

#5012 Store at -20°C

Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Biotinylated)

100 µl
 (10 western blots)



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rev. 03/04/10

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
W, F Endogenous	H, M, R, Dm, B, Z	60 kDa	Rabbit IgG

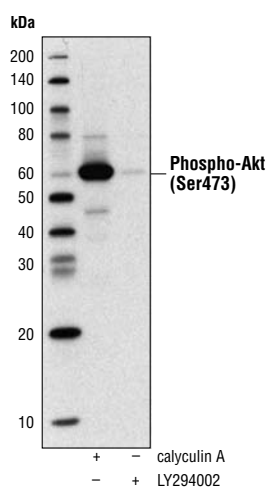
Description: This Cell Signaling Technology (CST) antibody is conjugated to biotin under optimal conditions. The unconjugated Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 reacts with human, mouse, rat, *Drosophila melanogaster*, hamster, bovine and zebrafish phospho-Akt (Ser473) protein. CST expects that Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Biotinylated) will also recognize phospho-Akt (Ser473) 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).

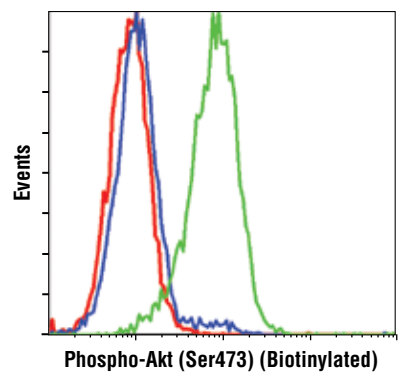
Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

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 tuberlin (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).

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb detects endogenous levels of Akt only when phosphorylated at Ser473.



Western blot analysis of extracts of Jurkat cells, treated with either Calyculin A #9902 or LY294002 #9901, using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Biotinylated) #5012 and detected with Streptavidin-HRP #3999.



◀ Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002 #9901, Wortmannin #9951, and U0126 #9903 treated (blue), using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Biotinylated) compared to XP™ Rabbit (DA1E) mAb IgG Isotype Control (Biotinylated) #4096 (red).

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

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.

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