

Product Specification

CAMK1G, active

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

Catalog #: 7736
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 151 nmol/min/mg

Quality Control Analysis

Activity assessment

1 µl of CAMK1G protein (~100 ng protein) was diluted to 50ng/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 CAMK1G 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, 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.

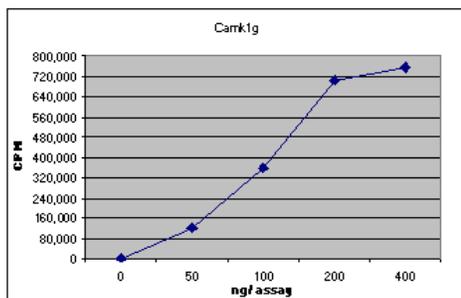


Fig. 1 CAMK1G activity assay

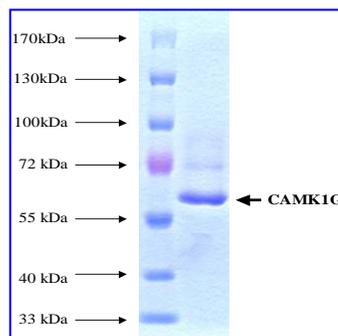


Fig. 2 CAMK1G protein gel

Purity assessment

1 µg of CAMK1G protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the CAMK1G band product, and the band was at ~60 kDa (Fig. 2).

Product Description

Recombinant full-length human CAMK1G containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_020439.

This material is sold for research purposes only.

Specific Activity

151 nmol phosphate incorporated into Autocamtide 2 per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/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

CLICK-III/CaMKIG is a novel membrane-anchored neuronal Ca²⁺/calmodulin-dependent protein kinase (CaMK), an isoform of the CaMKI family with an extended C-terminal domain ending with CAAX motif (where AA is aliphatic acid). As expected from the similarity of its kinase domain with the other CaMKI isoforms, full activation of CLICK-III/CaMKIG required both Ca(2+)/CaM and phosphorylation by CaMKK. Ca(2+)/cAMP-response element-binding protein (CREB) was a good substrate for CLICK-III/CaMKIG, at least in vitro. Interestingly enough, CLICK-III/CaMKIG transcripts were most abundant in neurons, with the highest levels in limited nuclei such as the central nucleus of the amygdala (CeA) and the ventromedial hypothalamus. Consistent with the presence of the CAAX motif, CLICK-III/CaMKIG was found to be anchored to various membrane compartments, especially to Golgi and plasma membranes. Both point mutation in the CAAX motif and treatment with compactin, a HMG-CoA reductase inhibitor, disrupted such membrane localization, suggesting that membrane localization of CLICK-III/CaMKIG occurred in a prenylation-dependent way. These findings provide a novel mechanism by which neuronal CaMK activity could be targeted to specific membrane compartments.

References

1. Takemoto-Kimura, S., Terai, H., Takamoto, M., Ohmae, S., Kikumura, S., Segi, E., Arakawa, Y., Furuyashiki, T., Narumiya, S. and Bito, H. Molecular cloning and characterization of CLICK-III/CaMKIgamma, a novel membrane-anchored neuronal Ca²⁺/calmodulin-dependent protein kinase (CaMK). *J. Biol. Chem.* 278 (20), 18597-18605 (2003)