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Product Specification

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

Catalog #:	7714-5
Lot #:	_____
Aliquot size:	5 μg protein in 50 μl
Specific activity:	69 nmol/min/mg

Quality Control Analysis
Activity assessment
PKCα 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, 30 mM $MgCl_2$, 0.05 mM DTT), 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 PKCα protein
7.5 μl CREBtide (1 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 CaCl2). 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 30ºC 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.

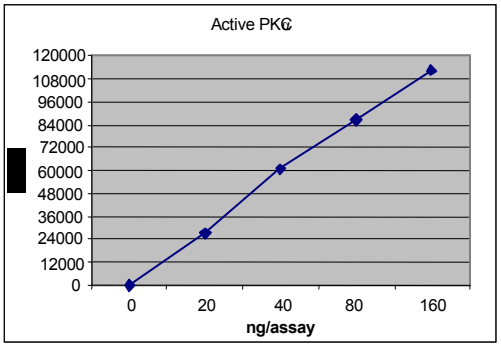

Fig. 1 PKCα activity assay

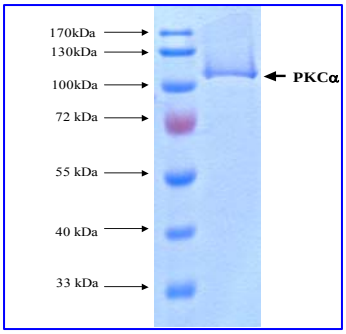

Fig. 2 PKCα protein gel

Purity assessment
0.5 μg of PKCα protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKCα product, and the band was at ~103 kDa, (Fig. 2).

Product Description
Recombinant full-length xenopus PKCα containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is BC078065.
This material is sold for research purposes only.

Specific Activity
69 nmol phosphate incorporated into CREBtide 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 -70ºC. Stable for 1 year at -70ºC as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. PKC family members also serve as major receptors for phorbol esters, a class of tumor promoters. PKC-alpha has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control. Coussens defined a new family of PKC-related genes and termed alpha, beta, and gamma (1). Latos-Bielenska refined the assignment of PRKCA1 to 17q22-q23.2 (2). Braz identified PKC-alpha as a fundamental regulator of cardiac contractility and Ca(2+) handling in myocytes in study of knockout mice (3).

References
1. Coussens, L.; Parker, P. J.; Rhee, L.; Yang-Feng, T. L.; Chen, E.; Waterfield, M. D.; Francke, U.; Ullrich, A.: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233: 859-866, 1986.
2. Latos-Bielenska, A.; Klett, C.; Just, W.; Hameister, H.: Refinement of localization of the human genes for myeloperoxidase (MPO), protein kinase C, alpha polypeptide, PRKCA, and the DNA fragment D17S21 on chromosome 17q. *Hereditas* 115: 69-72, 1991.
3. Braz, J. C.; Gregory, K.; Pathak, A.; Zhao, W.; Sahin, B.; Klevitsky, R.; Kimball, T. F.; Lorenz, J. N.; Nairn, A. C.; Liggett, S. B.; Bodi, I.; Wang, S.; and 9 others: PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nature Med.* 10: 248-254, 2004.