Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Akt (pS473)

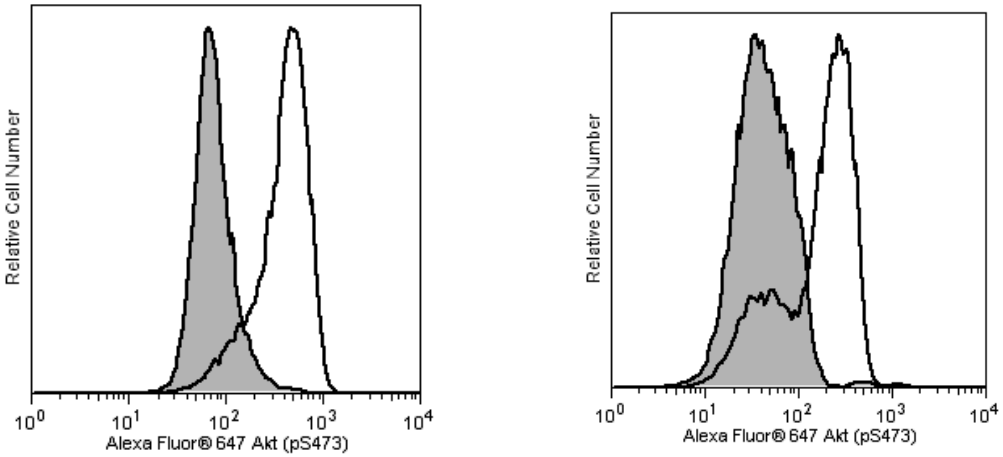
Product Information

Material Number:	**560343**
Alternate Name:	Akt1, Akt2, Akt3, PKBα, PKBβ, PKBγ, RAC-PKα, RAC-PKβ, RAC-PKγ, STK-2
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	M89-61
Immunogen:	Phosphorylated Human Akt1 (pS473) 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

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: Analysis of Akt (pS473) in mouse embryonic fibroblasts. *Serum-starved NIH/3T3 cells (ATCC CRL-1658) were either stimulated with PDGF-BB (Cat. No. 354051, open histogram) or unstimulated (shaded histogram). The cells were fixed (BD Cytofix™ 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 Alexa Fluor® 647 Mouse anti-Akt (pS473). Flow cytometry was performed on a BD FACSArray™ flow cytometry system.*

RIGHT: Analysis of Akt (pS473) in human T-cell leukemia. *Jurkat cells (ATCC TIB-152) were either treated with 1 µM Wortmannin (Invitrogen, Cat. No. PHZ1301) for 2 hours at 37°C (shaded histogram) or left untreated (open histogram). The cells were fixed (BD Cytofix™ 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 Alexa Fluor® 647 Mouse anti-Akt (pS473). The data demonstrates that the level of phosphorylation of Akt (pS473) decreases when phosphatidylinositol 3-kinase activity is inhibited by the treatment. Flow cytometry was performed on a BD FACSArray™ flow cytometry system.*

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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.

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

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 mL	(none)
554655	Fixation Buffer	100 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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. 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.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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