Akt Antibody

#9272 Store at –20°C

✔ 100 µl
(10 Western mini-blots)

![Cell Signaling Technology®]

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Support ■ 877-678-TECH (8324)
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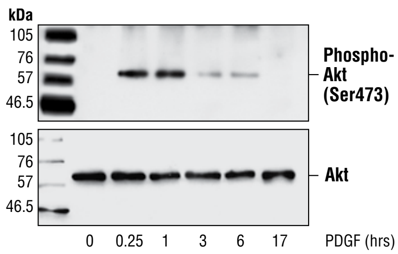
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC, F	H, M, R, C, Hm, Dr, Mk, Pg, Guinea Pig	60 kDa	Rabbit**

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).

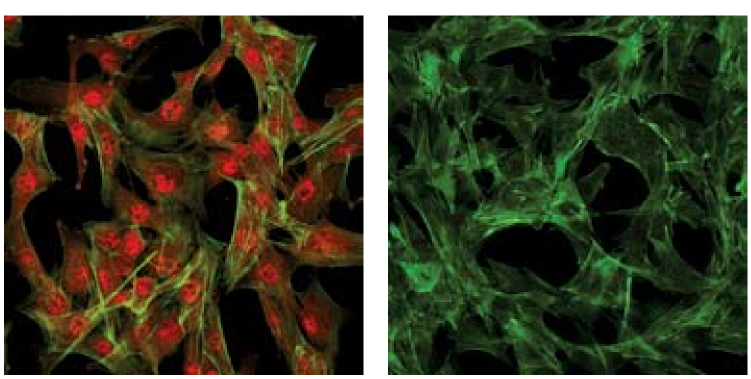
In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (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).



Western blot analysis of extracts from NIH/3T3 cells, untreated or PDGF-treated (50 ng/ml) for the indicated times, using Phospho-Akt (Ser473) Antibody #9271 (upper) or Akt Antibody (lower).

Specificity/Sensitivity: Akt Antibody detects endogenous levels of total Akt1, Akt2 and Akt3 proteins. The antibody does not cross-react with related kinases.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the carboxy-terminal sequence of mouse Akt. Antibodies are purified by protein A and peptide affinity chromatography.



Confocal immunofluorescent images of C2C12 cells showing nuclear and cytoplasmic localization with Akt Antibody (left, red) compared to an isotype control (right). Actin filaments have been labeled with fluorescein phalloidin.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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	1:50
Immunofluorescence (IF-IC)	1:100
Flow Cytometry:	1:100

For application specific protocols please see the web page for this product at www.cellsignal.com.

Companion Products:

Akt (pan) (C67E7) Rabbit mAb #4691

Akt Control Cell Extracts #9273

Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060

Phospho-Akt (Ser473) (736E11) Rabbit mAb (IHC Specific) #3787

Phospho-Akt (Thr308) (C31E5E) Rabbit mAb #2965

Phospho-Akt (Thr308) (244F9H2) Rabbit mAb (IHC Specific) #9266

Akt2 (5B5) Rabbit mAb #2964

Akt2 (54G8) Rabbit mAb (IHC Specific) #4057

Akt3 (62A8) Rabbit mAb #3788

Phospho-Akt Pathway Sampler Kit #9916

Immobilized Akt (1G1) Mouse mAb #9279

PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160

PathScan® Total Akt1 Sandwich ELISA Kit #7170

SignalSilence® Akt siRNA Kit #6210

SignalSilence® Akt2 siRNA Kit #6395

LY294002 (PI3 Kinase Inhibitor) #9901

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

Biotinylated Protein Ladder #7727

Prestained Protein Marker, Broad Range (Premixed Format) #7720

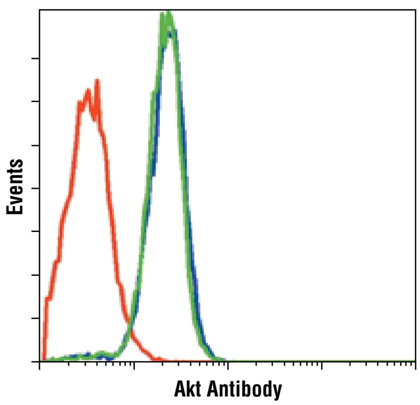
20X LumiGLO® Reagent and 20X Peroxide #7003

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.

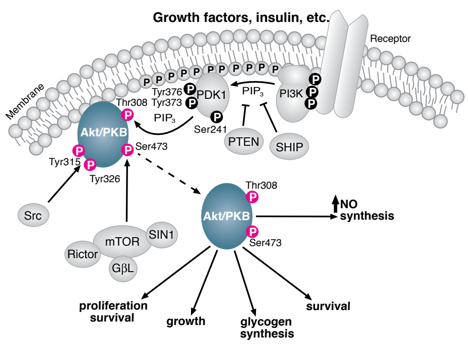
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **E-P**—ELISA Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected   Species enclosed in parentheses are predicted to react based on 100% homology.

Cell Signaling TECHNOLOGY®



Flow cytometric analysis of Jurkat cells, untreated (blue) or LY294002-treated (green), using Akt Antibody compared to a nonspecific negative control antibody (red).



Selected Application References:

Asselin, E. et al. (2001) XIAP regulates Akt activity and caspase-3-dependent cleavage during cisplatin-induced apoptosis in human ovarian epithelial cancer cells. *Cancer Res.* 61, 1862–1868. Application: W.

Bommakanti, R.K. et al. (2000) Dual regulation of Akt/protein kinase B by heterotrimeric G protein subunits. *J. Biol. Chem.* 275, 38870–38876. Application: W.

Campbell, R.A. et al. (2001) Phosphatidylinositol 3-kinase/Akt-mediated activation of estrogen receptor α: a new model for anti-estrogen resistance. *J. Biol. Chem.* 276, 9817–9824. Application: W.

Otero, D.C. et al. (2000) CD19-dependent activation of Akt kinase in B lymphocytes. *J. Biol. Chem.* 276, 1474–1478. Application: W.

Fukuda, T. et al. (2003) PINCH-1 is an obligate partner of integrin-linked kinase (ILK) functioning in cell shape modulation, motility, and survival. *J. Biol. Chem.* 278, 51324–51333. Application: W.

Patrucco, E. et al. (2004) PI3Kγ modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* 118, 375–387. Application: W.

Huang, H. et al. (2001) PTEN Induces Chemosensitivity in PTEN-mutated Prostate Cancer Cells by Suppression of Bcl-2 Expression. *J. Biol. Chem.* 276, 38830–38836. Application: W.

Meng, F. et al. (2002) Akt Is a Downstream Target of NF-κB. *J. Biol. Chem.* 277, 29674–29680. Application: W.

Zubiaur, M. et al. (2002) CD38 Is Associated with Lipid Rafts and upon Receptor Stimulation Leads to Akt/Protein Kinase B and Erk Activation in the Absence of the CD3-ζ Immune Receptor Tyrosine-based Activation Motifs. *J. Biol. Chem.* 277, 13–22. Application: W.

Radisavljevic, Z. et al. (2000) Vascular endothelial growth factor up-regulates ICAM-1 expression via the phosphatidylinositol 3 OH-kinase/AKT/nitric oxide pathway and modulates migration of brain microvascular endothelial cells. *J. Biol. Chem.* 275, 20770–20774. Application: W.

Background References:

(1) Franke, T.F. et al. (1997) *Cell* 88, 435–7.

(2) Burgering, B.M. and Coffer, P.J. (1995) *Nature* 376, 599–602.

(3) Franke, T.F. et al. (1995) *Cell* 81, 727–36.

(4) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541–51.

(5) Sarbassov, D.D. et al. (2005) *Science* 307, 1098–101.

(6) Jacinto, E. et al. (2006) *Cell* 127, 125–37.

(7) Cardone, M.H. et al. (1998) *Science* 282, 1318–21.

(8) Brunet, A. et al. (1999) *Cell* 96, 857–68.

(9) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741–4.

(10) Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240–5.

(11) Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241–8.

(12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199–203.

(13) Cross, D.A. et al. (1995) *Nature* 378, 785–9.

(14) Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499–511.

(15) Gesbert, F. et al. (2000) *J Biol Chem* 275, 39223–30.

(16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245–52.

(17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427–31.

(18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648–57.

(19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151–62.