

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

- Small 100 µl (10 western blots)
- Large 300 µl (30 western blots)

rev. 12/10/09

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #207  
Swiss-Prot Acc. #P31749

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F Endogenous	H, M, R	60 kDa	Rabbit IgG**

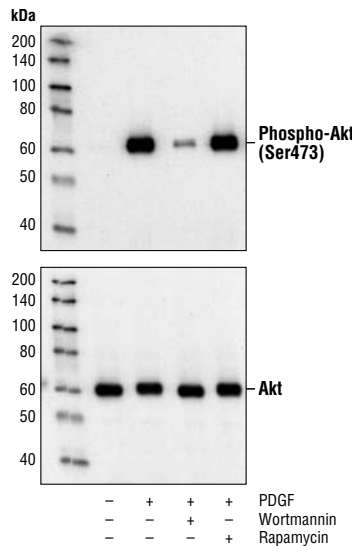
**Background:** Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid 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 by phosphorylating and inactivating 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-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

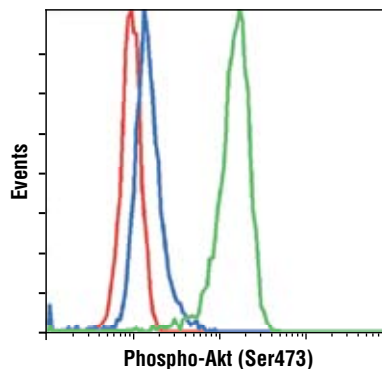
In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (16). Akt also plays a critical 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). Inhibition of mTOR stops the protein synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-EP1), an inhibitor of translation (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 (KLH-coupled) corresponding to residues around Ser473 of mouse Akt.



Western blot analysis of extracts from untreated or PDGF-treated NIH/3T3 cells, pretreated with wortmannin and/or rapamycin 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 and Wortmannin treated (blue), using Phospho-Akt (Ser473) (193H12) Rabbit mAb compared to a nonspecific negative control antibody (red).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/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 application 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:**

- (1) Franke, T.F. et al. (1997) *Cell* 88, 435-7.
- (2) Burgering, B.M. and Coffey, P.J. (1995) *Nature* 376, 599-602.
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- (16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
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- (18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
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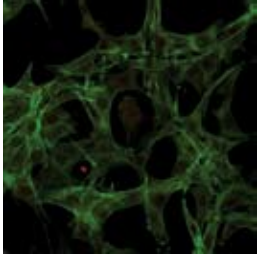
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

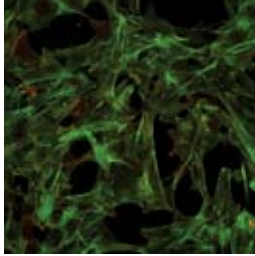
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

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.

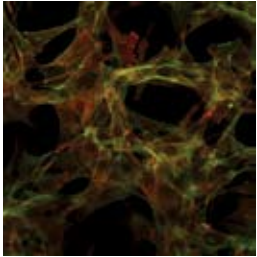
Untreated



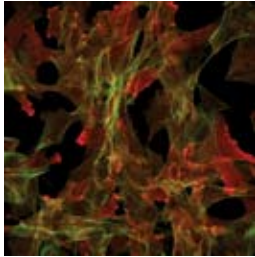
Insulin, 2 min



Insulin, 5 min



Insulin, 15 min



*Confocal immunofluorescent images of C2C12 cells either serum starved or treated with insulin as indicated and labeled with Phospho-Akt (Ser473) (193H12) Rabbit mAb (red). Actin filaments have been labeled with fluorescein phalloidin.*