Technical Data Sheet

PE Mouse Anti-Akt (pT308)

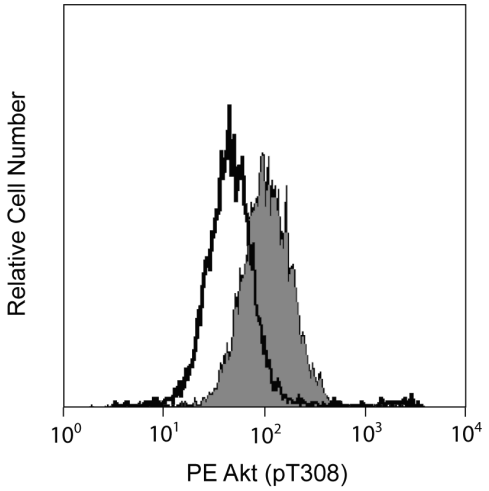
Product Information

Material Number:	**558275**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J1-223.371
Immunogen:	Phosphorylated Human Akt1 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human
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 (T308) 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 J1-223.371 antibody recognizes Akt phosphorylated at T308.



Analysis of Akt (pT308) in activated mouse embryonic fibroblasts. *NIH/3T3 cells (ATCC CR:-1658) were serum starved overnight and either stimulated with Platelet-Derived Growth Factor-BB (Cat. No. 354051) at 37˚C for 30 minutes (filled histogram) or unstimulated (open histogram). The cells were fixed with BD Phosflow™ Fix Buffer I (Cat. No. 557870) at 37˚C for 10 minutes, permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes (or overnight at -20˚C), and stained with PE anti-Akt (pT308, Cat. No. 558275). Flow cytometry was performed on a BD FACSCalibur™ 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody detects Akt (pT308) in human and mouse cell lines. We have been unable to detect upregulated phosphorylation of Akt in activated leukocytes, such as whole blood and peripheral blood mononuclear cells.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 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. 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)

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)

De Falco E, et al.. Altered SDF-1-mediated differentiation of bone marrow-derived endothelial progenitor cells in diabetes mellitus.. *J Cell Mol Med.* 2009; 13(9B):3405-3414. (Clone-specific: Flow cytometry)

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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