

Certificate of Analysis

CaM Kinase IV, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-547, 14-547-K, 14-547M

Parent Lot #1838057

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: C-terminal 6His-tagged, recombinant, full-length, human CaM Kinase IV, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Activated with CaM Kinase Kinase and repurified using glutathione-agarose and Ni²⁺/NTA-agarose. Purity 86.2% by SDS-PAGE and Coomassie blue staining. MW = 52.9kDa.

Specific Activity (Parent lot# 1838057): 1785U/mg, where one unit of CaM Kinase IV activity is defined as 1nmol phosphate incorporated into 30µM substrate peptide (KKLNRTLVA), cat# 12-240) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.698mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

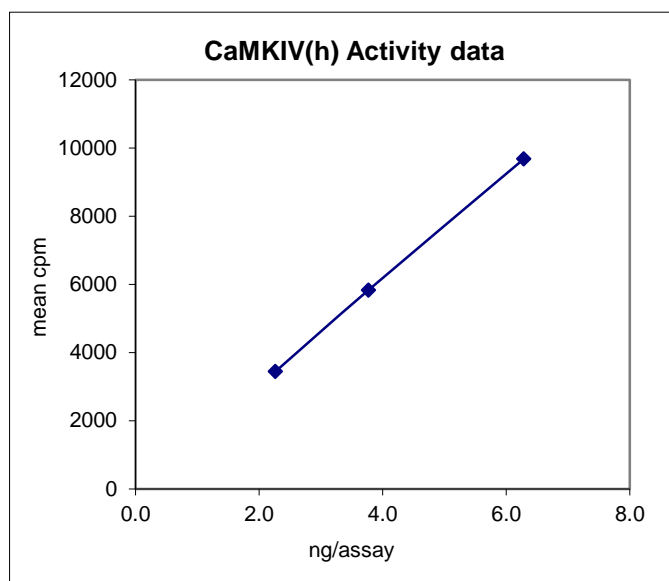
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

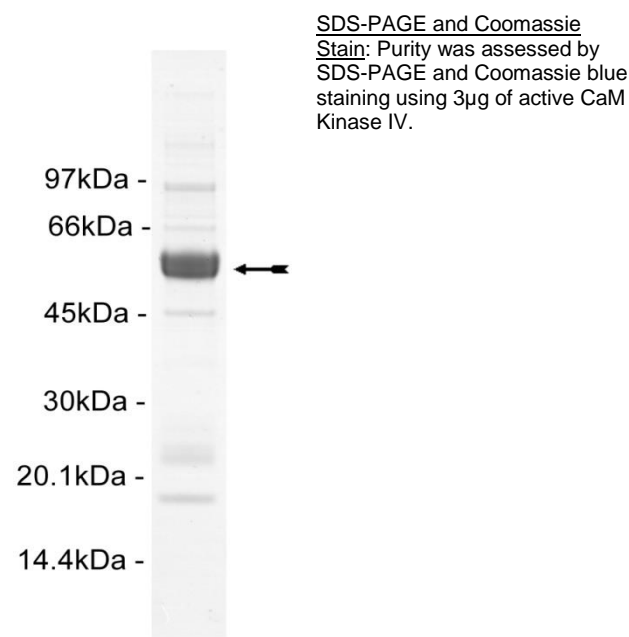
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.3–6.3ng of this lot of enzyme phosphorylated 30µM substrate peptide (KKLNRTLVA), cat# 12-240) 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 CaM Kinase IV with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 200mM HEPES pH7.4.
2. **CaCl₂:** Use at a final assay concentration of 5mM. Prepare a 50mM stock and add 2.5µl of stock per assay point.
3. **Calmodulin (Catalogue# 14-368):** Supplied as a 10 x stock solution. Add 2.5µl of stock per assay point.
4. **(KKLNRTLVA):** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.
5. **CaM Kinase IV, active:** Dilute in 40mM HEPES pH7.4, 1 mg/ml BSA. Use 2.3–6.3ng per assay point.
6. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKLNRTLVA)**.
3. Add 2.5µl of 50mM CaCl₂.
4. Add 2.5µl of calmodulin.
5. Add **2.5µl (2.3–6.3ng) CaM Kinase IV, active**.
6. Add 10µl of diluted [γ-³³P] ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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CAM Kinase IV Sequence Information

<u>Protein</u>	Human CaM Kinase IV
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human CaM Kinase IV
<u>Accession number</u>	GenBank D30742

Recombinant CaM Kinase IV amino acid sequence:

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1 MLKVTVPSCS ASSCSSVTAS AAPGTASLVP DYWIDGSNRD ALSDFFEVEES ELGRGATSIV
61 YRCKQKGTQK PYALKVLKKT VDKKIVRTEI GVLLRLSHPN IIKLKEIFET PTEISLVLEL
121 VTGGELFDRI VEKGYYSERD AADAVKQILE AVAYLHENGI VHRDLKPENL LYATPAPDAP
181 LKIADFGLSK IVEHQVLMKT VCGTPGYCAP EILRGCAYGP EVDMMWSVGII TYILLCGFEP
241 FYDERGDQFM FRRILNCEYY FISPWWEVS LNAKDLVRKL IVLDPKKRLT TFQALQHPWV
301 TGKAANFVHM DTAQKKLQEF NARRKLKAAV KAVVASSRLG SASSSHGSIQ ESHKASRDPS
361 PIQDGNEDMK AIPEGEKIQQ DGAQAAVKGA QAEMLKVQAL EKVKGADINA EEAPKMVPKA
421 VEDGIKVADL ELEEGLAEK LKTVEEAAAP REGQGSSAVG FEVPQQDVIL PEYHHHHHH

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Recombinant CaM Kinase IV nucleotide sequence:

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1 atgctcaaag tcacggtgcc ctctgctcc gcctcgctct gctcttcggt caccgccagt
61 gcggccccgg ggaccgcgag cctcgctccg gattactgga tcgacggctc caacagggat
121 gcgctgagcg atttcttcga ggtggagtcg gagctgggac ggggtgctac atccattgtg
181 tacagatgca aacagaaggg gaccagaag ccttatgctc tcaaagtgtt aaagaaaaca
241 gtggacaaaa aaatcgtaag aactgagata ggagttcttc ttcgcctctc acatccaaac
301 attataaaac ttaaagagat atttgaaacc cctacagaaa tcagtctggt cctagaactc
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1321 ctgaagactg tggaggagc agcagctccc agagaagggc aaggaagctc tgctgtgggt
1381 tttgaagtcc cacagcaaga tgtgatcctg ccagagtacc atcacatca ccatcattaa

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