

## 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<sub>2</sub>, 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 (KRREILSRPSYR) in the following assay condition:

- 10 µl diluted PKA cb protein
- 10 µl CREBtide substrate (1 mg/ml stock)
- 5 µl [<sup>32</sup>P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30°C and the reaction started by the addition of [<sup>32</sup>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.

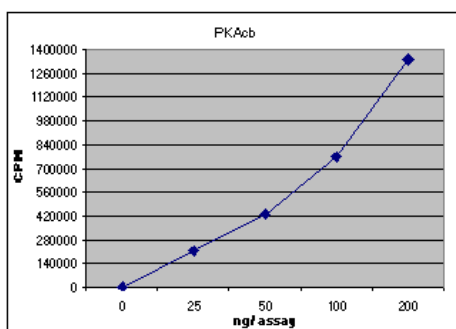


Fig. 1 PKAcb activity assay

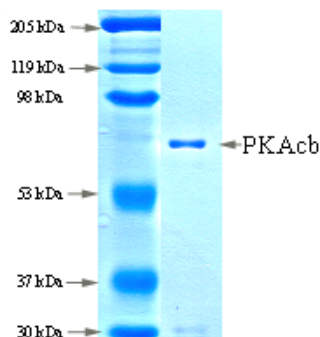


Fig. 2 PKAcb protein gel

#### 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 umol 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 (PKAcβ) is a member of the Ser/Thr protein kinase family and is a catalytic subunit C-beta of AMPK. Berube et al. assigned the PKAcβ 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.