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# Phospho-Akt (Ser473) (193H12) Rabbit mAb



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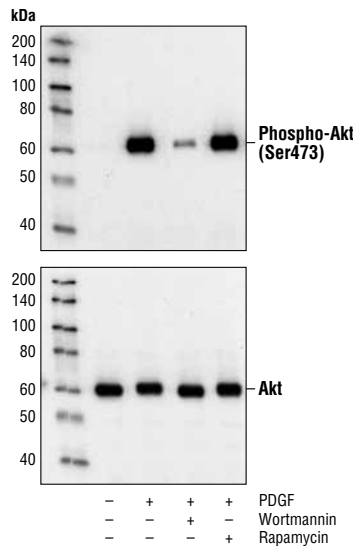
**For Research Use Only. Not For Use In Diagnostic Procedures.**

Applications W, IP, IF-IC, F Endogenous	Species Cross-Reactivity* H, M, R	Molecular Wt. 60 kDa	Isotype Rabbit IgG**
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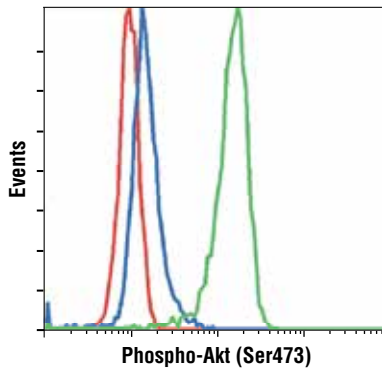
**Background:** Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9), and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 $\alpha$  and  $\beta$  (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 $\beta$ -mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip1 (15) and p21 Waf1/Cip1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

**Specificity/Sensitivity:** Phospho-Akt (Ser473) (193H12) Rabbit mAb detects endogenous levels of Akt only when phosphorylated at Ser473.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of mouse Akt.



Western blot analysis of extracts from untreated or PDGF-treated NIH/3T3 cells, pretreated with wortmannin #9951 and/or rapamycin #9904 as indicated, using Phospho-Akt (Ser473) (193H12) Rabbit mAb (upper) or Akt Antibody #9272 (lower).



Flow cytometric analysis of Jurkat cells, untreated (green) or LY294002 #9901 and Wortmannin #9951 treated (blue), using Phospho-Akt (Ser473) (193H12) Rabbit mAb compared to a nonspecific negative control antibody (red).

**Entrez Gene ID** # 207  
**UniProt ID** # P31749

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by western blot.**  
**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:200
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:400

For product specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

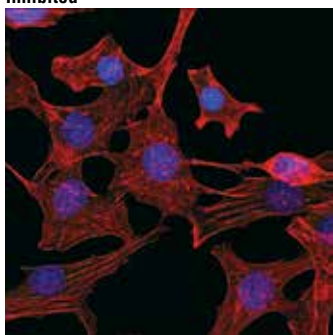
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U.S. Patent No. 5,675,063.  
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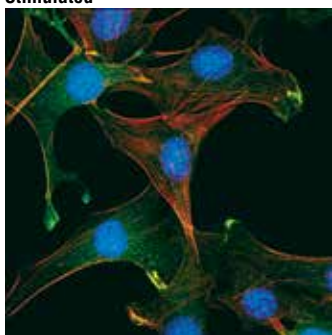
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween<sup>®</sup>20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

**Inhibited**



**Stimulated**



*Confocal immunofluorescent analysis of C2C12 cells, U0126/LY294002/Rapamycin-treated (left) or insulin-treated (right) using Phospho-Akt (Ser473) (193H12) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*