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CAMK2γ, Active

Recombinant protein expressed in Sf9 cells

Catalog # C14-10G
Lot # Q271-2

Product Description

Recombinant human CAMK2γ (c-terminal truncation) was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_172169.

Gene Aliases

CAMKG, CAMK, CAMK-II, MGC26678

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.

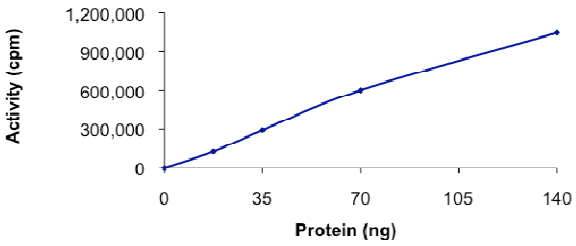
Scientific Background

CAMK2γ is a member of the CAMKII family which are ubiquitous serine/threonine protein kinases that have been implicated in diverse effects of hormones and neurotransmitters. CAMK2γ has six alternatively spliced variants that encode six different isoforms. Some of these variants have been identified in human tumors (1). Transgenic mice expressing a partially calcium-independent mutant form of CAMK2γ showed 1.5- to 2-fold increase in the thymus of these mice, at least in part due to an increase in the life span of double-positive thymocytes (2). There was an increase in the number of T cells in the secondary lymphoid organs that had acquired an antigen-dependent memory phenotype.

References

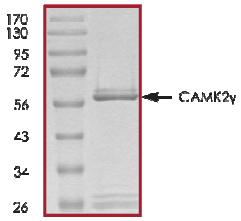
1. Tombes, R. M. et al: Identification of novel human tumor cell-specific CaMK-II variants. Biochim. Biophys. Acta 1355: 281-292, 1997.
2. Bui, J. D. et al: A role for CaMKII in T cell memory. Cell 100: 457-467, 2000.

Specific Activity



The specific activity of CAMK2γ was determined to be **259 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CAMK2γ was determined to be **>80%** by densitometry.
Approx. MW **60kDa**.

CAMK2γ, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	C14-10G
Specific Activity	259 nmol/min/mg
Specific Lot Number	Q271-2
Purity	>80%
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 #: C14-10G)

Active CAMK2γ (0.1µg/µl) 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 CAMK2γ 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/µl BSA 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 #: A15-58)

Autocamtide 2 synthetic peptide substrate (KKALRRQETVDAL-amide) diluted in distilled H_2O to a final concentration of 1mg/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 CAMK2γ, 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 CAMK2γ (Catalog #C14-10G)
- **Component 2.** 7.5µl of 1mg/ml stock solution of substrate (Catalog #A15-58)
- **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 5µl [^{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 20µl 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 5µl [^{33}P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

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

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