Product No. P 8333
Lot 057H4811

Anti-Protein Kinase C δ
Produced in Rabbit
Delipidized, Whole Antiserum

Anti-Protein Kinase C δ (PKC δ) is generated in rabbits using a synthetic peptide Lys-Ser-Phe-Val-Asn-Pro-Lys-Tyr-Glu-Gln-Phe-Leu-Glu corresponding to the C-terminal variable (V5) region (amino acids 662-673 with N-terminal added Lys) of rat PKC δ. The peptide, coupled to KLH with glutaraldehyde, is used as immunogen. The antiserum is treated to remove lipoproteins. Rabbit Anti-Protein Kinase C δ is supplied liquid containing 0.1% sodium azide (see MSDS)* as preservative.

Specificity

Anti-Protein Kinase C δ (PKC δ) reacts in immunoblotting (SDS-PAGE) with PKC δ (80 kD polypeptide) from rat brain extract. Staining of the PKC δ 80 kD band is specifically inhibited with PKC δ peptide (662-673), but not with peptides corresponding to C-terminal sequences of PKC α (659-672), PKC β_1 (658-671), PKC β_2 (660-673), PKC γ(684-697), PKC ϵ (726-737), or PKC ζ (577-592). Anti-Protein Kinase C δ specifically reacts in dot-blot immunoassay with PKC δ peptide conjugated to BSA with 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDCI).

Protein Concentration: 66.7 mg/ml by Biuret.

Working Dilution

1. A working dilution of 1:10,000 was determined by immunoblotting using rat brain extract. Specific staining of PKC δ (80 kD band) is blocked by incubating prediluted antibody with PKC δ peptide (concentration 0.5 µg/ml), for 2 hours at room temperature or overnight at 4°C.
2. A working dilution of 1:50,000 was determined by indirect dot blot immunoassay using PKC δ peptide conjugated to BSA with EDCI (conjugate concentration 125 ng/dot).

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 playing a crucial role in signal transduction leading to cellular regulation, cell growth and differentiation, oncogenesis, and modulation of neurotransmission.[1] PKC is a phospholipid-dependent enzyme, activated by the lipid 1,2-diacylglycerol (DAG), an intracellular second messenger produced as a result of 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 ester derivatives. PKC action is 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 kD) and a fragment containing the C-terminal kinase domain (50 kD) which is catalytically active.[7,8] Molecular cloning has established that PKC consists of at least 9 different isoenzymes which can be subdivided into two major classes based on their primary structure and activation requirements: conventional (cPKC) isoforms (α, β_1, β_2 and γ) and novel (nPKC) isoforms (δ, ϵ, ζ, ή(L) and θ). The cPKC isoforms have four conserved regions (C1 to C4) separated by five variable regions (V1 to V5) and require Ca^{2+}, DAG and phosphatidylserine (PtdSer) for activity. The nPKC isoforms lack the C2 region presumably involved in Ca^{2+} binding. These isoforms have kinase activities regulated by DAG or PtdSer but are Ca^{2+} independent. The PKC δ isoenzyme appears to be widely expressed in the brain, lung, heart, spleen, liver, ovary, pancreas, thymus, adrenal gland, skin and rat embryonic fibroblasts, and is expressed in lower levels in certain mouse fibroblasts.[3,9,10,11,12] Overexpression and stimulation of PKC δ leads to cell division arrest in CHO cells and growth inhibition of NIH3T3 cells.[12,13] Antibodies that react specifically with PKC isoenzymes may be used to study the specific activation requirements, differential tissue expression, intracellular

localization, of these isoenzymes.

Uses

Anti-Protein Kinase C δ (PKC δ) may be used for the detection of PKC δ isozyme by various immunochemical methods including immunoblotting, immunoprecipitation, ELISA and immunohistochemistry. The antibody may be used for detecting PKC δ by 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., J. Biol. Chem., **266**, 14866 (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. Ogita, K., et al., Proc. Natl. Acad. Sci. USA, **89**, 1592 (1992).
10. Borner, C., et al., J. Biol. Chem., **267**, 12892 (1992).
11. Wetsel, W., et al., J. Cell. Biol., **117**, 121 (1992).
12. Mischak, H., et al., J. Biol. Chem., **268** 6090 (1993).
13. Watanabe, T. et al., Proc. Natl. Acad. Sci. USA, **89**, 10159 (1992).