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# **Product Information**

# Anti-Protein Kinase Cα

produced in rabbit, delipidized, whole antiserum

Catalog Number P4334

Synonym: Anti-PKCa

#### **Product Description**

Anti-Protein Kinase  $C\alpha$  is produced in rabbit using a synthetic peptide conjugated to KLH as the immunogen. The peptide corresponds to the C-terminal variable (V5) region of rat PKC $\alpha$ . The antiserum has been treated to remove lipoproteins.

Anti-Protein Kinase  $C\alpha$  reacts in immunoblotting (SDS-PAGE) with PKC $\alpha$  (80 kDa) in rat brain extract or NIH 3T3 mouse fibroblast lysate. A minor band at 45 kDa may be observed. Staining of the PKC $\alpha$  80 kDa band is inhibited with PKC $\alpha$  peptide.

Anti-Protein Kinase  $C\alpha$  may be used to detect the PKC $\alpha$  isoenzyme using brain tissue and cell culture extracts. The antibody may be used to detect PKC $\alpha$  in chemiluminescence detection systems.

Protein Kinase C (PKC, 76-93 kDa), is a family of serine/threonine (Ser/Thr) specific protein kinases which are key enzymes considered to play a crucial role in signal transduction leading to cellular regulation, cell growth and differentiation, oncogenesis, and modulation of neurotransmission. PKC is a phospholipid dependent enzyme, activated by the lipid 1,2-diacylglycerol (DAG), an intracellular second messenger produced from hydrolysis of inositol phospholipids, in response to a variety of hormones, growth factors, and neurotransmitters. 1-3 PKC is also the major cellular receptor for the tumor promoting phorbol esters. PKC action is mediated by binding to specific receptors for activated C-kinase (RACKs) and through the phosphorylation of several cellular substrates.4-6 Proteolysis of PKC in vivo is mediated by calpains I and II. Calpains cleave PKC in the V3 hinge region to produce two distinct fragments, one comprising the N-terminal regulatory domain (30 kDa) and a fragment containing the C-terminal kinase domain (50 kDa) which is catalytically active. 7,8

Molecular cloning has established that the PKC family of isoenzymes consists of at least 9 different subtypes that can be subdivided in two major classes based on their primary domain structure and activation requirements: conventional (cPKC) isoforms  $(\alpha,\,\beta_1,\,\beta_2,\,$  and  $\gamma)$  and novel (nPKC) isoforms  $(\delta,\,\epsilon,\,\zeta,\,\eta,\,$  and  $\theta). The cPKC isoforms have four conserved regions (C1 to C4) separated by five variable regions (V1 to V5) and require Ca²+, DAG, and phosphatidylserine (PtdSer) for activity. The nPKC isoforms lack the C2 region presumably involved in Ca²+ binding. These isoforms have kinase activities regulated by DAG or PtdSer but are Ca²+ independent. The PKC<math display="inline">\alpha$  isoenzyme is ubiquitously expressed in most tissues, and appears to be the major PKC isoform in fibroblasts.  $^{1.3}$ 

Overexpression and stimulation of PKC $\alpha$  leads to enhanced growth rate of cells in culture. In various cell lines, PKC $\alpha$  is located in the cytosol and is translocated to the cellular membrane or nuclear membrane, upon activation by growth factors, and downregulated by the phorbol ester TPA. 9.10,11 PKC $\alpha$  directly phosphorylates and activates Raf-1 in NIH 3T3 fibroblasts. 12 Antibodies that react specifically with PKC isoenzymes may be used to study the specific activation requirements, differential tissue expression, and intracellular localization of these isoenzymes. Antibodies to PKC $\alpha$  may also be used to study the expression of PKC $\alpha$  in normal and neoplastic tissue.

### Reagent

Supplied as a liquid containing 15 mM sodium azide as preservative.

# **Precautions and Disclaimer**

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

# Storage/Stability

For continuous use, store at 2–8 °C. For extended storage freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

#### **Product Profile**

Protein Concentration is determined by Biuret.

Indirect immunoblotting: a minimum working dilution of 1;50,000 was determined using rat brain extract. Staining of an 80 kDa band was observed.

Indirect immunoblotting: a minimum working dilution of 1:20,000 was determined using mouse NIH 3T3 fibroblast lysate. Staining of an 80 kDa band was observed.

<u>Note</u>: In order to obtain best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration test.

#### References

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VS,DS,KAA,PHC,MAM 06/19-1