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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 Ni^{2+}/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 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.

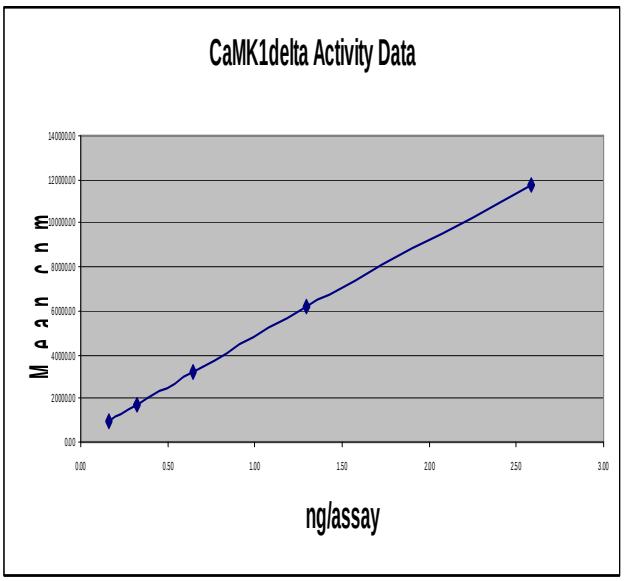
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

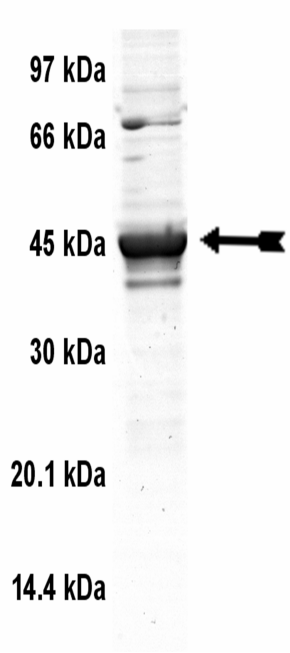
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN H UMANS OR ANIMALS

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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CaM Kinase Iδ active.

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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 150µM. Make up a 1.5mM stock. Use 2.5µl of stock per assay point.
3. **$CaCl_2$:** Use at a final assay concentration of 500µM. Make up a 5mM stock. Add 2.5µl of stock per assay point.
4. **Calmodulin:** Use at a final assay concentration of 1 µM. Make up a 0.3mg/ml stock. Add 1.33µl of stock per assay point.
5. **CaM Kinase Iδ, 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [$\gamma^{3\text{-}3}$P]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 **(KKLRRTLSVA)**
3. Add **2.5µl (0.16–2.59ng)**, **CaM Kinase Iδ active**.
4. Add 1.17µl of dH_2O.
5. Add 2.5µl of $CaCl_2$.
6. Add 1.33µl of Calmodulin.
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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CaM Kinase Iδ Sequence Information

Protein Human CaM Kinase Iδ

Tags *N*-terminal 6His

Native sequence M31 of recombinant protein is equivalent to M1 of native Human CaMKIδ

Accession Number GenBank NM_153498

Recombinant CaM Kinase Iδ amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MARENGESSS SWKKQAEDIK KIFEFKETLG
 61 TGAFSEVVLA EEKATGKLFA VKCIPKKALK GKESSIENEI AVLRKIKHEN IVALEDIYES
121 PNHLYLVMQL 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 SNASVSSSLS LASQKDCLAP STLCSFISSS 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 gcgccatgga tccggaattc atggcccggg agaacggcga gagcagctcc
 121 tcctggaaaa agcaagctga agacatcaag aagatcttcg agttcaaaga gaccctcgga
 181 accggggcct tttccgaagt ggttttagct gaagagaagg caactggcaa gctctttgct
 241 gtgaagtgta tccctaagaa ggcgctgaag ggcaaggaaa gcagcataga gaatgagata
 301 gccgtcctga gaaagattaa gcatgaaaat attgttgccc tggaagacat ttatgaaagc
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1201 agacccaggc ccaccactgt gacggcagtg cactctggaa gcaagtga
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Reviewed and approved by site quality representative.