Technical Data Sheet

PE Mouse anti-PKCα (pT497)

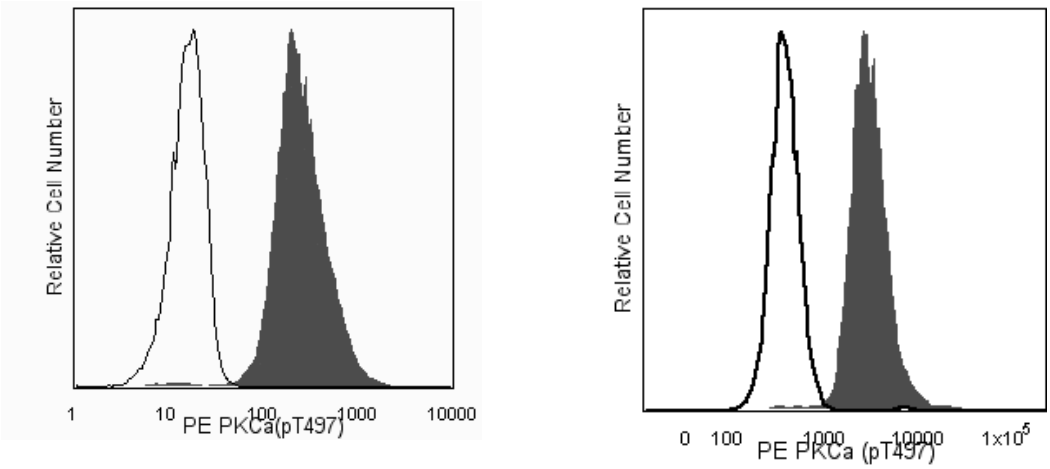
Product Information

Material Number:	**560141**
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	K14-984
Immunogen:	Phosphorylated Human PKCα Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Protein Kinase C (PKC) family of serine/threonine protein kinases is involved in a number of processes such as growth, differentiation, and cytokine secretion. Three categories exist, conventional PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC). All have C-terminal kinase domains, which are closely related to those of protein kinases A and B (Akt), and variable N-terminal regulatory domains that give them different modes of activation. For example, cPKC (α, βI, βII, and γ isoforms) are calcium-activated, phospholipid-dependent serine/threonine-specific enzymes that can also be activated by phorbol esters. However, nPKC (δ, ε, η, and θ isoforms) and aPKC (ζ, ι, and λ isoforms) are Ca2+-independent. aPKC are unique in that their activity is independent of diacylglycerols and phorbol esters. Phosphorylation at three conserved sites in the kinase domain is required for catalytic activity. Specifically, the threonine 497 (T497) of PKCα is in the activation loop of the kinase domain and is phosphorylated by the constitutively active phosphoinositide-dependent kinase-1 (PDK-1).

The K14-984 monoclonal antibody recognizes the phosphorylated T497 in the kinase domain of human PKCα.



Analysis of PKCα (pT497) in human peripheral blood lymphocytes and vascular endothelium.
LEFT PANEL: Human peripheral blood mononuclear cells were either treated with 50 nM calyculin A for 30 minutes at 37ºC (shaded histogram) or untreated (open histogram). For data analysis, lymphocytes were selected by their scatter profile.
RIGHT PANEL: After serum starvation overnight, EA.hy926 cells (see reference Edgell, McDonald, Graham, 1983; ATCC CRL-2922) were detached with trypsin, washed, resuspended in serum-free DMEM, and either treated with 50 nM calyculin A (shaded histogram) for 30 minutes at 37°C or untreated (open histogram).
The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for 30 minutes, and then stained with PE Mouse anti-PKCα (pT497). The data demonstrates that the level of phosphorylation of PKCα increases when phosphatase activity is inhibited by the treatment. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. BD Phosflow™ Fix Buffer I (Cat. No 557870) may be used for cell fixation.

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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	EA-hy 926	Calyculin A	Cytofix or Fix I	Perm III	Up-regulation
	Human	PBMC	Calyculin A	Cytofix or Fix I	Perm III	Up-regulation
	Human	Jurkat	Phospho peptide	Cytofix or Fix I	Perm III	Blocking
	Human	Jurkat	Irrelevant peptide	Cytofix or Fix I	Perm III	No blocking
	Human	Jurkat	Lambda phosphatase	Cytofix or Fix I	Perm III	Signal reduction
	Human	Jurkat	Kinase inhibitors	Cytofix or Fix I	Perm III	No change
WB	Human	EA-hy 926	Calyculin A			Up-regulation
	Human	PBMC	Calyculin A			Up-regulation
	Human	Jurkat	Lambda phosphatase			Signal reduction
	Mouse	Splenocytes	Calyculin A			Up-regulation

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
557870	Fix Buffer I	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

Edgell C-JS, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. *Proc Natl Acad Sci U S A.* 1983; 80:3734-3737. (Methodology)

Newton AC. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. *Biochem J.* 2003; 370:361-371. (Biology)

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