CAMK2β, Active
Full-length recombinant protein expressed in Sf9 cells
Catalog # C12-10H
Lot # G179-1

Product Description
Recombinant full-length human CAMK2β was expressed by baculovirus in Sf9 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

Specific Activity
The specific activity of CAMK2β was determined to be 5670 nmol/min/mg as per activity assay protocol.

Purity
The purity of Camk2β was determined to be >75% by densitometry. Approx. MW 58kDa.
**Activity Assay Protocol**

**Reaction Components**

**Active Kinase** (Catalog #: C12-10H)
Active CAMK2β (0.1 µg/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 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.

**Kinase Assay Buffer II** (Catalog #: K01-09)
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 #: 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 CAMK2β, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

**Step 3.** In a pre-cooled microtube, 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 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 5µl [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 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 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 [P³²]-ATP Specific Activity (SA) (cpm/pmol)**

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

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