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CAMKK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C17-10G
Lot # V322-1

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.

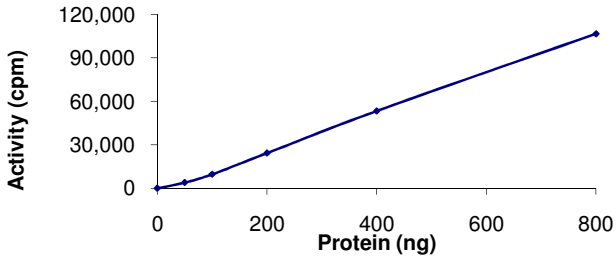
Scientific Background

CAMKK1 or CAMKKα 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 CAMKKα 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 CAMKKα (2).

References

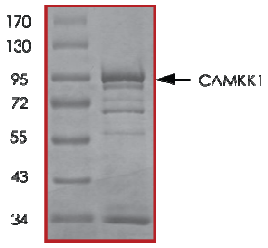
1. Okuno, S. et al: Regulation of Ca(2+)/calmodulin-dependent protein kinase kinase alpha by cAMP-dependent protein kinase: I. Biochemical analysis. J Biochem (Tokyo). 2001 Oct;130(4):503-13.
2. Hurley, R L. et al: The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem. 2005 Aug 12;280(32):29060-6.

Specific Activity



The specific activity of CAMKK1 was determined to be **9 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CAMKK1 was determined to be **>75%** by densitometry, approx. MW **94 kDa**.

CAMKK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	C17-10G
Specific Activity	9 nmol/min/mg
Specific Lot Number	V322-1
Purity	>75%
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 #: 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 $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-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 H_2O to a final concentration of 0.2mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-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 Ca^{2+}/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 H_2O.

Step 5. Initiate the reaction by the addition of 5 µl [^{33}P]-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 H_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [^{33}P]-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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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