**Technical Data Sheet**

# PE Mouse anti-Akt (pS473)

## Product Information

**Material Number:** 560378  
**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  
**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.](image1)

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™ 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 PE 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.](image2)

Jurkat cells (ATCC TIB-152) were either treated with 1 µM Wortmannin (Life Technologies, Cat. No. PHZ1301) for 2 hours at 37°C (shaded histogram) or left untreated (open 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 PE 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.
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.

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Cells</th>
<th>Treatment</th>
<th>Fixation</th>
<th>Perm buffer</th>
<th>Result</th>
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<tbody>
<tr>
<td>Flow</td>
<td>Human</td>
<td>Jurkat</td>
<td>none</td>
<td>Cytofix</td>
<td>Perm III</td>
<td>expression observed</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Wortmannin</td>
<td>Cytofix</td>
<td>Perm III</td>
<td>Down-regulated expression</td>
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<tr>
<td>Mouse</td>
<td>NIH/3T3</td>
<td>PDGF</td>
<td>Cytofix</td>
<td>Perm III</td>
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<td>Up-regulated expression</td>
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<td></td>
<td></td>
<td></td>
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<td>60-kDa band</td>
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<tr>
<td>WB</td>
<td>Human</td>
<td>Jurkat</td>
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<td></td>
<td>1 µM Wortmannin for 2 hours</td>
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<td></td>
<td></td>
<td></td>
<td>phosphopeptide</td>
<td>blocking of 60-kDa band</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>non-phospho peptide or unrelated phospho peptide</td>
<td>no blocking</td>
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<tr>
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<td></td>
<td></td>
<td>lambda phosphatase</td>
<td>loss of signal</td>
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Application Notes
Application
Intracellular staining (flow cytometry) Routinely Tested

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
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<tbody>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
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<td>(none)</td>
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<tr>
<td>558050</td>
<td>Perm Buffer III</td>
<td>125 ml</td>
<td>(none)</td>
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Product Notices
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use $1 \times 10^6$ cells in a 100-µl experimental sample (a test).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. All other brands are trademarks of their respective owners.

References