Technical Data Sheet

Purified Mouse anti-Akt (pS473)

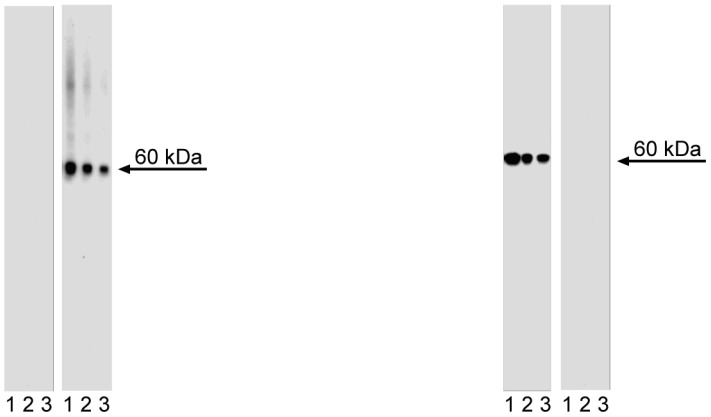
Product Information

Material Number:	**560397**
Alternate Name:	Akt1, Akt2, Akt3, PKBα, PKBβ, PKBγ, RAC-PKα, RAC-PKβ, RAC-PKγ, STK-2
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	M89-61
Immunogen:	Phosphorylated Human Akt1 (pS473) Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse Predicted due to immunogen sequence identity: Cow, Rat
Target MW:	60 kDa
Storage Buffer:	Aqueous buffered solution containing ≤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.



LEFT: ***Western blot analysis of AKT (pS473) in mouse embryonic fibroblasts.*** *Lysates from control (left blot) and PDGF-treated (Sigma Cat. No. P8147, right blot) NIH/3T3 cells (ATCC CRL-1658) were probed with Purified Mouse anti-Akt (pS473) monoclonal antibody at the following concentrations: 12.5 (lanes 1), 6.25 (lanes 2), and 3.125 ng/ml (lanes 3). AKT (pS473) is identified as a 60-kDa band in the treated cells. RIGHT:* ***Western blot analysis of AKT (pS473) in human T leukemia.*** *Lysates from control (left panel) and Wortmannin-treated (Invitrogen, Cat. No. PHZ1301, right panel) Jurkat cells (ATCC TIB152) were probed with Purified Mouse anti-Akt (pS473) monoclonal antibody at the following concentrations: 2.0 (lanes 1), 1.0 (lanes 2), and 0.5 µg/ml (lanes 3). The data demonstrates that the level of phosphorylation of Akt (pS473) decreases when phosphatidylinositol 3-kinase activity is inhibited.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

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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	Jurkat	none	Cytofix	Perm III	expression observed
			Wortmannin	Cytofix	Perm III	Down-regulated expression
	Mouse	NIH/3T3	PDGF	Cytofix	Perm III	Up-regulated expression
WB	Human	Jurkat	none			60-kDa band
			1 μM Wortmannin for 2 hours			signal reduced
			phospho peptide			blocking of 60-kDa band
			non-phospho peptide or unrelated phospho peptide			no blocking
			lambda phosphatase			loss of signal

Application Notes

Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. An isotype control should be used at the same concentration as the antibody of interest.

References

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Datta SR, Dudek H, Tao X, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell.* 1997; 91:231-241. (Biology)

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)

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