CAMK2α, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C11-10G
Lot # C090-1

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, 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

CAMK2α is a ser/thr protein kinase that is a member of the Ca^{2+}/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 Ca^{2+}/calmodulin-dependent activity, CAMK2α can undergo autophosphorylation, resulting in Ca^{2+}/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


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Activity Assay Protocol

Reaction 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.

**[32P]-ATP Assay Cocktail**
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

1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active CAMK2α, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
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. 7.5µl of 1mg/ml stock solution of substrate (Catalog #:A15-58)
   - Component 3. 2.5µl of Ca²⁺/Calmodulin Solution, 10x (Catalog #:C02-39)
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.
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.
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.
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.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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 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 [32P]-ATP in cpm/pmol)*[Reaction time in min]*[Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]