Technical Data Sheet

Alexa Fluor® 647 Mouse anti-PKCα

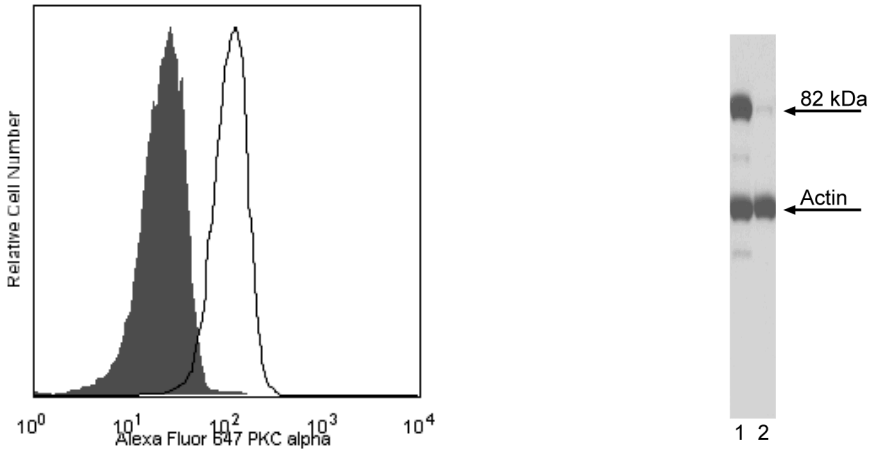
Product Information

Material Number:	**560243**
Alternate Name:	Protein Kinase C α, PKC-α, PKC-A, PKCA, PRKCA
Size:	50 tests
Vol. per Test:	20 µl
Clone:	3/PKCα
Immunogen:	Human PKCα aa. 270-427 Recombinant Protein
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The Protein Kinase C (PKC) family of homologous serine/threonine protein kinases is involved in a number of processes, such as growth, differentiation, and cytokine secretion. At least eleven isozymes have been described. These proteins are products of multiple genes and alternative splicing. Conventional PKC (cPKC) subfamily members (α, β, and γ isoforms) consists of a single polypeptide chain containing four conserved regions (C) and five variable regions (V). The N-terminal half containing C1, C2, V1, and V2 constitutes the regulatory domain and interacts with the PKC activators Ca2+, phospholipid, diacylglycerol, or phorbol ester. However, the the C2-like domains of novel PKC (nPKC) subfamily members (δ, ε, η, and θ isoforms) are Ca2+-independent. The atypical PKC (aPKC) subfamily members (ζ, ι, and λ isoforms) lack the C2 domain and are unique in that their activity is independent of diacylglycerols and phorbol esters. They also lack one repeat of the cysteine-rich sequences that are conserved in cPKC and nPKC members. The C-terminal region of PKC contains the catalytic domain. The PKC pathway represents a major signal transduction system that is activated following ligand-stimulation of transmembrane receptors by hormones, neurotransmitters, and growth factors. PKCα regulates a wide variety of functions such as cellular growth, apoptosis, cardiomyocyte function, and brain cognitive functions.

The 3/PKCα monoclonal antibody recognizes PKCα, regardless of phosphorylation status, and has been reported to crossreact with PKCβ.



Analysis of PKCa in lymphocytes. *[Left Panel] Human peripheral blood mononuclear cells (PBMC) were either stimulated with 10 µM Phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, Cat. No. P8139) for 24 hours (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-PKCα. Lymphocytes were selected by scatter profile. The data demonstrates that the expression of PKCα decreases when the lymphocytes are stimulated by PMA. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. [Right Panel] The specificity of mAb 3/PKCa was confirmed by western blot analysis using unconjugated antibody (Cat. No. 610107 or 610108) at 0.063 µg/ml on lysates from control (lane 1) and PMA-treated (lane 2) PBMC. PKCa is identified as a band of 82 kDa, which decreases in intensity in the treated cells. Purified Mouse anti-Actin monoclonal antibody (Cat. No. 612656 or 612657) was the gel-loading control.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation. Any of the three BD Phosflow™ permeabilization buffers may be used.

The purified format for this antibody has been reported to be reactive to human, mouse, rat, chicken, dog, and frog by western blotting (Cat. No. 610107 or 610108).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
610107	Purified Mouse Anti-PKCα	50 µg	3/PKCα
610108	Purified Mouse Anti-PKCα	150 µg	3/PKCα

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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