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Product Specification

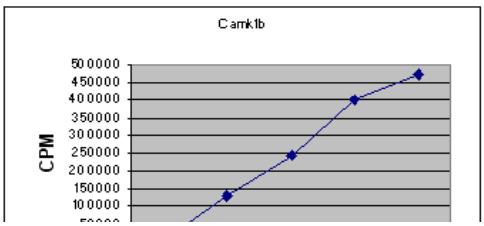
CAMK1b, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7729
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 223 nmol/min/mg

Quality Control Analysis
Activity assessment
CAMK1b protein (100 ng/µl concentration) was diluted to 25ng/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 $MgCl_2$, 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 CAMK1b protein
7.5 µl Autocamtide 2 (1mg/ml stock)
2.5 µl Calmodulin (0.3 mg/ml in 5mM CaCl2)
5 µl [^{32}P] ATP mixture (250 µM ATP stock, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] 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.



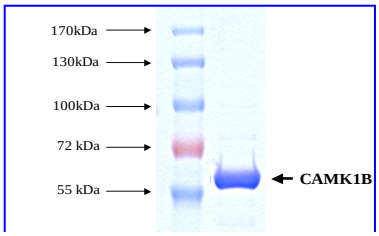


Fig. 1 CAMK1b activity assay

Fig. 2 CAMK1b protein gel

Purity assessment
2 µg of CAMK1b protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the CAMK1b band product, and the band was at ~64 kDa (Fig. 2).

Product Description
Recombinant full-length mouse CAMK1b containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_012040.
This material is sold for research purposes only.

Specific Activity
223 nmol phosphate incorporated into Autocamtide 2 per minute per mg protein at 30ºC 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
Many of the effects of calcium are mediated via its interaction with calmodulin and the subsequent activation of Ca(2+)/calmodulin-dependent (CaM) kinases. CaM kinases are involved in a wide variety of cellular processes including muscle contraction, neurotransmitter release, cell cycle control, and transcriptional regulation. While CaMKII has been implicated in learning and memory, the biological role of the other multifunctional CaM kinases, CaMKI and CaMKIV, is largely unknown. CaMKIbeta, or pregnancy upregulated non-ubiquitously expressed CaM kinase (PNCK), is a 38-kDa serine/threonine kinase whose catalytic domain shares 45-70% identity with members of the CaM kinase family. The gene for CaMKIbeta localizes to mouse chromosome X. CaMKIbeta is upregulated during intermediate and late stages of murine fetal development with highest levels of expression in developing brain, bone, and gut. CaMKIbeta is also expressed in a tissue-specific manner in adult mice with highest levels of expression detected in brain, uterus, ovary, and testis. Interestingly, CaMKIbeta expression in these tissues is restricted to particular compartments and appears to be further restricted to subsets of cells within those compartments. The chromosomal localization of CaMKIbeta, along with its tissue-specific and restricted pattern of spatial expression during development, suggests that CaMKIbeta may be involved in a variety of developmental processes including development of the central nervous system (1). Also CaMKIbeta2, an isoform of mCaMKIbeta, was mainly identified in the nervous system, including brain, spinal cord, trigeminal ganglion, and retina. Within the CNS, the expression of CaMKIbeta2 is detected in the mantle zone, but not in the ventricular zone, suggesting its possible involvement in the differentiation of neurons (2).

References
1. Gardner,H.P., Rajan,J.V., Ha,S.I., Copeland,N.G., Gilbert,D.J., Jenkins,N.A., Marquis,S.T. and Chodosh,L.A. Cloning, characterization, and chromosomal localization of Pnck, a Ca(2+)/calmodulin-dependent protein kinase. Genomics 63 (2), 279-288 (2000)
2. Ueda,T., Sakagami,H., Abe,K., Oishi,I., Maruo,A., Kondo,H., Terashima,T., Ichihashi,M., Yamamura,H. and Minami,Y. Distribution and intracellular localization of a mouse homologue of