

## Product Specification

### **PKA cg, active**

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

Catalog #: 7728  
 Lot #: \_\_\_\_\_  
 Aliquot size: 5 µg protein in 50 µl  
 Specific activity: 156 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

PKA cg protein (~100 ng/µl concentration) was diluted to 50ng/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 cg protein
- 10 µl CREBtide (1mg/ml stock)
- 5 µl [<sup>32</sup>P] ATP mixture (250 µM ATP stock, 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 PKA<sub>cg</sub> activity assay

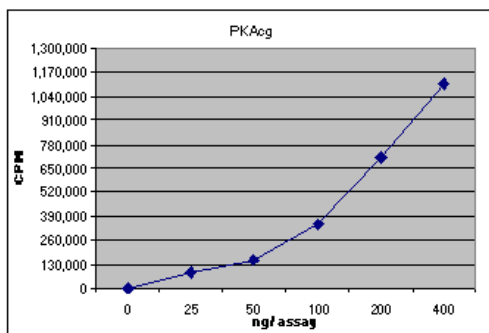
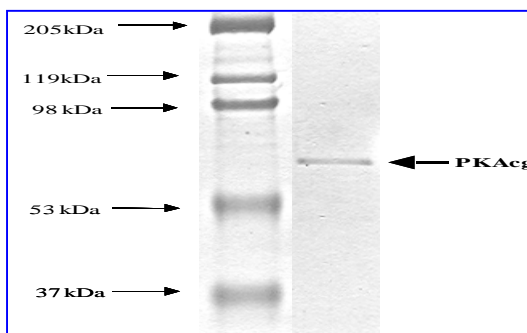


Fig. 2 PKA<sub>cg</sub> protein gel



#### Purity assessment

0.5 µg of PKA cg protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKA cg protein, and the band was at ~65 kDa, (Fig. 2).

#### **Product Description**

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

The gene accession number is NM\_002732.

This material is sold for research purposes only.

#### Specific Activity

156 nmol phosphate incorporated into CREBtide per minute per mg protein at 30°C for 15 minutes using a final concentration of 50  $\mu$ M ATP (0.83  $\mu$ Ci/assay).

#### Formulation

Recombinant proteins 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

The inactive holoenzyme of the cAMP-dependent protein kinase (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. cAMP causes the dissociation of the inactive holoenzyme into a dimer of regulatory subunits bound to four cAMP and two free monomeric catalytic subunits. PKA C-gamma (PKA cg) is a third isoform of the catalytic subunit of cAMP-dependent protein kinase (C-alpha:PRKACA and C-beta:PRKACB had been previously characterized). It was isolated from a human testis cDNA library and was clearly derived from a gene distinct from C-alpha and C-beta and showed tissue-specific expression. Whereas at the amino acid level C-alpha and C-beta showed 93% homology, C-gamma showed only about 80% homology to both C-alpha and C-beta (1). Reinton isolated the entire human PRKACG genomic sequence (2). The PRKACG gene is intronless, contains remnants of a poly(A) tail, is flanked by direct repeats, and is co-linear with the PRKACA gene. Thus, it was concluded that the PRKACG gene is a PRKACA-derived retroposon. Northern blot analysis detected PRKACG expression in fractionated germ cells of human testes. Foss et al (3).

#### References

1. Beebe, S. J.; Oyen, O.; Sandberg, M.; Froysa, A.; Hansson, V.; Jahnsen, T.: Molecular cloning of a tissue-specific protein kinase (C gamma) from human testis--representing a third isoform for the catalytic subunit of cAMP-dependent protein kinase. *Molec. Endocr.* 4: 465-475, 1990.
2. Reinton, N.; Haugen, T. B.; Orstavik, S.; Skalhegg, B. S.; Hansson, V.; Jahnsen, T.; Tasken, K.: The gene encoding the C gamma catalytic subunit of cAMP-dependent protein kinase is a transcribed retroposon. *Genomics* 49: 290-297, 1998.
3. Foss, K. B.; Berube, D.; Simard, J.; Sandberg, M.; Grzeschik, K.-H.; Gagne, R.; Hansson, V.; Jahnsen, T. : Localization of the catalytic subunit C-gamma of cAMP-dependent protein kinase on human chromosome 9q13. (Abstract) *Cytogenet. Cell Genet.* 58: 1937-1938, 1991.