Technical Data Sheet

PE Mouse anti-Akt1

Product Information

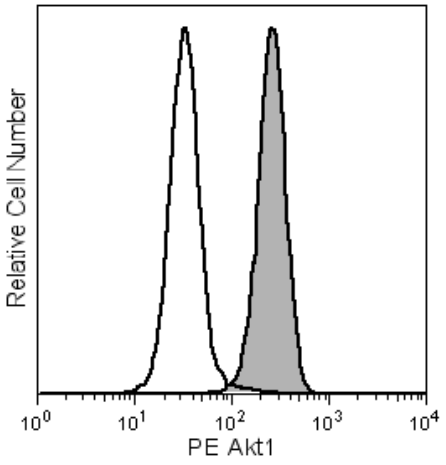
Material Number:	**560049**
Alternate Name:	PKBα, RAC-PK
Size:	50 tests
Vol. per Test:	20 µl
Clone:	55/PKBa/Akt
Immunogen:	Human Akt1 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed by flow cytometry: Human Confirmed by western blot using purified antibody (Cat. No. 610860 or 610861): Dog, Human, Mouse, Rat
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 kinase 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. 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 55/PKBa/Akt monoclonal antibody recognizes Akt1, regardless of phosphorylation status.

The specificity of this antibody conjugate for flow cytometric analysis was validated by confirming that RNA-mediated interference (RNAi) of the specific protein reduced the staining of the cells (see figure). Furthermore, the capacity of the RNAi to down-regulate the expression of the relevant protein was confirmed by western blot analysis.



Analysis of Akt1 in human epithelioid carcinoma. *HeLa S3 cells (ATCC CCL 2.2) were either transfected with Akt1 RNAi (open histogram) or untreated (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 30 minutes, and then stained with PE Mouse anti-Akt1. Down-regulation of Akt1 expression is evident in the RNAi-transfected cells. Flow cytometry was performed on a BD FACSArray™ bioanalyzer 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
557870	Fix Buffer I	250 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry 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.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Alessi DR, Andjelkovic M, Caudwell B, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J.* 1996; 15(23):6541-6551. (Biology: Activation)

Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A.* 1999; 96(8):4240-4245. (Biology)

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)

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)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**