

CAMKK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # **C17-10G**

Lot # U183-1

Product Description

Recombinant full-length human CAMKK1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_032294](#).

Gene Aliases

CAMKKA, MGC34095, DKFZp761M0423

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

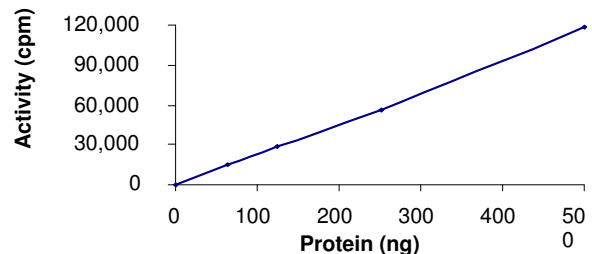
CAMKK1 or CAMKKalpha is a Ca^{2+} /calmodulin-dependent protein kinase that activates CAM-kinases I and IV via phosphorylation of their Thr(177) and Thr(196) residues, respectively. Recent studies have shown that the activity of CAMKK1 is decreased upon phosphorylation by cAMP-dependent protein kinase (PKA) (1) The CAMKKalpha has been identified in intact cells as AMPKKs, predicting a significant role for this kinase in regulating AMPK activity in vivo. It has been shown that 2-deoxyglucose- and ionomycin-stimulated AMPK activity is substantially reduced in HeLa cells transfected with small interfering RNAs specific for CAMKKalpha (2).

References

- Okuno, S. et al: Regulation of Ca^{2+} /calmodulin-dependent protein kinase kinase alpha by cAMP-dependent protein kinase: I. Biochemical analysis. J Biochem (Tokyo). 2001 Oct;130(4):503-13.
- Hurley, R L. et al: The Ca^{2+} /calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem. 2005 Aug 12;280(32):29060-6.

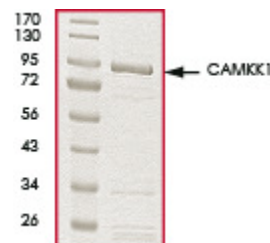
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Specific Activity



The specific activity of CAMKK1 was determined to be **10 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CAMKK1 was determined to be **>90%** by densitometry, approx. MW **94 kDa**.

CAMKK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	C17-10G
Specific Activity	10 nmol/min/mg
Specific Lot Number	U183-1
Purity	>90%
Concentration	0.1 $\mu\text{g}/\mu\text{l}$
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 #: C17-10G)

Active CAMKK1 (0.1µg/µl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) 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 CAMKK1 for optimal results).

Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl 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.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 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 200µl aliquots at -20°C.

Substrate (Catalog #: M42-54G)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 0.2mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active CAMKK1, 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 20µl:
 - Component 1.** 10µl of diluted Active CAMKK1 (Catalog #C17-10G)
 - Component 2.** 7.5µl of 0.2mg/ml stock solution of substrate (Catalog #M42-54G)
 - Component 3.** 2.5µl 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 µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl 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 µl 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 µl [³²P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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