Technical Data Sheet

PE-CF594 Mouse Anti-Akt (pS473)

Product Information

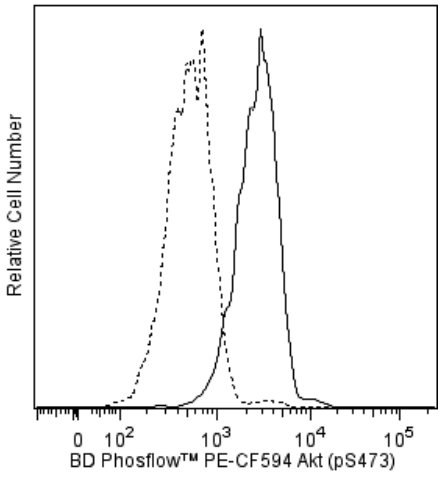
Material Number:	**562465**
Alternate Name:	Akt1, Akt2, Akt3, PKBα, PKBβ, PKBγ, RAC-PKα, RAC-PKβ, RAC-PKγ, STK-2
Size:	50 tests
Vol. per Test:	5 µl
Clone:	M89-61
Immunogen:	Phosphorylated Human Akt1 (pS473) Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Clone tested in development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Akt [also known as PKB (Protein kinase B) or RAC-PK (Related to the A and C kinases)] is a family of serine/threonine kinases that contains a pleckstrin homology (PH) domain. PH domains play important roles in signal transduction. There are three known isoforms of Akt in mammalian cells [Akt1 (α), Akt2 (β) and Akt3 (γ)]; they are thought to be regulated similarly. Akt is activated by insulin and growth factors by a mechanism involving phosphoinositide 3-OH kinase. Phosphoinositide 3-OH kinases products bind to the PH domain, resulting in translocation of Akt to the plasma membrane and activation of Akt to phospho-Akt by upstream kinases. Akt is phosphorylated within the activation loop at threonine 308 and the C-terminus at serine 473 (S473). Phospho-Akt promotes cell survival by inhibiting apoptosis. Specifically, phospho-Akt1 has been shown to phosphorylate Bad, a member of the Bcl-2 family that promotes cell death. This phosphorylation results in the inactivation of the proapoptotic function of Bad. The Akt molecule is thus considered to link extracellular survival signals (growth factors) with the apoptotic machinery (BAD). Akt is also a key mediator of the metabolic effects of insulin. Additionally, Akt has been referred to as an oncogene because it has increased activity in a number of tumors.

The M89-61 antibody recognizes Akt phosphorylated at S473. This phosphorylation site is shared by all three isoforms of Akt. The homologous phosphorylation sites in Akt2 and Akt3 are S474 and S472, respectively.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Flow cytometric analysis of Akt (pS473) expression in human T-cell leukemia cells. *Human Jurkat cells (ATCC TIB-152) were either treated with 1 µM Wortmannin (Life Technologies, Cat. No. PHZ1301) for 2 hours at 37°C (dotted line histogram) or left untreated (solid line histogram). The cells were fixed (BD Cytofix™ Fixation Buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with BD Phosflow™ PE-CF594 Mouse Anti-Akt (pS473) (Cat. No. 562465). The data demonstrates that the level of phosphorylation of Akt (pS473) decreases when phosphatidylinositol 3-kinase activity is inhibited by the treatment of Jurkat cells with Wortmannin. The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed on a BD™ LSR II Flow Cytometry System.*

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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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	Jurkat	Wortmannin	Cytofix	Perm III	Phosphorylation reduced
	Human	Whole blood	PMA	Lyse/Fix	Perm I, II, III, or IV	Induced
	Human	PBMC	PMA	Cytofix	Perm I, II, III, or IV	Induced
	Mouse	NIH/3T3	PDGF	Cytofix	Perm III	Induced
	Mouse	Spleen cells	PMA	Lyse/Fix	Perm I, II, III, or IV	Induced
WB	Human	Jurkat	Wortmannin			60-kDa band reduced
			peptide blocking			Blocked by pS473 phospho peptide but not by non-phospho or unrelated phospho peptide
			lambda phosphatase			Loss of signal
	Mouse	NIH/3T3	PDGF			60-kDa band induced

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 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. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. CF™ is a trademark of Biotium, Inc.
9. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
10. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
11. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
12. 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

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Ferrigno P, Silver PA. Regulated nuclear localization of stress-responsive factors: how the nuclear trafficking of protein kinases and transcription factors contributes to cell survival. *Oncogene.* 1999; 18(45):6129-6134. (Biology)
Kandel ES, Hay N. The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. *Exp Cell Res.* 1999; 253(1):210-229. (Biology)

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