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Certificate of Analysis

CaM Kinase Iγ, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-967, 14-967-K, 14-967M
Parent Lot # D15MP006N

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 GST-tagged, recombinant, human CaM Kinase Iγ full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 80kDa.

Specific Activity (Parent lot# D15MP006N): 2386U/mg, where one unit of CaM Kinase Iγ, active activity is defined as 1nmol phosphate incorporated into 250µM KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.70mg/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.

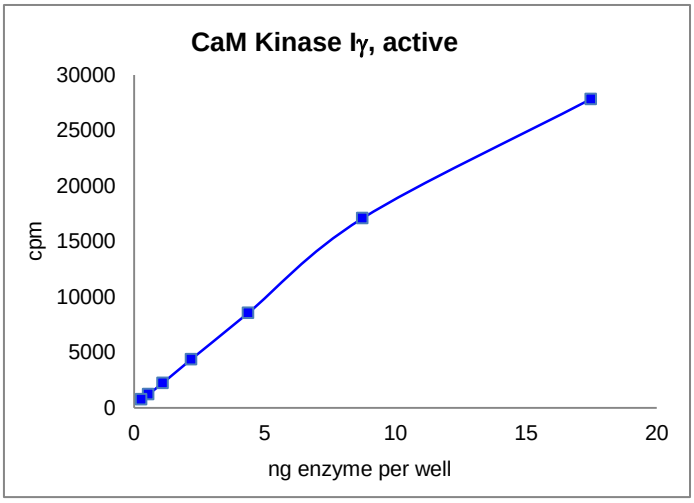
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

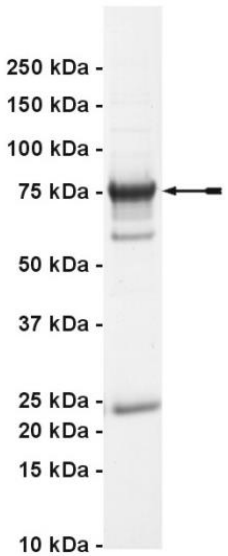
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0–17ng 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 Iγ with the translated 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 Iγ, 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 assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Calcium Chloride**: Use at a final assay concentration of 0.5mM. Prepare a 50mM stock and use 0.25µl per assay point.
4. **Calmodulin**: Use at a final assay concentration of 0.016mg/ml. Prepare a 0.3mg/ml stock and use 1.325µl per assay point
5. **CaM Kinase Iγ, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0–17ng per assay point.
6. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 3.425µl of dH_2O.
3. Add 0.25µl of 50mM calcium chloride.
4. Add 1.325µl of 0.3mg/ml calmodulin.
5. Add 2.5µl of **KKLNRTLSFAEPG**
6. Add **2.5µl (0–17ng) CaM Kinase Iγ, active.**
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 Iγ, active Sequence Information

Protein	Human CaM Kinase Iγ
Tags	*N*-terminal GST
Native sequence	M231 of the recombinant protein is equivalent to M1 of human CaM Kinase Iγ
Accession number	GenBank NM_020439.2

Recombinant CaM Kinase Iγ amino acid sequence:

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MGRKEEDDCS
241 SWKKQTTNIR KTFIFMEVLG SGAFSEVFLV KQRLTGKLFA LKCIKKSPAF RDSSLENEIA
301 VLKKIKHENI VTLEDIYEST THYYLVMQLV SGGELFDRIL ERGVYTEKDA SLVIQQVLSA
361 VKYLHENGIV HRDLKPENLL YLTPEENSKI MITDFGLSKM EQNGIMSTAC GTPGYVAPEV
421 LAQKPYSKAV DCWSIGVITY ILLCGYPPFY EETESKLFEK IKEGYYEFES PFWDDISESA
481 KDFICHLLEK DPNERYTCEK ALSHPWIDGN TALHRDIYPS VSLQIQKNFA KSKWRQAFNA
541 AAVVHHMRKL HMNLHSPGVR PEVENRPPET QASETSRPSS PEITITEAPV LDHSVALPAL
601 TQLPCQHGRR PTAPGGRSLN CLVNGSLHIS SSLVPMHQGS LAAGPCGCCS SCLNIGSKGK
661 SSYCSEPTLL KKANKKQNFK SEVMVPVKAS GSSHCRAGQT GVCLIM
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Recombinant CaM Kinase Iγ nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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 721 tcctggaaga aacagaccac caacatccgg aaaaccttca tttttatgga agtgctggga
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 961 acccactact acctggtcat gcagcttgtt tctggtgggg agctctttga ccggatcctg
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1081 gtgaaatacc tacatgagaa tggcatcgtc cacagagact taaagcccga aaacctgctt
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1201 gaacagaatg gcatcatgtc cactgcctgt gggaccccag gctacgtggc tccagaagtg
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1381 atcaaggagg gctactatga gtttgagtct ccattctggg atgacatttc tgagtcagcc
1441 aaggacttta tttgccactt gcttgagaag gatccgaacg agcggtacac ctgtgagaag
1501 gccttgagtc atccctggat tgacggaaac acagccctcc accgggacat ctacccatca
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1561 gtcagcctcc agatccagaa gaactttgct aagagcaagt ggaggcaagc cttcaacgca
1621 gcagctgtgg tgcaccacat gaggaagcta cacatgaacc tgcacagccc gggcgtccgc
1681 ccagaggtgg agaacaggcc gcctgaaact caagcctcag aaacctctag acccagctcc
1741 cctgagatca ccatcaccga ggcacctgtc ctggaccaca gtgtagcact ccctgccctg
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2041 tcggaggtca tggtaccagt taaagccagt ggcagctccc actgccgggc agggcagact
2101 ggagtctgtc tcattatgtg a
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Reviewed and approved by site quality representative.