

Product Specification

CAMK4, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7740
 Lot #: _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 83 nmol/min/mg

Quality Control Analysis

Activity assessment

CAMK4 protein (~100 ng/µl concentration) was diluted to 20ng/µl in assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 30 mM MgCl₂, 0.05 mM DTT and 40ng/ul BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Autocamtide 2 (KKALRRQETVDAL-amide) in the following assay condition:

- 10 µl diluted CAMK4 protein
- 7.5 µl Autocamtide 2 (1mg/ml stock)
- 2.5 µl Calmodulin (0.3 mg/ml in 5mM CaCl₂)
- 5 µl [³²P] ATP mixture (250 µM ATP stock, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

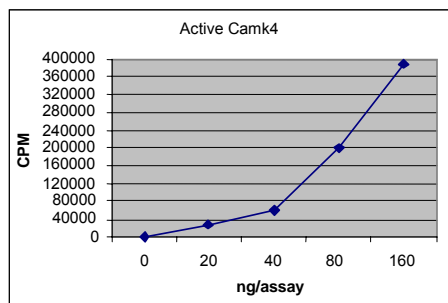


Fig. 1 CAMK4 activity assay

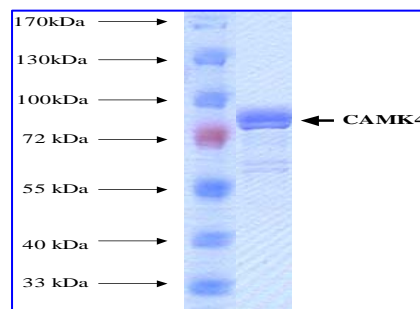


Fig. 2 CAMK4 protein gel

Purity assessment

1 µg of CAMK4 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the CAMK4 band product, and the band was at ~79 kDa (Fig. 2).

Product Description

Recombinant full-length human CAMK4 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_001744.

This material is sold for research purposes only.

Specific Activity

83 nmol phosphate incorporated into Autocamtide 2 per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ Ci/assay).

Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

CAMK4 belongs to the serine/threonine protein kinase family and Ca(2+)/calmodulin-dependent protein kinase subfamily. This enzyme is a multifunctional serine/threonine protein kinase with limited tissue distribution. Sikela et al. identified that brain CaM kinase IV (CAMK4) appeared to be restricted to brain in the rat; interestingly, it was not detected in the brain of the newborn, but became detectable within a few days after birth. They also raised the possibility that a defect in Camk4 may be responsible for 1 of the mutant phenotypes in the centromeric region near 2 mutations, which known to affect neurologic function and fertility (1). Wei et al. found Camk4-labeled neurons in the hippocampus, amygdala, anterior cingulate cortex, somatosensory cortex, and insular cortex (2). Kang et al. generated transgenic mice in which the expression of a dominant-negative form of Camk4 was restricted to the postnatal forebrain. In these transgenic mice, activity-induced Creb phosphorylation and Fos expression were significantly attenuated. The results indicated that neural activity-dependent CAMK4 signaling in the neuronal nucleus plays an important role in the consolidation/retention of hippocampus-dependent long-term memory (3). Wu et al. generated transgenic mice that selectively express in skeletal muscle a constitutively active form of CAMK4 and concluded that a calcium-regulated signaling pathway controls mitochondrial biogenesis in mammalian cells (4). Wei et al. studied pain and fear memory in Camk4-null mice and concluded that Camk4 is crucial in the trapping of Ca2(+)/calmodulin complexes in neuronal nuclei and in Creb phosphorylation and activation (5).

References

1. Sikela, J. M.; Law, M. L.; Kao, F.-T.; Hartz, J. A.; Wei, Q.; Hahn, W. E.: Chromosomal localization of the human gene for brain Ca(2+)/calmodulin-dependent protein kinase type IV. *Genomics* 4: 21-27, 1989.
2. Wei, F.; Qiu, C.S.; Liauw, J.; Robinson, D. A.; Ho, N.; Chatila, T.; Zhuo, M.: Calcium-calmodulin-dependent protein kinase IV is required for fear memory. *Nature Neurosci.* 5: 573-579, 2002.
3. Kang, H.; Sun, L. D.; Atkins, C. M.; Soderling, T. R.; Wilson, M. A.; Tonegawa, S.: An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. *Cell* 106: 771-783, 2001.
4. Wu, H.; Kanatous, S. B.; Thurmond, F. A.; Gallardo, T.; Isotani, E.; Bassel-Duby, R.; Williams, R. S.: Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 296: 349-352, 2002.
5. Wei, F.; Qiu, C.S.; Liauw, J.; Robinson, D. A.; Ho, N.; Chatila, T.; Zhuo, M.: Calcium-calmodulin-dependent protein kinase IV is required for fear memory. *Nature Neurosci.* 5: 573-579, 2002.