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Certificate of Analysis

CaM Kinase IIγ, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-719, 14-719-K, 14-719M
Parent Lot # D8NN026U

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, CaM Kinase IIγ, amino acids 1–330, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 91.2% by SDS-PAGE and Coomassie blue staining. MW = 41.2kDa.

Specific Activity (Parent lot# D8NN026U): 20010U/mg, where one unit of CaM Kinase IIγ activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.885mg/ml of enzyme in 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

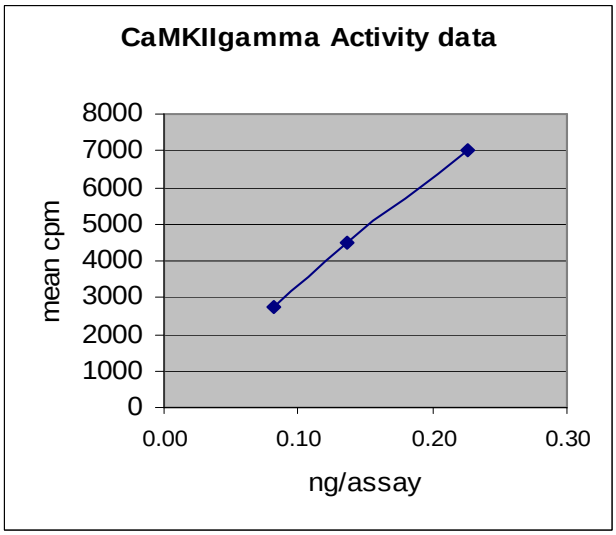
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

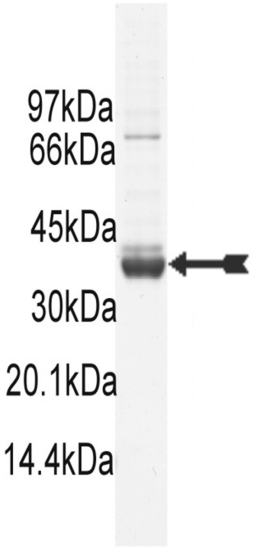
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.08–0.23ng 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 IIγ 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 IIγ, 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. 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 1µM. Make up a 0.3 mg/ml Stock. Add 1.33µl of stock per assay point.
5. **CaM Kinase IIγ, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.08–0.23ng 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 (0.08–0.23ng) CaM Kinase IIγ 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 IIγ Sequence Information

Protein Human CaM Kinase IIγ

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of CaM Kinase IIγ

Accession number GenBank NM_172171

Recombinant CaM Kinase IIγ amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MATTATCTRF TDDYQLFEEL GKGAFSVVRR
  61 CVKKTSTQEY AAKIINTKKL SARDHQKLER EARICRLLKH PNIVRLHDSI SEEGFHYLVF
 121 DLVTGGELFE DIVAREYYSE ADASHCIHQI LESVNHIHQH DIVHRDLKPE NLLLASKCKG
 181 AAVKLADFGL AIEVQGEQQA WFGFAGTPGY LSPEVLRKDP YGKPVDIWAC GVILYILLVG
 241 YPPFWDEDQH KLYQQIKAGA YDFPSPEWDT VTPEAKNLIN QMLTINPAKR ITADQALKHP
 301 WVCQRSTVAS MMHRQETVEC LRKFNARRKL KGAILTTMLV SRNFSAAKSL LNKKSDGGVK
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Recombinant CaM Kinase IIγ nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggccacca ccgccacctg cacccgtttc
 121 accgacgact accagctctt cgaggagctt ggcaagggtg ctttctctgt ggtccgcagg
 181 tgtgtgaaga aaacctccac gcaggagtac gcagcaaaaa tcatcaatac caagaagttg
 241 tctgcccggg atcaccagaa actagaacgt gaggctcgga tatgtcgact tctgaaacat
 301 ccaaacatcg tgcgcctcca tgacagtatt tctgaagaag ggtttcacta cctcgtgttt
 361 gaccttgtta ccggcgggga gctgtttgaa gacattgtgg ccagagagta ctacagtgaa
 421 gcagatgcca gccactgtat acatcagatt ctggagagtg ttaaccacat ccaccagcat
 481 gacatcgtcc acagggacct gaagcctgag aacctgctgc tggcgagtaa atgcaagggt
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1021 tccaggaact tctcagctgc caaaagccta ttgaacaaga agtcggatgg cggtgtcaag
1081 taa
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Reviewed and approved by site quality representative.