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Certificate of Analysis

CaM Kinase IIδ, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-723, 14-723-K, 14-723M
Parent Lot # 1838058

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 IIδ, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 90.3% by SDS-PAGE and Coomassie blue staining. MW = 58kDa.

Specific Activity (Parent lot# 1838058): 16527U/mg, where one unit of CaM Kinase IIδ activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.38mg/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.

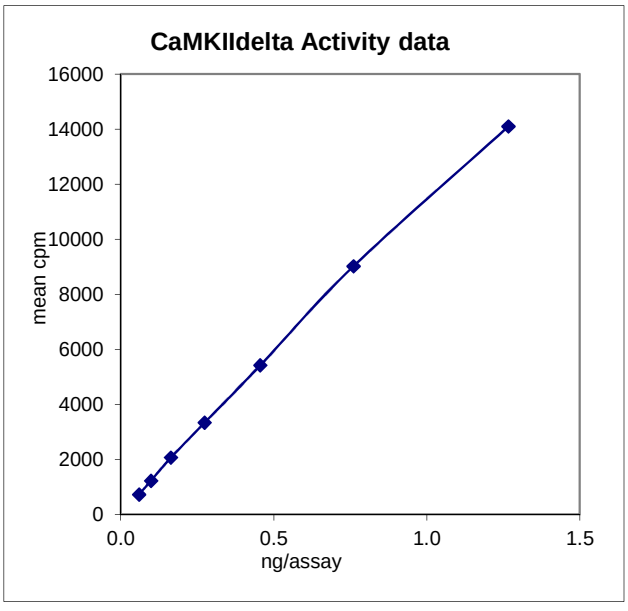
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

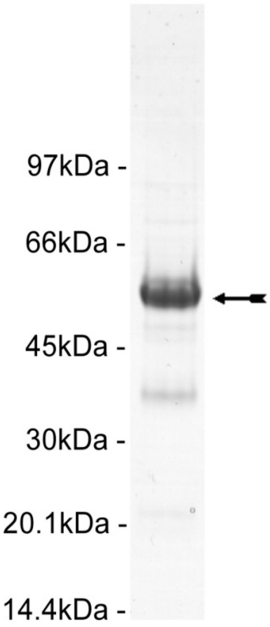
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.1–1.3ng of this lot of enzyme phosphorylated 250µM (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 IIδ 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 IIδ active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSFAEPG):** Use at a final concentration of 250µM. Make up a 2.5mM stock. Add 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 1µM. Make up a 0.3mg/ml stock. Add 1.33µl of stock per assay point.
5. **CaM Kinase IIδ, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.1–1.3ng 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 [γ-^{33}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 **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (0.1–1.3ng) CaM Kinase IIδ 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 IIδ Sequence Information

Protein Human CaM Kinase IIδ

Tags *N*-terminal 6His

Native sequence M31 of recombinant protein is equivalent to M1 of human CaM Kinase IIδ

Accession number GenBank NM_172115

Recombinant CaM Kinase IIδ amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MASTTTCTRF TDEYQLFEEL GKGAFSVVRR
  61 CMKIPTGQEY AAKIINTKKL SARDHQKLER EARICRLLKH PNIVRLHDSI SEEGFHYLVF
 121 DLVTGGELFE DIVAREYYSE ADASHCIQQI LESVNHCHLN GIVHRDLKPE NLLLASKSKG
 181 AAVKLADFGL AIEVQGDQQA WFGFAGTPGY LSPEVLRKDP YGKPVDMWAC GVILYILLVG
 241 YPPFWDEDQH RLYQQIKAGA YDFPSPEWDT VTPEAKDLIN KMLTINPAKR ITASEALKHP
 301 WICQRSTVAS MMHRQETVDC LKKFNARRKL KGAILTTMLA TRNFSAAKSL LKKPDGVKES
 361 TESSNTTIED EDVKARKQEI IKVTEQLIEA INNGDFEAYT KICDPGLTAF EPEALGNLVE
 421 GMDFHRFYFE NALSKSNKPI HTIILNPHVH LVGDDAACIA YIRLTQYMDG SGMPKTMQSE
 481 ETRVWHRRDG KWQNVHFHRS GSPTVPIK
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Recombinant CaM Kinase IIδ nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggcttcga ccacaacctg caccaggttc
 121 acggacgagt atcagctttt cgaggagctt ggaaaggggg cattctcagt ggtgagaaga
 181 tgtatgaaaa ttcctactgg acaagaatat gctgccaaaa ttatcaacac caaaaagctt
 241 tctgctaggg atcatcagaa actagaaaga gaagctagaa tctgccgtct tttgaagcac
 301 cctaatattg tgcgacttca tgatagcata tcagaagagg gctttcacta cttggtgttt
 361 gatttagtta ctggaggtga actgtttgaa gacatagtgg caagagaata ctacagtgaa
 421 gctgatgcca gtcattgtat acagcagatt ctagaaagtg ttaatcattg tcacctaaat
 481 ggcatagttc acagggacct gaagcctgag aatttgcttt tagctagcaa atccaaggga
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 601 tggtttggtt ttgctggcac acctggatat ctttctccag aagttttacg taaagatcct
 661 tatggaaagc cagtggatat gtgggcatgt ggtgtcattc tctatattct acttgtgggg
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1201 aaaatctgtg acccaggcct tactgctttt gaacctgaag ctttgggtaa tttagtggaa
1261 gggatggatt ttcaccgatt ctactttgaa aatgctttgt ccaaaagcaa taaaccaatc
1321 cacactatta ttctaaaccc tcatgtacat ctggtagggg atgatgccgc ctgcatagca
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1441 gagactcgtg tgtggcaccg ccgggatgga aagtggcaga atgttcattt tcatcgctcg
1501 gggtcaccaa cagtacccat caagtaa
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