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Product Specification

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

Catalog #: 7744-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 342 nmol/min/mg

Quality Control Analysis
Activity assessment
PKA cb protein (~100 ng/µl concentration) was diluted to 25ng/µl with storage buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the CREBtide (KRREILSRRPSYR) in the following assay condition:

10 µl diluted PKA cb protein
10 µl CREBtide substrate (1 mg/ml stock)
5 µl [^{32}P] ATP mixture (250 µM ATP, 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.

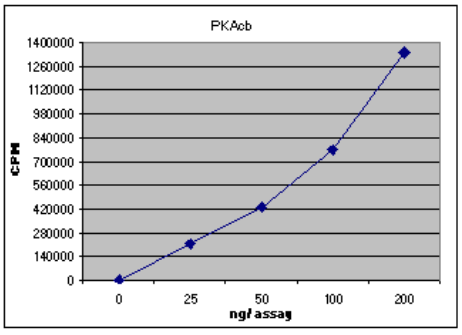

Fig. 1 PKAcb activity assay

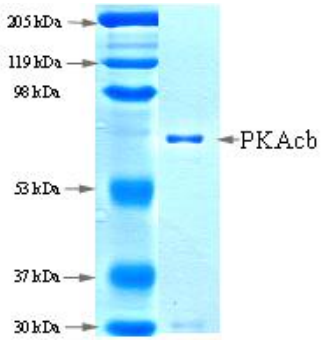

Fig. 2 PKAcb protein gel

Purity assessment
1.25 µl of PKAcb protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKAcb protein, and the band was at ~65 kDa (Fig. 2)

Product Description
Recombinant full-length human PKA cb containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_002731.
This material is sold for research purposes only.

Specific Activity
342 µmol phosphate incorporated into CREBtide substrate 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
Most of the effects of cAMP are mediated through the phosphorylation of target proteins on serine or threonine residues by the cAMP-dependent protein kinase (PKA). The inactive holoenzyme of AMPK is a tetramer composed of two regulatory and two catalytic subunits. The mammalian catalytic subunit has been shown to consist of three PKA gene products: C-alpha, C-beta, and C-gamma. Two PKA isoforms exist, designated types I and II, which differ in their dimeric regulatory subunits, designated RI and RII, respectively. Furthermore, there are at least four different regulatory subunits: RI-alpha, RI-beta, RII-alpha, and RII-beta. The cAMP causes the dissociation of the inactive holoenzyme into a dimer of regulatory subunits bound to four cAMP and two free monomeric catalytic subunits. The catalytic subunit C-beta of PKA (PKAcb) is a member of the Ser/Thr protein kinase family and is a catalytic subunit C-beta of AMPK. Berube et al. assigned the PKAcb to human chromosome 1 by Southern blot analysis of somatic cell hybrids (1) and Simard et al located it to 1p36.1 by in situ hybridization (2).

References
1. Berube, D.; Simard, J.; Sandberg, M.; Grzeschik, K.-H.; Gagne, R.; Hansson, V.; Jahnsen, T.: Assignment of the gene encoding the catalytic subunit C(beta) of cAMP-dependent protein kinase to the p36 band on chromosome 1. (Abstract) *Cytogenet. Cell Genet.* 58: 1850 only, 1991.
2. Simard, J.; Berube, D.; Sandberg, M.; Grzeschik, K.-H.; Gagne, R.; Hansson, V.; Jahnsen, T.: Assignment of the gene encoding the catalytic subunit C-beta of cAMP-dependent protein kinase to the p36 band on chromosome 1. *Hum. Genet.* 88: 653-657, 1992.