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CAMK2β, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C12-10H
Lot # Q102-2

Product Description

Recombinant full-length human CAMK2β was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_172081.

Gene Aliases

CAMKB, CAM2, CAMK2, MGC29528

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.2mM DTT, 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.

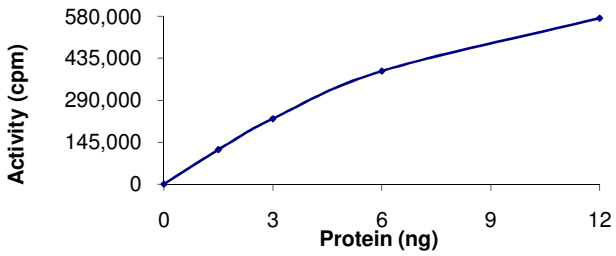
Scientific Background

CAMK2β belongs to the serine/threonine protein kinase family and to the type II multifunctional Ca(2+)/calmodulin-dependent protein kinase subfamily. CAMK2β showed wide tissue and cell distribution, and one of CAMK2β variant predominated in adult brain (1). The ratio of CAMK2α and CAMK2β protein levels were inversely related during activity in hippocampal neurons (2). CAMK2β is a prominent kinase in the central nervous system and may function in long-term potentiation and neurotransmitter release.

References

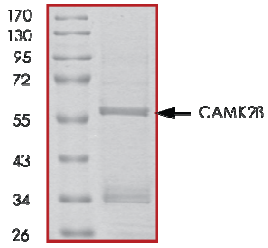
1. Tombes, R. M. et al: Identification of novel human tumor cell-specific CaMK-II variants. *Biochim. Biophys. Acta* 1355: 281-292, 1997.
2. Thiagarajan, T. C. Et al: Alpha- and beta-CaMKII: inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron* 36: 1103-1114, 2002.

Specific Activity



The specific activity of CAMK2β was determined to be **5700 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CAMK2β was determined to be **>75%** by densitometry.
Approx. MW **58kDa**.

CAMK2β, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	C12-10H
Specific Activity	5700 nmol/min/mg
Specific Lot Number	Q102-2
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 #: C12-10H)

Active CAMK2β (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 CAMK2β 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 [^{33P}]-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 CAMK2β, 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 CAMK2β (Catalog #C12-10H)
- **Component 2.** 7.5μl of 1mg/ml stock solution of substrate (Catalog #A15-58)
- **Component 3.** 2.5μl of Ca^{2+}/Calmodulin, 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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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