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Product Specification

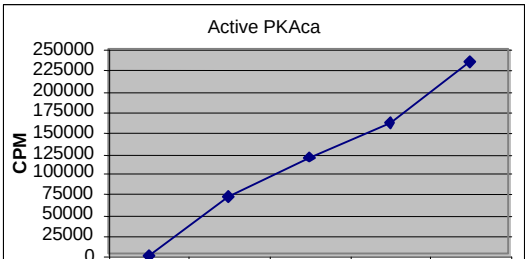
PKAca, active
(Full-length recombinant protein expressed in Sf 9 cells)

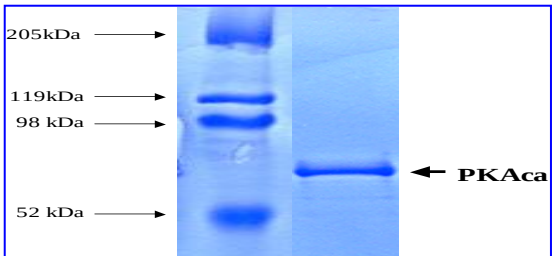
Catalog #: 7743-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 137 nmol/min/mg

Quality Control Analysis
Activity assessment
PKAca protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution 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 Histone H1 protein in the following assay condition:

10 µl diluted PKAca protein
7.5 µl Histone H1 (5 mg/ml stock)
2.5 µl lipid activators (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, 25 mM beta-glycerophosphate, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM $CaCl_2$). Sonicate for 1 minute prior to use.
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.





Purity assessment
1 µg of PKAca protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKAca product, and the band was at ~69 kDa (Fig. 2).

Product Description

Recombinant full-length human PKAca containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_002730.
This material is sold for research purposes only.

Specific Activity
137 nmol phosphate incorporated into Histone H1 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 (AMPK). 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. 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-alpha of PKA (PKAca) is a member of the Ser/Thr protein kinase family and is a catalytic subunit C-beta of AMPK. Tasken et al. assigned the PKAca gene to 19p13.1 (1). Yasuda et al found that protein kinase A is required for long-term potentiation in neonatal tissue and suggested that developmental changes in synapse morphology may underlie the changes in the kinase activity (2). Skalhegg et al generated a null mutation in the major catalytic subunit of PKAca, and observed early postnatal lethality in the majority of C-alpha knockout mice. Surprisingly, a small percentage of C-alpha knockout mice, although runted, survived to adulthood. In these animals, compensatory increases in C-beta levels occurred in brain whereas many tissues, including skeletal muscle, heart, and sperm, contained less than 10% of the normal PKA activity (3).

References
1. Tasken, K.; Solberg, R.; Zhao, Y.; Hansson, V.; Siciliano, M. J.: The gene encoding the catalytic subunit C-alpha of cAMP-dependent protein kinase (locus PRKACA) localizes to human chromosome region 19p13.1. *Genomics* 36: 535-538, 1996.
2. Yasuda, H.; Barth, A. L.; Stellwagen, D.; Malenka, R. C.: A developmental switch in the signaling cascades for LTP induction. *Nature Neurosci.* 6: 15-16, 2003.
3. Skalhegg, B. S.; Huang, Y.; Su, T.; Idzerda, R. L.; McKnight, G. S.; Burton, K. A.: Mutation of the C-alpha subunit of PKA leads to growth retardation and sperm dysfunction. *Molec. Endocr.* 16: 630-639, 2002.

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