

Certificate of Analysis

CK1ε, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-045, 16-045-K, 16-045M

Parent Lot # D17NP008N

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 CK1ε amino acids 1-348 expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by size exclusion chromatography. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 67kDa.

Specific Activity (Parent lot# D17NP008N): 1462U/mg, where one unit of CK1ε activity is defined as 1nmol phosphate incorporated into 200μM KRRRALS(p)VASLPGL per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.17mg/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.

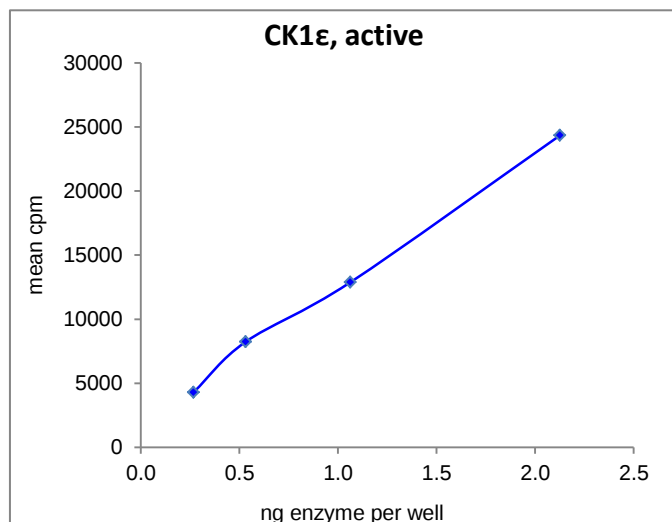
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

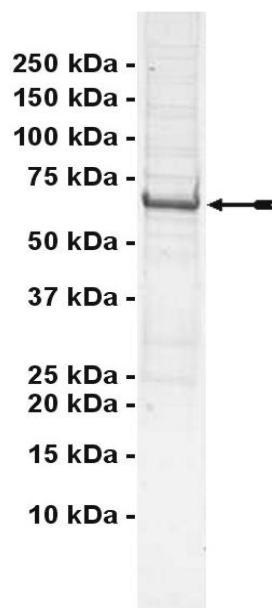
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.3–2.1ng of this lot of enzyme phosphorylated 200μM KRRRALS(p)VASLPGL 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 CK1ε 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 CK1ε, active..

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KRRRALS(p)VASLPGL:** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock and add 2.5 μ l of stock per assay point.
- 3. CK1 ϵ , active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–2.1ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KRRRALS(p)VASLPGL.
3. Add **2.5 μ l (0.3–2.1ng) CK1 ϵ , active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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CK1ε, active Sequence Information

<u>Protein</u>	Human CK1ε
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human CK1ε
<u>Accession number</u>	GenBank NM_001894.4

Recombinant CK1ε 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 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKELM ELRVGNKYRL
241 GRKIGSGSFG DIYLGANIAS GEEVAIKLEC VKTKHPQLHI ESKFYKMMQG GVGIPSIKWC
301 GAEGDYNVMV MELLGPSLED LFNFCSRKFS LKTVLLLDQ MISRIEYIHS KNFIHRDVKP
361 DNFLMGLGKK GNLVYIIDFG LAKKYRDART HQHIPYRENK NLTGTARYAS INTHLGIEQS
421 RRDDLES LGY VLMYFNLGSL PWQGLKAATK RQKYERISEK KMSTPIEVLK KGYPSEFSTY
481 LNFCSRSLRF DKPDYSYLRQ LFRNLFHRQG FSYDYVFDWN MLKFGAARNP EDVDRERREH
541 EREERMGQLR GSATRALPPG PPTGATANRL RSAAPV

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Recombinant CK1ε nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatatc ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggttg gagtttcca atcttcctta ttatattgat
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1381 aagatgtcaa cgcccatcga ggtcctctgc aaaggctatc cctccgaatt ctcaacatac
1441 ctcaacttct gccgctccct gcggtttgac gacaagcccg actactctta cctacgtcag

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1621 gaacgcgagg agaggatggg gcagctacgg gggtcgcgca cccgagccct gccccctggc
1681 ccaccacagg gggccactgc caaccggctc cgcagtgccg ccgagcccgt gtaa
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