Eurofins Discovery Products France
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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.

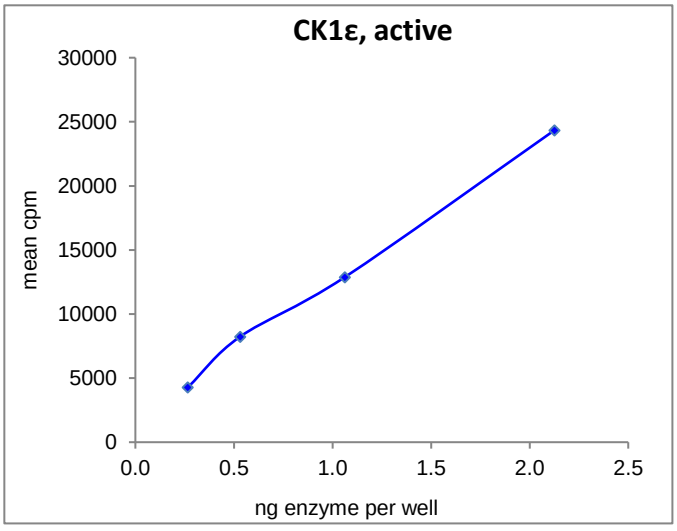
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

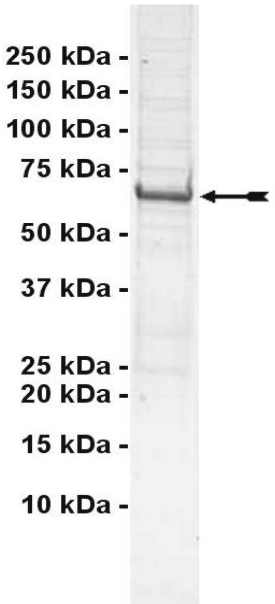
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 200µM. Prepare a 2mM stock and add 2.5µl of stock per assay point.
3. **CK1ε, 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-^{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 2.5µl of KRRRALS(p)VASLPGL.
3. Add **2.5µl (0.3–2.1ng) CK1ε, active.**
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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CK1ε, active Sequence Information

Protein	Human CK1ε
Tags	*N*-terminal GST
Native sequence	M230 of the recombinant protein is equivalent to M1 of human CK1ε
Accession number	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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKELM ELRVGNKYRL
241 GRKIGSGSFG DIYLGANIAS GEEVAIKLEC VKTKHPQLHI ESKFYKMMQG GVGIPSIKWC
301 GAEGDYNVMV MELLGPSLED LFNFCSRKFS LKTVLLLADQ MISRIEYIHS KNFIHRDVKP
361 DNFLMGLGKK GNLVYIIDFG LAKKYRDART HQHIPYRENK NLTGTARYAS INTHLGIEQS
421 RRDDLESLGY VLMYFNLGSL PWQGLKAATK RQKYERISEK KMSTPIEVLC KGYPSEFSTY
481 LNFCRSLRFD DKPDYSYLRQ LFRNLFHRQG FSYDYVFDWN MLKFGAARNP EDVDRERREH
541 EREERMGQLR GSATRALPPG PPTGATANRL RSAAEPV
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Recombinant CK1ε 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
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccaa ggaattgatg gagctacgtg tggggaacaa gtaccgcctg
 721 ggacggaaga tcgggagcgg gtccttcgga gatatctacc tgggtgccaa catcgcctct
 781 ggtgaggaag tcgccatcaa gctggagtgt gtgaagacaa agcaccccca gctgcacatc
 841 gagagcaagt tctacaagat gatgcagggt ggcgtgggga tcccgtccat caagtggtgc
 901 ggagctgagg gcgactacaa cgtgatggtc atggagctgc tggggcctag cctcgaggac
 961 ctgttcaact tctgttcccg caaattcagc ctcaagacgg tgctgctctt ggccgaccag
1021 atgatcagcc gcatcgagta tatccactcc aagaacttca tccaccggga cgtcaagccc
1081 gacaacttcc tcatggggct ggggaagaag ggcaacctgg tctacatcat cgacttcggc
1141 ctggccaaga agtaccggga cgcccgcacc caccagcaca ttccctaccg ggaaaacaag
1201 aacctgaccg gcacggcccg ctacgcttcc atcaacacgc acctgggcat tgagcaaagc
1261 cgtcgagatg acctggagag cctgggctac gtgctcatgt acttcaacct gggctccctg
1321 ccctggcagg ggctcaaagc agccaccaag cgccagaagt atgaacggat cagcgagaag
1381 aagatgtcaa cgcccatcga ggtcctctgc aaaggctatc cctccgaatt ctcaacatac
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1441 ctcaacttct gccgctccct gcggtttgac gacaagcccg actactctta cctacgtcag
1501 ctcttccgca acctcttcca ccggcagggc ttctcctatg actacgtctt tgactggaac
1561 atgctgaaat tcggtgcagc ccggaatccc gaggatgtgg accgggagcg gcgagaacac
1621 gaacgcgagg agaggatggg gcagctacgg gggtccgcga cccgagccct gccccctggc
1681 ccacccacgg gggccactgc caaccggctc cgcagtgccg ccgagcccgt gtaa
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Reviewed and approved by site quality representative.