CAMK2α, Active
Full-length human recombinant protein expressed in Sf9 cells

Catalog # C11-10G
Lot # I243-2

Product Description
Recombinant full-length human CAMK2α was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_171825.

Gene Aliases
CAMKA; KIAA0968

Formulation
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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
CAMK2α is a ser/thr protein kinase that is a member of the Ca²⁺/calmodulin-dependent protein kinase family. CAMK2α is abundant in the brain as a major constituent of the postsynaptic density and is required for hippocampal long-term potentiation (LTP) and spatial learning. In addition to its Ca²⁺/calmodulin-dependent activity, CAMK2α can undergo autophosphorylation, resulting in Ca²⁺/calmodulin-independent activity. The protein level of CAMK2α fluctuates during neuronal activity in cultured rat pup hippocampal neurons. The levels of CAMK2α increased with heightened neuronal activity (2).

References

Specific Activity
The specific activity of CAMK2α was determined to be 294 nmol/min/mg as per activity assay protocol.

Purity
The purity of Camk2α was determined to be >85% by densitometry. Approx. MW 74kDa.

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Specific Activity 294 nmol/min/mg
Specific Lot Number I243-2
Purity >85%
Concentration 0.1 µg/µl

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.

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Activity Assay Protocol

Reactions Components

**Active Kinase (Catalog #: C11-10G)**
Active CAMK2α (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 CAMK2α 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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[33P]-ATP Assay Cocktail**
Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by following the 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 H₂O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active CAMK2α, 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 CAMK2α (Catalog #C11-10G)
   - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #A15-58)
   - Component 3. 2.5µl of Ca²⁺/Calmodulin Solution, 10x (Catalog #C02-39)
   - Component 4. 2.5µl distilled H₂O (4°C)
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 [33P]-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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)**
Specific activity (SA) = cpm for 5µl [³³P]-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 ³³P-ATP in cpm/pmol]*[Reaction time in min]*[Enzyme amount in µg or mg])*[(Reaction Volume) / (Spot Volume)]

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