

## Certificate of Analysis

### CaM Kinase I, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-663, 14-663-K, 14-663M

Parent Lot # 0610042334

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 I, amino acids 2–end, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 83.7% by SDS-PAGE and Coomassie blue staining. MW = 68kDa.

**Formulation:** 3.04mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

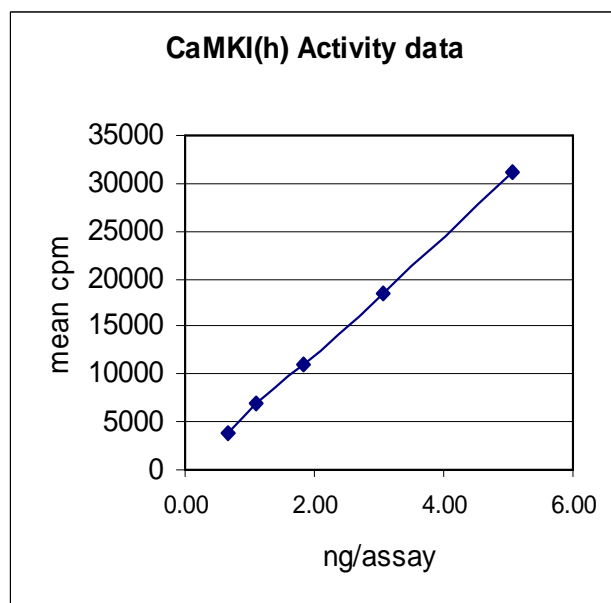
**Specific Activity (Parent lot# 0610042334):** 2960U/mg, where one unit of CaM Kinase I activity is defined as 1nmol phosphate incorporated into 250 $\mu$ M (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Storage and Stability:** On receipt of material store at -20°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.

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

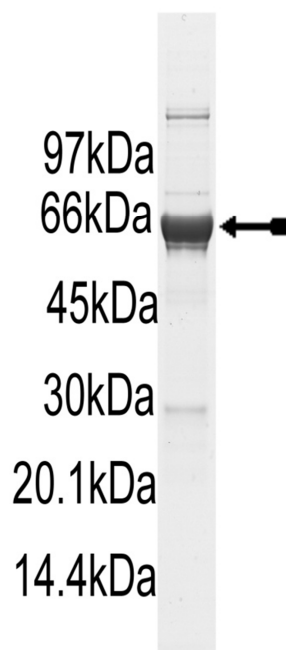
### Quality Control Testing

**Kinase Assay:** 0.7–5.1ng of this lot of enzyme phosphorylated 250 $\mu$ M (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 I 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 3 $\mu$ g of CaM Kinase I, active.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KCLNRTL SFAEPG):** Use at a final assay concentration of 250 $\mu$ M. Make a 2.5mM stock. Add 2.5 $\mu$ l of stock per assay point.
3. **CaCl<sub>2</sub>:** Use at a final assay concentration of 500 $\mu$ M. Make a 5mM stock. Add 2.5 $\mu$ l of stock per assay point.
4. **Calmodulin:** Use at a final assay concentration of 1 $\mu$ M. Make a 0.3mg/ml stock. Add 1.33 $\mu$ l of stock per assay point.
5. **CaM Kinase I, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.7–5.1 ng per assay point.
6. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **(KCLNRTL SFAEPG)**.
3. Add **2.5 $\mu$ l (0.7–5.1ng) CaM Kinase I, active**.
4. Add 2.5 $\mu$ l of 5mM CaCl<sub>2</sub>.
5. Add 1.33 $\mu$ l of 0.3mg/ml calmodulin.
6. Add 1.17 $\mu$ l of dH<sub>2</sub>O.
7. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
10. Transfer a 10 $\mu$ l 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 1 $\mu$ l of 30% phosphoric acid.

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### CaM Kinase I Sequence Information

<b><u>Protein</u></b>	Human CaM Kinase I
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	L232 of the recombinant protein is equivalent to L2 of human CaM Kinase I
<b><u>Accession number</u></b>	GenBank NM_003656

#### ***Recombinant CaM Kinase I amino acid sequence:***

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPLG  SLGAVEGPRW
241  KQAEDIRDIY  DFRDVLGTGA  FSEVILAEDK  RTQKLVAIKC  IAKEALEGKE  GSMENEIAVL
301  HKIKHPNIVA  LDDIYESGGH  LYLIMQLVSG  GELFDRIVEK  GFYTERDASR  LIFQVLDAVK
361  YLHDLGIVHR  DLKPENLLYY  SLDEDSKIMI  SDFGLSKMED  PGSVLSTACG  TPGYVAPEVL
421  AQKPYSKAVD  CWSIGVIAYI  LLCGYPPFYD  ENDAKLFEQI  LKAEYEFDSP  YWDDISDSAK
481  DFIRHLMKED  PEKRFTCEQA  LQHPWIAGDT  ALDKNIHQSV  SEQIKKNFAK  SKWKQAFNAT
541  AVVRHMRKLQ  LGTSQEGQGQ  TASHGELLTP  VAGGPAAGCC  CRDCCVEPGT  ELSPTLPHQL

```

#### ***Recombinant CaM Kinase I nucleotide sequence:***

```

1  atgtccocta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgtat
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1561  agtgagcaga  tcaagaagaa  ctttgccaag  agcaagtgga  agcaagcctt  caatgccacg
1621  gctgtggtgc  ggcacatgag  gaaactgcag  ctgggcacca  gccaggagg  gcaggggag
1681  acggcgagcc  atggggagct  gctgacacca  gtggctgggg  ggccggcagc  tggctgttgc

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## Certificate of Analysis

1741 tgtcgagact gctgcgtgga gccgggcaca gaactgtccc ccacactgcc ccaccagctc  
1801 tag

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