

## CAMK2α, Active

Full-length human recombinant protein expressed in Sf9 cells

**Catalog # C11-10G**

Lot # I339-1

### 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, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF and 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.

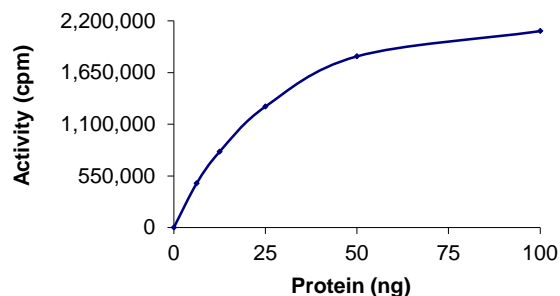
### Scientific Background

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

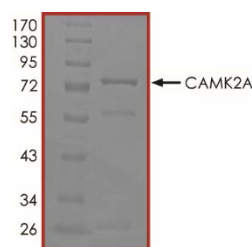
1. Silva, A J. et al: Impaired spatial learning in alpha-calcium calmodulin kinase II mutant mice. Science 257: 206-211, 1992.
2. Thiagarajan, T C. et al: Alpha- and beta-CaMKII: inverse regulation by neuronal activity and opposing effects on synaptic strength. Neuron 36: 1103-1114, 2002.

### Specific Activity



The specific activity of CAMK2α was determined to be **295 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>75%** by densitometry. Camk2α Approx. MW **74kDa**.

## CAMK2α, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	C11-10G
Specific Activity	295 nmol/min/mg
Lot #	I339-1
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.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: C11-10G)

Active CAMK2 $\alpha$  (0.1 $\mu$ g/ $\mu$ l) 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 CAMK2 $\alpha$  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/ $\mu$ l BSA solution.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ l), 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 200 $\mu$ l aliquots at -20°C.

### Substrate (Catalog #: A15-58)

Autocamtide 2 synthetic peptide substrate (KKALRRQET-VDAL-amide) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1. Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active CAMK2 $\alpha$ , 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 20 $\mu$ l:
  - Component 1. 10 $\mu$ l of diluted Active CAMK2 $\alpha$  (Catalog #C11-10G)
  - Component 2. 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog #A15-58)
  - Component 3. 2.5 $\mu$ l of Ca<sup>2+</sup>/Calmodulin solution (10X) (Catalog #C02-39)
  - Component 4. 2.5 $\mu$ l distilled H<sub>2</sub>O (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 H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5 $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 20 $\mu$ l 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<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 $\mu$ l [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 $\mu$ l of a 250 $\mu$ M ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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