

CAMK1δ, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # **C09-10G**

Lot # B066-1

Product Description

Recombinant full-length human CAMK1δ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_153498](#).

Gene Aliases

RP11-462F15.1, CKLiK, CaM-K1, CaMKID

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

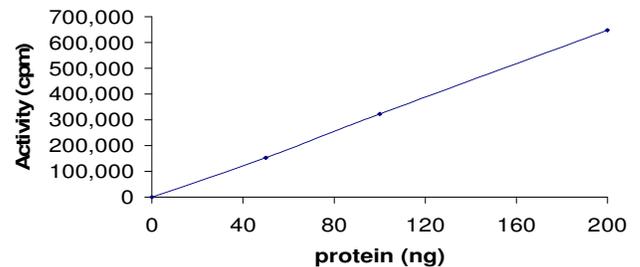
Scientific Background

CAMK1δ or Ca^{2+} /calmodulin-dependent kinase I-like kinase (CKLiK) is activated by Ca^{2+} and calmodulin and is detected in CD34+ -derived neutrophils and eosinophils, as well as in mature peripheral blood granulocytes (1). CAMK1δ exhibits Ca^{2+} /CaM-dependent activity that is enhanced in vitro by phosphorylation of its Thr180 by CaM-K kinase (CaMKK)α, consistent with detection of CAMK1δ-activating activity in HeLa cells (2).

References

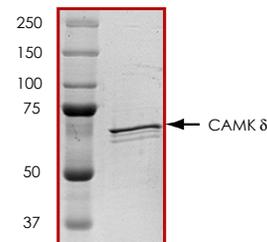
1. Verploegen, S. et al: Characterization of the role of CaMKI-like kinase (CKLiK) in human granulocyte function. *Blood* 106 (3), 1076-1083 (2005).
2. Ishikawa, Y. et al: Identification and characterization of novel components of a Ca^{2+} /calmodulin-dependent protein kinase cascade in HeLa cells. *FEBS Lett.* 550 (1-3), 57-63 (2003).

Specific Activity



The specific activity of CAMK1δ was determined to be **138 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CAMK1δ was determined to be **>90%** by densitometry. Approx. MW **68kDa**.

CAMK1δ, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number C09-10G
Specific Activity 138 nmol/min/mg
Specific Lot Number B066-1

Purity >90%
Concentration 0.1μg/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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C09-10G)

Active CAMK1 δ (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 CAMK1 δ for 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 50 ng/ μ l BSA 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.

[³²P]-ATP Assay Cocktail

Prepare 250 μ M [³²P]-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 [³²P]-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 #: A15-58)

Autocamtide 2 synthetic peptide substrate (KKALRRQETVDAL-amide) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active CAMK1 δ , 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 CAMK1 δ (Catalog #C09-10G)
 - Component 2.** 7.5 μ l of 1mg/ml stock solution of substrate (Catalog #A15-58)
 - Component 3.** 2.5 μ l of Ca²⁺/Calmodulin Solution, 10x (Catalog #C02-39)
- 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 [³²P]-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 [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³²P]-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 ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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