

CK1 epsilon (R178C), Active

Full length recombinant protein expressed in Sf9 cells

Catalog # C66-12BG

Lot # M2778-12

Product Description

Full length recombinant human CK1 ϵ (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, and 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

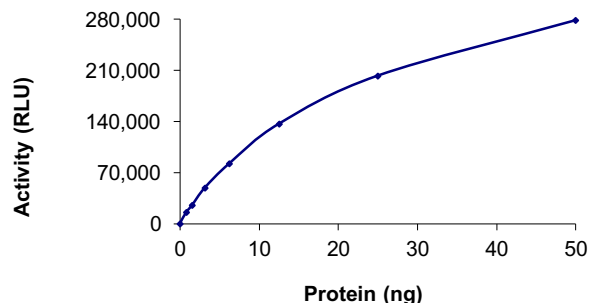
CK1 ϵ is a member of the CK1 family of serine/threonine protein kinases which play an important role in diverse cell processes, including DNA replication and repair. CK1 ϵ is a regulator of Yes-associated protein (YAP) transcription coactivator which is a key regulator of organ size and a candidate human oncogene. CK1 ϵ 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). CK1 ϵ can phosphorylate topoisomerase (topo) II α 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 II α at serine-1106 and modulates DNA cleavage activity. Nucleic Acids Res. 2009 Feb;37(2):382-92.

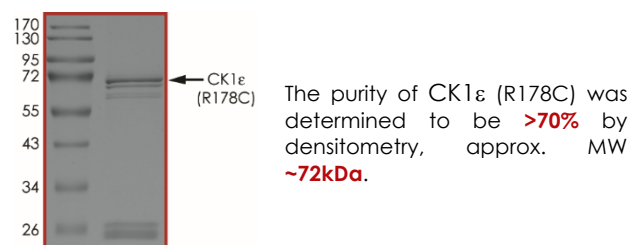
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Specific Activity



The specific activity of CK1 ϵ (R178C) was determined to be **36 nmol/min/mg** as per activity assay protocol, and was equivalent to **36 nmol/min/mg** as per radiometric assay.

Purity



CK1 epsilon (R178C), Active

Full length human recombinant protein expressed in Sf9 cells

Catalog #	C66-12BG
Specific Activity	36 nmol/min/mg
Lot #	M2778-12
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 epsilon (R178C) (0.1µg/µl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 epsilon (R178C) for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl₂ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250µM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50µM.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: C03-54BN)

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

Assay Protocol

The CK1 epsilon (R178C) assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the CK1 epsilon (R178C) reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active CK1 epsilon (R178C), Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 µM example):

Component	Amount (µL)	Component	Amount (µL)
10µM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	79		

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5µL:

Component 1.	3µl of diluted Active CK1 epsilon (R178C) (Catalog # C66-12BG).
Component 2.	2µl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5µl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10µl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]

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