eurofins | Discovery Services

Certificate of Analysis

CaM Kinase IIβ , active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-718, 14-718-K, 14-718M
Parent Lot # 31119U

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 IIβ, amino acids 1–315, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 86% by SDS-PAGE and Coomassie blue staining. MW = 39.6kDa.

Specific Activity (Parent lot# 31119U): 14744U/mg, where one unit of CaM Kinase IIβ activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.6mg/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.

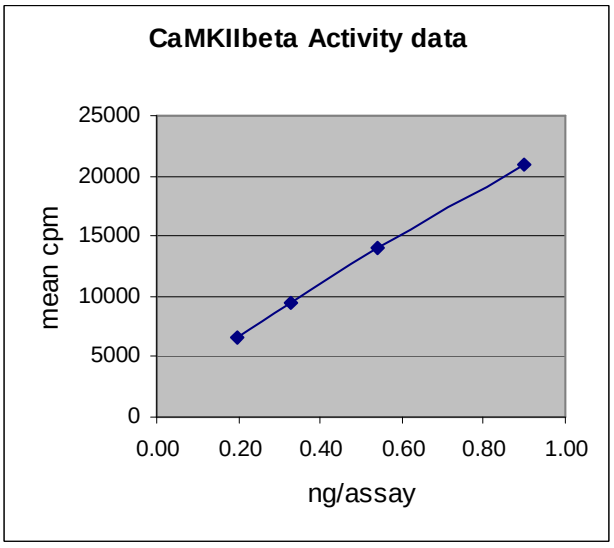
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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: 0.2–0.9ng 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 IIβ 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 IIβ , active

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

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 0.3mg/ml stock. Add 1.33µl of stock per assay point.
5. **CaM Kinase IIβ, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–0.9ng 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 (0.2–0.9ng) CaM Kinase IIβ 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.

eurofins | Discovery Services

Certificate of Analysis

CaM Kinase IIβ Sequence Information

Protein Human CaM Kinase IIβ

Tags *N*-terminal 6His

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

Accession number GenBank AF081572

Recombinant CaM Kinase IIβ amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MATTVTCTRF TDEYQLYEDI GKGAFSVVRR
  61 CVKLCTGHEY AAKIINTKKL SARDHQKLER EARICRLLKH SNIVRLHDSI SEEGFHYLVF
 121 DLVTGGELFE DIVAREYYSE ADASHCIQQI LEAVLHCHQM GVVHRDLKPE NLLLASKCKG
 181 AAVKLADFGL AIEVQGDQQA WFGFAGTPGY LSPEVLRKEA YGKPVDIWAC GVILYILLVG
 241 YPPFWDEDQH KLYQQIKAGA YDFPSPEWDT VTPEAKNLIN QMLTINPAKR ITAHEALKHP
 301 WVCQRSTVAS MMHRQETVEC LKKFNARRKL KGAILTTMLA TRNFS
```

Recombinant CaM Kinase IIβ nucleotide sequence:

```
   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggccacca cggtgacctg cacccgcttc
 121 accgacgagt accagctcta cgaggatatt ggcaaggggg ctttctctgt ggtccgacgc
 181 tgtgtcaagc tctgcaccgg ccatgagtat gcagccaaga tcatcaacac caagaagctg
 241 tcagccagag atcaccagaa gctggagaga gaggctcgga tctgccgcct tctgaagcat
 301 tccaacatcg tgcgtctcca cgacagcatc tccgaggagg gcttccacta cctggtcttc
 361 gatctggtca ctggtgggga gctctttgaa gacattgtgg cgagagagta ctacagcgag
 421 gctgatgcca gtcactgtat ccagcagatc ctggaggccg ttctccattg tcaccaaatg
 481 ggggtcgtcc acagagacct caagccggag aacctgcttc tggccagcaa gtgcaaaggg
 541 gctgcagtga agctggcaga cttcggccta gctatcgagg tgcaggggga ccagcaggca
 601 tggtttggtt tcgctggcac accaggctac ctgtcccctg aggtccttcg caaagaggcg
 661 tatggcaagc ctgtggacat ctgggcatgt ggggtgatcc tgtacatcct gctcgtgggc
 721 tacccaccct tctgggacga ggaccagcac aagctgtacc agcagatcaa ggctggtgcc
 781 tatgacttcc cgtcccctga gtgggacacc gtcactcctg aagccaaaaa cctcatcaac
 841 cagatgctga ccatcaaccc tgccaagcgc atcacagccc atgaggccct gaagcacccg
 901 tgggtctgcc aacgctccac ggtagcatcc atgatgcaca gacaggagac tgtggagtgt
 961 ctgaaaaagt tcaatgccag gagaaagctc aagggagcca tcctcaccac catgctggcc
1021 acacggaatt tctcataa
```

Reviewed and approved by site quality representative.