

**Product No. P-4334**

**Lot 085H4848**

**Anti-Protein Kinase C  $\alpha$  (PKC  $\alpha$ )**

Developed in Rabbit

Delipidized, Whole Antiserum

Anti-Protein Kinase C  $\alpha$  (PKC  $\alpha$ ) is developed in rabbit using a synthetic peptide (Lys-Val-Asn-Pro-Gln-Phe-Val-His-Pro-Ile-Leu-Gln-Ser-Ala-Val) conjugated to KLH as immunogen. The peptide corresponds to the C-terminal variable (V5) region (amino acids 659-672 with N-terminal added Lys) of rat PKC  $\alpha$ . The antiserum has been treated to remove lipoproteins. Rabbit Anti-Protein Kinase C  $\alpha$  is supplied as a liquid containing 0.1% sodium azide (see MSDS)\* as preservative.

**Specificity**

Anti-Protein Kinase C  $\alpha$  (PKC  $\alpha$ ) reacts in immunoblotting (SDS-PAGE) with PKC  $\alpha$  (80 kD polypeptide) in rat brain extract or NIH 3T3 mouse fibroblast lysate. A minor band at 45 kD may be observed. Staining of the PKC  $\alpha$  80 kD band is inhibited with PKC  $\alpha$  peptide (659-672), but not with peptides corresponding to C-terminal sequences of PKC  $\beta_1$  (658-671), PKC  $\beta_2$  (660-673), PKC  $\gamma$  (684-697), PKC  $\delta$  (662-673), PKC  $\epsilon$  (726-737), or PKC  $\zeta$  (577-592).

**Protein Concentration:** 59.6 mg/ml by Biuret.

**Working Dilution**

1. A working dilution of 1:50,000 was determined by indirect immunoblotting using rat brain extract. Staining of an 80 kD band was observed.
2. A working dilution of 1:20,000 was determined by indirect immunoblotting using mouse NIH 3T3 fibroblast lysate. Staining of an 80 kD band was observed.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

**Description**

Protein Kinase C (PKC, 76-93 kD), 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.<sup>1</sup> PKC is a phospholipid dependent enzyme, activated by the lipid 1,2-diacylglycerol (DAG), an intracellular second messenger produced as a result from hydrolysis of inositol phospholipids, in response to a variety of hormones, growth factors and neurotransmitters.<sup>1-3</sup> 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.<sup>4-6</sup> 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 (30kDa) and a fragment containing the C-terminal kinase domain (50kDa) which is catalytically active.<sup>7,8</sup> 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  $\text{Ca}^{2+}$ , DAG and phosphatidylserine (PtdSer) for activity. The nPKC isoforms lack the C2 region presumably involved in  $\text{Ca}^{2+}$  binding. These isoforms have kinase activities regulated by DAG or PtdSer but are  $\text{Ca}^{2+}$  independent. The PKC  $\alpha$  isoenzyme is ubiquitously expressed in most tissues, and appears to be the major PKC isoform in fibroblasts.<sup>1,3</sup> 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 down-regulated by the phorbol ester TPA.<sup>9,10,11</sup> PKC  $\alpha$  directly phosphorylates and activates Raf-1 in NIH 3T3

fibroblasts.<sup>12</sup> 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.

### Uses

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

### Storage

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

\* Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

### References

1. Nishizuka, Y., *Nature*, **334**, 661 (1988).
2. Nishizuka, Y., *Science*, **258**, 607 (1992).
3. Hug, H., and Sarre, T., *Biochem. J.*, **291**, 329 (1993).
4. Robinson, P., *Molecular Neurobiology*, **5**, 87 (1991).
5. Mochly-Rosen, D., et al., *Proc. Natl. Acad. Sci. USA*, **88**, 3997 (1991).
6. Huang, K., and Huang, F., *Neurochemistry Int.*, **22**, 417 (1993).
7. Kishimoto, A., et al., *J. Biol. Chem.*, **264**, 4088 (1989).
8. Pears, C., and Parker, P., *J. Cell Science*, **100**, 683 (1991).
9. Hocevar, B., and Fields, A., *J. Biol. Chem.* **266**, 28 (1991).
10. Borner, C., et al., *J. Biol. Chem.*, **267**, 12892 (1992).
11. Leach, K., et al., *J. Cell. Biol.* **109**, 685 (1989).
12. Kolch, W., et al., *Nature* **364**, 249 (1993).