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ProductInformation

ANTI-PROTEIN KINASE C η
Developed in Rabbit
Delipidized, Whole Antiserum

Product Number **P 8090**

Product Description

Anti-Protein Kinase C η (PKC η) is developed in rabbit immunized using a synthetic peptide corresponding to the C-terminal variable (V5) region (amino acids 670-683) of mouse PKC η coupled to KLH. This sequence is highly conserved in human PKC η (single substitution of C-terminal amino acid between human and mouse PKC η).

Protein Kinase C (PKC, 76-93 kDa) is a family of serine/threonine 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 from hydrolysis of inositol phospholipids, in response to a variety of hormones, growth factors and neurotransmitters.[1-3] PKC is the major cellular receptor for the tumor-promoting phorbol esters derivatives. 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 PKC consists of several different isoenzymes which can be subdivided in two major classes based on their primary structure and activation requirements: conventional (cPKC) isoforms (α, β_1, β_2 and γ), novel (nPKC) isoforms (δ, ε, η and θ), and atypical (aPKC) isoforms (λ and ζ).[2,3] 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 involved in Ca^{2+} binding. These isoforms have kinase activities regulated by DAG or PtdSer but are Ca^{2+} independent. The aPKC isoforms, which have only zinc finger-like domain, are unique in that their activity is independent of Ca^{2+}, DAG and phorbol esters. The PKC η isoform is expressed in skin, lung and heart and in very low levels in the brain.[3,] PKC η is also expressed in several skin-derived human cell lines.[9,10,11] PKC η has been reported to be localized specifically in the cell nucleus and is not down-regulated by phorbol ester treatment.[10] Antibodies that react specifically with PKC isoenzymes are useful for the study of the specific activation requirements, differential tissue expression, intracellular localization, of these isoenzymes.

Reagents

The product is provided as rabbit antiserum containing 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 MSDS for information regarding hazardous 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 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.

Procedure

Immunoblotting Procedure for PKC Isozymes Using NIH 3T3 (PKCη-3T3) Total Cell Lysate

Preparation of NIH 3T3 (PKC η-3T3) Cell Lysate

Reagents

1. NIH 3T3 mouse fibroblasts culture over-expressing human PKC η (PKC η-3T3 cell line).
1. Laemmli sample buffer (1X) containing 2-mercaptoethanol (5% v/v).
3. Dulbecco's Modified Eagle Medium (DMEM) (Product No. D 5671).
4. Fetal Bovine Serum (FBS) (Product No. F 2442).
5. Phosphate Buffered Saline (PBS) (Product No. P 3813).

Procedure

Note: Due to the rapid degradation of PKC η, the total cell lysate must be freshly prepared, just before use.

1. Grow cells to confluence in 10 cm plate containing 10% FBS in DMEM.
2. Remove medium from culture dish.
3. Rinse plates with ice cold PBS (2 x 10 ml).
4. Add 1.2 ml/plate of boiling sample buffer and scrape cells.
5. Remove cell lysate to appropriate vial and boil for 5 minutes at 95 °C.
6. Centrifuge lysate at 12,000 x *g* for 10 minutes at room temperature.
7. Store 0.1 ml of total cell lysate at –70 °C.

Immunoblotting

Reagents and Equipment

1. Freshly prepared NIH3T3 fibroblasts total cell lysate (PKC η-3T3 cell line).
2. 10% polyacrylamide slab minigel
3. Nitrocellulose membrane (0.45 μm).
4. Prestained LMW markers (Product No. C 3312).
5. Blocking Buffer : 10% dry milk (w/v) (Product No. M 7409) in 10 mM PBS pH 7.4 (Product No. P 3813).
6. Dilution Buffer: 1% BSA in PBS pH 7.4 (Product No. P 3688) containing 0.05% TWEEN-20 (Product No. P 1379).
7. Washing Buffer: PBS pH 7.4 containing 0.05% TWEEN-20 (Product No. P 3563).
8. PKC η peptide (PKC η 670-683). Dissolve peptide in dH_2O at 1 mg/ml by addition of 3N NaOH to final pH 9-11.
9. Primary antibody: Anti-Protein Kinase C η at working dilution.
10. Alkaline Phosphatase Anti-Rabbit IgG (Product No. A 9919) at appropriate dilution.
11. SIGMA *FAST*™ BCIP/NBT Tablets (Product No. B 5655).
12. Electrophoresis and transfer apparatus.

Procedure

To obtain best results in different preparations, optimize procedure conditions (dilutions, incubation times, blocking conditions etc.), for a specific application.

1. Resolve freshly prepared NIH 3T3 total cells lysate (PKC η-3T3 cell line), (15-30 μl/well) on precast 10% polyacrylamide minigel.
2. Run SDS-PAGE at room temperature.
3. Perform transfer to nitrocellulose at room temp.
4. Block nitrocellulose in blocking buffer for at least 1 hour at room temperature.
5. Incubate membrane with primary antibody dilutions for 2 hours at room temperature.[(a)]
6. Wash membrane with washing buffer 4 times, 5 minute each wash.
7. Incubate membrane with secondary antibody at recommended dilution for 1 hour at room temp.
8. Wash membrane as in step 6 then wash once for 5 minute in deionized water.
9. Incubate membrane with BCIP/NBT substrate.
10. Wash membrane thoroughly with deionized water.
11. Air-dry blots on filter paper.

[(a)]Note: For specific inhibition of PKC η (80 kD band) it is recommended to incubate prediluted antibody with PKC η peptide (concentration of 1.0 μg/ml) for 2 hours at room temperature or overnight at 4 °C.

Product Profile

By immunoblotting Anti-Protein Kinase C η (PKC η) reacts with PKC η (80 kDa protein) using a lysate of mouse NIH 3T3 cells over-expressing human PKC η. Staining of the PKC η 80 kDa band is specifically inhibited with PKC η peptide (670-683).

Anti-Protein Kinase C η (PKC η) may be used for the detection of PKC η isoenzyme by immunoblotting using cell culture extracts.

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

References

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