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# **ProductInformation**

# Anti-Protein Kinase Cg(PKC g)

Developed in Rabbit Affinity Isolated Antigen Specific Antibody

Product Number P 3328

### **Product Description**

Anti-Protein Kinase C  $\gamma$  (PKC  $\gamma$ ) is developed in rabbit using a synthetic peptide (Cys-Asp-Ala-Arg-Ser-Pro-Thr-Ser-Pro-Val-Pro-Val-Pro-Val-Met) conjugated to maleimide-activated KLH as the immunogen. The peptide corresponds to the C-terminal region of PKC  $\gamma$  (amino acid residues 684-697). The antibody is isolated from antiserum by immunospecific methods of purification. Antigen specific affinity isolation removes essentially all rabbit serum proteins, including immunoglobulins which do not specifically bind to protein kinase C  $\gamma$ .

Anti-Protein Kinase C  $\gamma$  recognizes PKC  $\gamma$  (80 kDa polypeptide) from rat brain extract in immunoblotting. The antibody does not react with PKC peptides corresponding to C-terminal sequences from PKC- $\beta_1$  (amino acid residues 658-671) and PKC- $\beta_2$  (amino acid residues 660-673) conjugated to BSA.

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 as a result from hydrolysis of inositol phospholipids, in response to a variety of hormones, growth factors and neurotransmitters. PKC is the major cellular receptor for the tumor-promoting phorbol esters. PKC action is thought to be mediated through the phosphorylation of several cellular substrates. 4-6 Proteolysis of PKC *in vivo* is thought to be 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) that is catalytically active. 7,8

Molecular cloning has established that the PKC family of isoenzymes consists of at least 9 subtypes that can be subdivided into 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( $\delta$ ,  $\epsilon$ ,  $\xi$ ,  $\eta$ (L), and  $\theta$ ). The cPKC isoforms have four conserved regions (C1 to C4) separated by five variable regions (V1 to V5) and require Ca<sup>2+</sup>, DAG and phosphatidylserine (PtdSer) for activity. The nPKC isoforms lack the C2 region, presumably involved in  $Ca^{2+}$  binding, and thus do not require  $Ca^{2+}$  for activity but require either DAG or expressed only in the central nervous system (i.e. the brain and spinal cord) and is restricted to a neuronal cell line (PC12). In the brain PKC is particularly concentrated in specific regions of the cerebral cortex, amygdala, hippocampus, and cerebellum (Purkinje cells). <sup>1,9,10</sup> Antibodies that react specifically with PKC isoenzymes are useful for the study of the differential tissue expression and intracellular and subcellular localization of these isoenzymes.

Anti-Protein Kinase C  $\gamma$  may be used for the detection of PKC  $\gamma$  using various immunochemical methods including immunoblotting, immunoprecipitation, and immunohistology.

#### Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

#### **Precautions and Disclaimer**

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

## Storage/Stability

For continuous use, store at 0-5 °C. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

#### **Product Profile**

- 1. A working dilution of 1:1,000 was determined by direct dot blot immunoassay using PKC  $\gamma$  peptide conjugated to BSA (0.25 0.50  $\mu$ g/dot).
- 2. A working dilution of 1:8,000 was determined by indirect immunoblotting using rat brain extract.

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

#### References

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JWM/KMR 08/02