquot onto the appropriate area of a P30 Filtermat.
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
- 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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CaM kinase II α Sequence Information

<u>Protein</u>	human CaM kinase II α
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human CaM kinase II α
<u>Accession number</u>	GenBank AF091486.1

Recombinant CaM kinase II α amino acid sequence:

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1 MHHHHHHMAT ITCTRFTEEY QLFEELGKGA FSVVRRCKV LAGQEYAAKI INTKCLSARD
61 HQKLEREARI CRLKHPNIV RLHDSISEEG HHYLIFDLVT GGELFEDIVA REYYSEADAS
121 HCIQQILEAV LHCHQMGVVH RDLKPENLLL ASKLKGA AVK LADFGLAIEV EGEQQAWFGF
181 AGTPGYLSPE VLRKDPYGKP VDLWACGVIL YILLVGYPPF WDEDQHRLYQ QIKAGAYDFP
241 SPEWDTVTPE AKDLINKMLT INPSKRITAA EALKHPWISH RSTVASCMMR QETVDCLKKF
301 NARRKLGAI LTTMLATRNF SGGKSGGNKK SDGVKESSES TNTTIEDEDT KVRKQEI IKV
361 TEQLIEAISN GDFESYTKMC DPGMTAFEPE ALGNLVEGLD FHRFYFENLW SRNSKPVHTT
421 ILNPHIHLMG DESACIAYIR ITQYLDAGGI PRTAQSEETR VWHRRDQKQW IVHFHRS GAP
481 SVLPH
  
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Recombinant CaM kinase II α nucleotide sequence:

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1 atgcatcatt accatcacca tatggccacc atcacctgca cccgcttcac ggaagagtac
61 cagctcttcg aggaattggg caaggaggcc ttctcgggtg tgcaaggtg tgtgaaggtg
121 ctggctggcc aggagtatgc tgccaagatc atcaacacaa agaagctgtc agccagagac
181 catcagaagc tggagcgtga agcccgcata tgccgcctgc taaagcacc caacatcgtc
241 cgactacatg acagcatctc agaggaggga caccactacc tgatcttcga cctggctcact
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1441 tccgtcctgc cccactga
  
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