

#4070 Store at -20°C

Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Sepharose Bead Conjugate)

400 µl
 (40 immunoprecipitations)



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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.

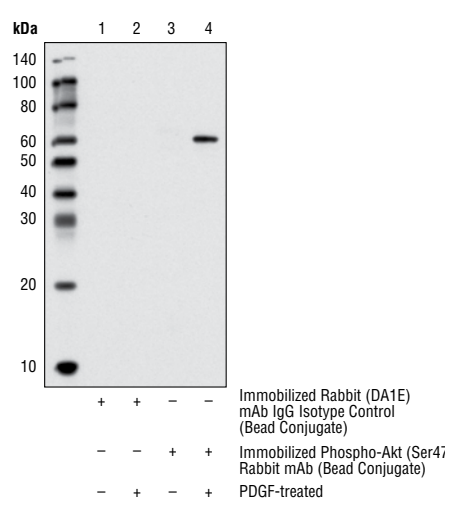
Applications	Species Cross-Reactivity	Molecular Wt.	Isotype
IP Endogenous	H, M, R, Hm, B, Dm, Z, (Mk, C, Dg).	60 kDa	Rabbit IgG

Description: This Cell Signaling Technology (CST) antibody is immobilized via covalent binding of primary amino groups to N-hydroxysuccinimide (NHS)-activated sepharose beads. Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Sepharose Bead Conjugate) is useful for immunoprecipitation assays. The unconjugated Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 reacts with human, mouse, rat, hamster, *Drosophila melanogaster*, bovine and zebrafish phospho-Akt protein. CST expects that Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Sepharose Bead Conjugate) will also recognize phospho-Akt in these species.

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/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 tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery by inactivating p70 S6 kinase and activating the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation (18,19).



Immunoprecipitation of extracts from NIH/3T3 cells, untreated or PDGF-treated, using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Sepharose Bead Conjugate) (lanes 3 and 4). XP™ Rabbit IgG (DA1E) mAb Isotype Control (Sepharose Bead Conjugate) #3423 was used as a negative control (lanes 1 and 2). The western blot was probed using Phospho-Akt (Ser473) (587F11) Mouse mAb #4051.

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Sepharose Bead Conjugate) immunoprecipitates 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 human Akt.

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

Storage: Supplied in 10mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 ug/ml BSA, 50% glycerol. Store at -20°C. Do not aliquot the antibody.

Directions for Use: Add 10 µl of well-vortexed beads to 200 µl of cell lysate at 1 mg/ml in 1X Cell Lysis Buffer (10X) #9803. See protocol for more details.

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

Please visit 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.
- (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.

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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.