

CK1 epsilon (R178C), Active

Full length recombinant protein expressed in Sf9 cells

Catalog # C66-12BG

Lot # U1582-1

Product Description

Full length recombinant human CK1ε (R178C) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_001894](#).

Gene Aliases

CSNK1E, HCKIE, MGC10398

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

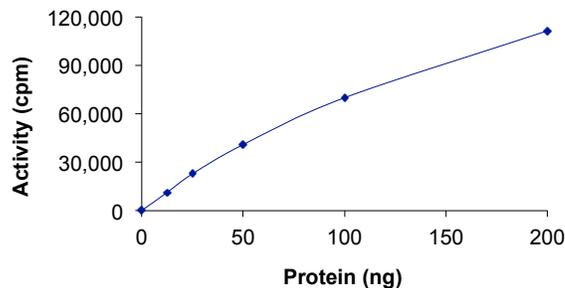
CK1ε is a member of the CK1 family of serine/threonine protein kinases which play an important role in diverse cell processes, including DNA replication and repair. CK1ε is a regulator of Yes-associated protein (YAP) transcription coactivator which is a key regulator of organ size and a candidate human oncogene. CK1ε is activated by CCK2R and this then phosphorylates PKD2 at Ser244. Phosphorylation of PKD2 leads to its nuclear accumulation and efficient phosphorylation of nuclear PKD2 substrates in human gastric cancer cells (1). CK1ε can phosphorylate topoisomerase (topo) IIα at serine-1106 and this regulates the enzyme activity and sensitivity to topo II-targeted drugs (2).

References

1. von Blume J. et al: Phosphorylation at Ser244 by CK1 determines nuclear localization and substrate targeting of PKD2. EMBO J. 2007 Nov 14;26(22):4619-33.
2. Grozav, A G. et al: Casein kinase I delta/epsilon phosphorylates topoisomerase IIalpha at serine-1106 and modulates DNA cleavage activity. Nucleic Acids Res. 2009 Feb;37(2):382-92.

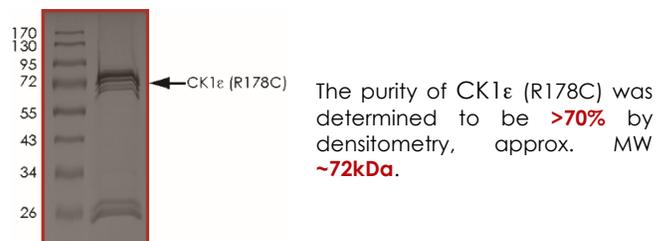
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Specific Activity



The specific activity of CK1ε (R178C) was determined to be **42 nmol/min/mg** as per activity assay protocol.

Purity



The purity of CK1ε (R178C) was determined to be **>70%** by densitometry, approx. MW **~72kDa**.

CK1 epsilon (R178C), Active

Full length human recombinant protein expressed in Sf9 cells

Catalog #	C66-12BG
Specific Activity	42 nmol/min/mg
Lot #	U1582-1
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C66-12BG)

Active CK1 ϵ (R178C) (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active CK1 ϵ (R178C) or optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/ μ l BSA and 5% glycerol solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: C03-54BN)

Casein, Dephosphorylated, a protein substrate, was diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active CK1 ϵ (R178C), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active CK1 ϵ (R178C) (Catalog #C66-12BG)
 - Component 2.** 5 μ l of 1mg/ml stock solution of substrate (Catalog #C03-54BN)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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