Product No. A-4210
Monoclonal Anti-Protein Kinase C-Agarose
Purified Mouse Immunoglobulin
Clone MC5

Monoclonal Anti-Protein Kinase C (PKC) (mouse IgG2a isotype) is derived from the MC5 hybridoma produced by fusion of mouse myeloma cells and splenocytes from an immunized mouse. Purified bovine brain protein kinase C was used as the immunogen.[1] Monoclonal Anti-Protein Kinase C-Agarose consists of affinity-purified monoclonal antibody to PKC coupled to agarose. The product is supplied as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Monoclonal Anti-Protein Kinase C (clone MC5) recognizes an epitope located within the amino acid residues 296-317 of protein kinase C (PKC).[1] It has been reported to bind to the α, β_1 and β_2 PKC isoforms, but not to the γ isoform (80 kD polypeptides). Monoclonal Anti-Protein Kinase C conjugated to agarose immunoprecipitates specifically the PKC α isoform from a NIH 3T3 mouse fibroblast cell lysate. This cell line has been reported to contain mainly the PKC α isoform.[7]

Description

Protein kinase C (PKC, 77-90 kD), is a family of homologous serine-threonine protein kinases, which are key regulatory enzymes in signal transduction, cellular regulation, tumor promotion and oncogenesis.[1] PKC is a calcium-dependent and phospholipid-dependent enzyme that is activated *in vivo* by the lipid diacylglycerol, produced in response to a variety of hormones and growth factors.[1-3] PKC consists of a single polypeptide chain, containing four conserved regions and five variable regions. Sequence information defined a putative domain structure for the enzyme which can be divided into an amino-terminal regulator and a carboxy-terminal catalytic domain joined by a hinge region. Proteolysis of purified native PKC by trypsin yields two major fragments, representing the regulatory and kinase domains of the enzyme, due to cleavage in a proposed hinge region between residue 292 and residue 317.[4] There is evidence that *in vivo* agonist-induced generation of a catalytic fragment of the enzyme occurs as well.[1] Molecular cloning has established that PKC consists of several different isoenzymes which can be subdivided in three 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 (ζ, λ and ι).[5-7] 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. Antibodies that react specifically with PKC are useful for the study of the specific activation requirements, subcellular distribution, variation of mode of action and substrate specificities of these isoenzymes. Furthermore, they also allow the detection and localization of PKC in normal and malignant tissues. Monoclonal Anti-Protein Kinase C-Agarose is particularly useful for immunoprecipitation and immunoaffinity purification procedures of PKC.

Uses

Monoclonal Anti-Protein Kinase C-Agarose may be used for the immunoprecipitation of protein kinase C α isoform from cell culture lysates.

Antibody-Agarose Performance

Anti-Protein Kinase C-Agarose performance is determined by immunoprecipitation of PKC α from NIH 3T3 mouse fibroblasts cell lysate and its detection by immunoblotting. For microscale immunoprecipitation or immunopurification of PKC α, it is recommended to determine the optimal amount of antibody-agarose conjugate and cell lysate. As a guideline 50 µl of antibody-agarose per 250-500µg cell lysate may be used.

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

Storage

For continuous use, store at 2-8°C. **Do Not Freeze.**

* 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

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Immunoprecipitation and Western Blotting Procedure of PKC Isozymes Using NIH 3T3 Lysate

Reagents and Equipment

1. NIH3T3 mouse fibroblasts culture.
2. Phenylmethyl-sulfonylfluoride (PMSF) (Sigma Product No. P-7626), 0.5 M in EtOH.
3. Leupeptin (Sigma Product No. L-2884).
4. Aprotinin (Sigma Product No. A-4529).
5. IGEPAL (Sigma Product No. I-3021).
6. Anti-PKC- Agarose (Sigma Product No. A-4210).

Note: Add all protease inhibitors freshly before cell lysis.

7. 10 mM PBS pH 7.4, (Sigma Product No. P-3813)
8. Lysis Buffer SBN, Ice cold: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% (w/v) glycerol, 1% (w/v) IGEPAL, 1 mM EGTA, 10 µg/ml aprotinin, 10 µg/ml leupeptin and 2 mM PMSF.
9. Washing Buffer HNTG, Ice cold: 20 mM HEPES buffer pH 7.5, 150 mM NaCl containing 0.1% (w/v) Triton X-100 and 10% (w/v) glycerol.
10. Refrigerated Eppendorf microcentrifuge
11. Protein assay kit.
12. Laemmli sample (3X) buffer with 2-mercaptoethanol.

Procedure

All steps are carried out at 2-8°C, unless otherwise noted. Prewash of the antibody-agarose conjugate can be performed one day prior to the IP procedure. Immunoprecipitation must be completed and immunoprecipitates run on SDS-PAGE on the same day.

Preparation of a NIH 3T3 Cell Lysate

1. Grow cells to confluence at 37°C in 10 cm plate containing 10% FCS in DMEM.
2. Place plates containing NIH 3T3 cells on ice.
3. Gently suction growing medium from each plate and replace with ice-cold PBS, pH 7.4 (10 ml/plate).
5. Gently suction PBS from each plate.
6. Replace with ice-cold lysis buffer (0.55 ml/plate).
7. Scrape cells from plates with rubber policeman
8. Remove cell lysate to appropriate vial, vortex, and leave for 15 min. on ice with occasional mixing.
9. Centrifuge lysate at 12,000 x g for 10 min at 4°C, to remove nuclei and cell debris.
10. Determine protein concentration of lysate.

Immunoprecipitation

1. Wash the appropriate amount of antibody-agarose (50 µl/vial) with HNTG buffer (0.75 ml/vial), by re-suspension and centrifugation at 12,000 x g for 10 sec. at room temp. Remove buffer. Repeat 4 times.
2.. Add 0.25 mg of lysate to Anti-PKC-Agarose (50 µl).
3. Incubate for 90 minutes at 2-8°C with mixing.
4. Wash the antibody-agarose with HNTG buffer (0.75 ml/vial), by resuspension and centrifugation at 12,000 x g for 10 sec at 4°C. Remove HNTG buffer. Repeat this step at least 4 times.
5. Resuspend the pellet in 50 µl HNTG. Add 25 µl of 3x sample buffer.
6. Heat samples for 5 min. at 95°C. Centrifuge samples at 12,000 x g for 30 sec. at room temperature. Collect supernatant and subject it immediately to SDS-PAGE.

Immunoblotting

Reagents and Equipment

1. Immunoprecipitate of NIH3T3 fibroblasts lysate.
2. 10% polyacrylamide slab minigel with 5% stacking gel
3. Nitrocellulose membrane (0.45µm).
4. Prestained markers (Sigma Product No. C-3312).
5. Blocking Buffer: 10% dry milk (w/v) in TBS.
6. Dilution Buffer: 1% BSA in PBS, pH 7.4 with 0.05% Tween-20.
7. Washing Buffer: PBS, pH 7.4 with 0.05% Tween-20.
8. PKCα peptide (amino acids 659-672) 0.5 mg/ml in dH_2O. Store aliquots at −20°C.
9. Anti-Protein Kinase C α (Sigma Product No. P-4334).
10. Alkaline Phosphatase conjugated secondary at appropriate dilution (Sigma Product No. A-9919).
11. BCIP/NBT Tablets (Sigma Product No. B-5655).
12. Electrophoresis and transfer apparatus.

Procedure

1. Resolve freshly prepared immunoprecipitate (10-30 µl/well) on precast 10% polyacrylamide gel.
2. Run SDS-PAGE at room temperature.
3. Perform transfer at room temperature to nitrocellulose.
4. Block membrane in blocking buffer for at least 1 hour
5. Incubate membrane with diluted primary antibody for 2 hours at room temperature. For specific inhibition of PKCα (80 kD) incubate prediluted antibody with PKCα peptide (2.0 µg/ml) overnight at 2-8°C.
6. Wash membrane with washing buffer 4x, 5 min each.
7. Incubate membrane with secondary antibody at recommended dilution for 1 hour at room temperature.
8. Wash membrane with washing buffer 4x, 5 min each. Wash 1x 5 min. in deionized water.
9. Incubate the membrane with BCIP/NBT substrate.
10. Wash membrane thoroughly with dH_20 then air dry.