Technical Data Sheet

PE Mouse anti-PDPK1 (pS241)

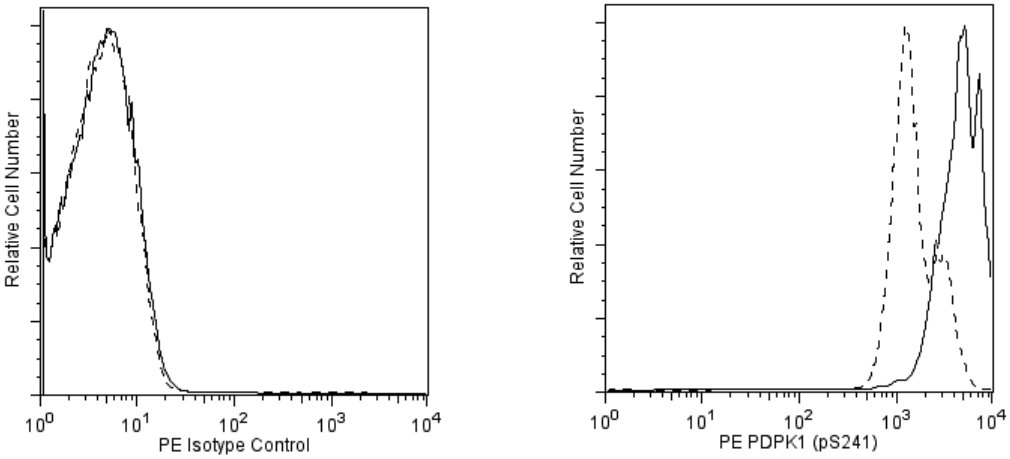
Product Information

Material Number:	**560092**
Alternate Name:	PDK1 (pS241), PKB Kinase (pS241)
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J66-653.44.17
Immunogen:	Phosphorylated Human PDPK1 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The serine/threonine kinase 3-*P*hosphoinositide-*D*ependent *P*rotein *K*inase-1 (PDPK1, also known as PDK1) contributes to the activation of many important kinases in the insulin and IGF-1 signaling pathways. It acts downstream of phosphatidylinositol 3-kinase (PI3-kinase) to phosphorylate residues in the activation loops of many cellular kinases, including protein kinase B (PKB/Akt), PKC isoforms, p70 S6 kinase, and PDPK1 itself. The autophosphorylation of PDPK1 at serine 241 (S241) has recently been suggested to play a role in the regulation of PDPK1. It has been proposed that PDPK1 activity plays a key role in the regulation of various cellular events such as cell proliferation, differentiation, and apoptosis.

The J666-653.44.17 monoclonal antibody recognizes the phosphorylated S241 in the activation loop of human PDPK1. The orthologous phosphorylation site in mouse and rat PDPK1 is S244.



Analysis of PDPK1 (pS241) in human peripheral blood mononuclear cells (PBMC). *PBMC were either treated with calyculin A plus okadaic acid for 30 minutes at 37ºC or untreated. The cells were fixed with pre-warmed BD™ Phosflow Lyse/Fix Buffer (Cat. No. 558049) for 15 minutes at 37ºC and permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for 30 minutes. LEFT PANEL: The treated (solid line) and untreated (dashed line) PBMC were stained with PE Mouse IgG1, κ isotype control mAb MOPC-21 (Cat. No. 559320). RIGHT PANEL: The treated (solid line) and untreated (dashed line) PBMC were stained with PE Mouse anti-PDPK1 (pS241). The data demonstrates that the phosphorylation of PDPK1 is constitutive in all PBMC and that the level of phosphorylation increases when phosphatase activity is inhibited by the treatment. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

Preparation and Storage

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.

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

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This mAb was characterized by flow cytometry (Flow) and western blot analysis (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	Jurkat	Calyculin A + Okadaic Acid	Lyse/Fix or Cytofix	III	Up-regulation
Flow	Human	Jurkat	Calyculin A + Okadaic Acid	Lyse/Fix	I or II	Unsatisfactory
Flow	Human	PBMC	Calyculin A + Okadaic Acid	Lyse/Fix	III	Up-regulation
WB	Human	Jurkat	Calyculin A + Okadaic Acid			63 kDa

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Komander D, Kular G, Deak M, Alessi DR, van Aalten DM. Role of T-loop phosphorylation in PDK1 activation, stability, and substrate binding. *J Biol Chem.* 2005; 280(19):18797-18802. (Biology)

Wick MJ, Ramos FJ, Chen H, et al. Mouse 3-phosphoinositide-dependent protein kinase-1 undergoes dimerization and trans-phosphorylation in the activation loop. *J Biol Chem.* 2003; 278(44):42913-42919. (Biology)