

Certificate of Analysis

CaM Kinase Iδ, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-731, 14-731-K, 14-731M

Parent Lot # 31350U

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 CaM Kinase Iδ, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 87.6% by SDS-PAGE and Coomassie blue staining. MW = 46.7kDa.

Specific Activity (Parent lot# 31350U): 17815U/mg, where one unit of CaM Kinase Iδ, active activity is defined as 1nmol phosphate incorporated into 150μM (KKLRRTLSVA) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 4.14mg/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.

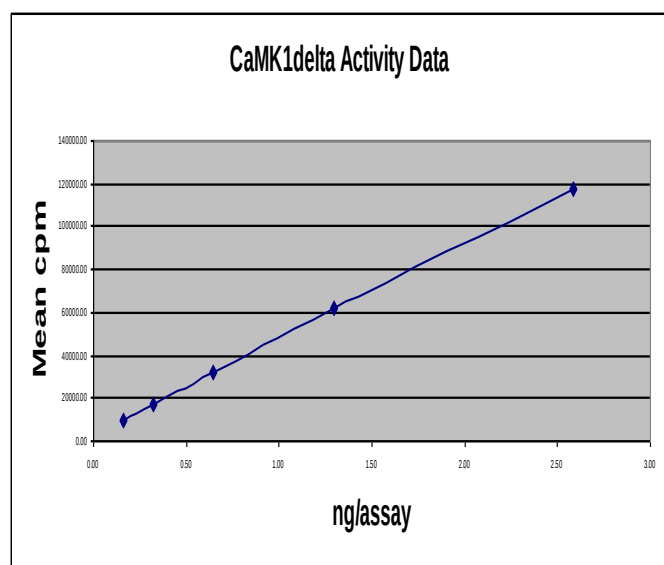
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

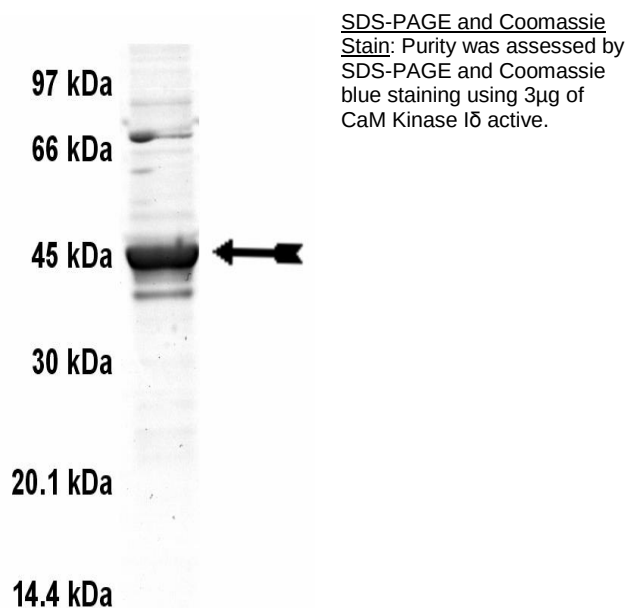
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.16–2.59ng of this lot of enzyme phosphorylated 150μM (KKLRRTLSVA) 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 Iδ with the translated native sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKLRRTLSVA):** Use at a final concentration of 150 μ M. Make up a 1.5mM stock. Use 2.5 μ l of stock per assay point.
3. **CaCl₂:** Use at a final assay concentration of 500 μ M. Make up a 5mM stock. Add 2.5 μ l of stock per assay point.
4. **Calmodulin:** Use at a final assay concentration of 1 μ M. Make up a 0.3mg/ml stock. Add 1.33 μ l of stock per assay point.
5. **CaM Kinase I δ , active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.16–2.59ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLRRTLSVA)**
3. Add **2.5 μ l (0.16–2.59ng), CaM Kinase I δ active.**
4. Add 1.17 μ l of dH₂O.
5. Add 2.5 μ l of CaCl₂.
6. Add 1.33 μ l of Calmodulin.
7. Add 10 μ l of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
10. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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 Iδ Sequence Information

<u>Protein</u>	Human CaM Kinase Iδ
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of recombinant protein is equivalent to M1 of native Human CaMKIδ
<u>Accession Number</u>	GenBank NM_153498

Recombinant CaM Kinase Iδ amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MARENGESS SWKKQAEDIK KIFEFKETLG
61 TGAFSEVVLV EEKATGKLFA VKCIPKKALK GKESSEIENEI AVLRKIKHEN IVALEDIYES
121 PNHLVLMQQL VSGGELFDRI VEKGFYTEKD ASTLIRQVLD AVYYLHRMGI VHRDLKPENL
181 LYYSQDEESK IMISDFGLSK MEGKGDVMST ACGTPGYVAP EVLAQKPYSK AVDCWSIGVI
241 AYILLCGYPP FYDENDSKLF EQILKAEYEF DSPYWDDISD SAKDFIRNLM EKDPNKRYTC
301 EQAARHPWIA GDTALNKNIH ESVSAQIRKN FAKSKWRQAF NATAVVRHMR KLHLGSSLDS
361 SNASVSSSL LASQKDCCLAP STLCSFISS SGVSGVGAER RPRPTTVTAV HSGSK

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Recombinant CaM Kinase Iδ nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgcatgga tccggaattc atggcccggg agaacggcga gaggagctcc
121 tcctggaaaa agcaagctga agacatcaag aagatcttcg agttcaaaga gaccctcgga
181 accggggcct tttccgaagt ggtttttagct gaagagaagg caactggcaa gctctttgct
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1201 agaccagcg ccaccactgt gacggcagtg cactctggaa gcaagtga

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