

# Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb

- ☐ Small 100 µl  
(20 western blots)
- ☐ Large 300 µl  
(60 western blots)

rev. 06/15/11



**Orders** ■ 877-616-CELL (2355)  
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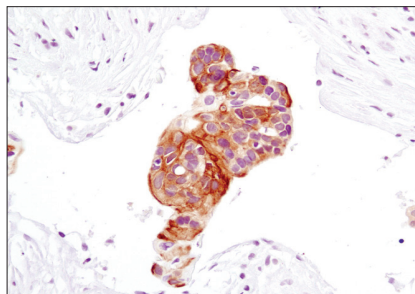
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IHC-F, IF-IC, F Endogenous	H, M, R, Mk, Hm, B, Dm, Z, (Pg, C, X, Dg)	60 kDa	Rabbit IgG**

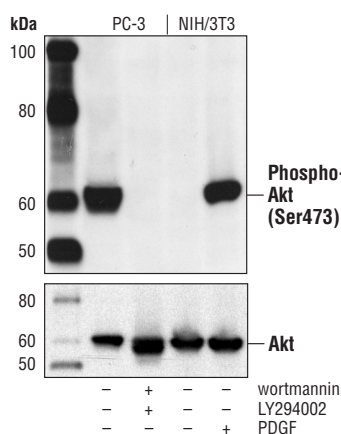
**Background:** Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid 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/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).



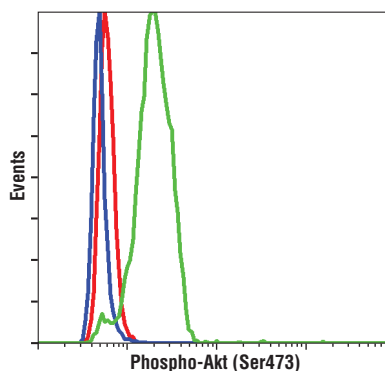
Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb.



Western blot analysis of extracts from PC-3 cells, untreated or LY294002/wortmannin-treated, and NIH/3T3 cells, serum-starved or PDGF-treated, using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (upper) or Akt (pan) (C67E7) Rabbit mAb #4691 (lower).

**Specificity/Sensitivity:** Phospho-Akt (Ser473) (D9E) XP® 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 human Akt.



Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002, wortmannin and U0126 (blue), using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb compared to a nonspecific negative control antibody (red).

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

**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	1:2000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunohistochemistry (Frozen)	1:50†
Fixative:	3% Formaldehyde/MeOH
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:100

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

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

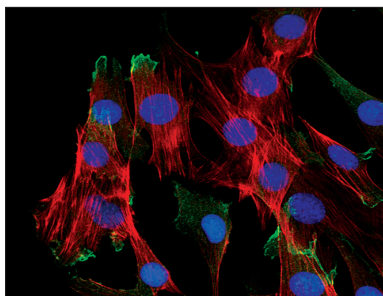
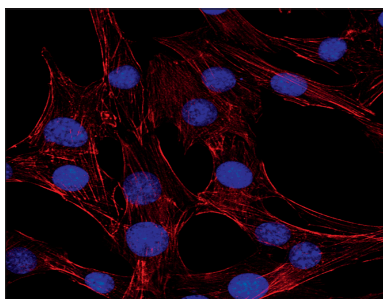
DRAQ5® is a registered trademark of Biostatus Limited.

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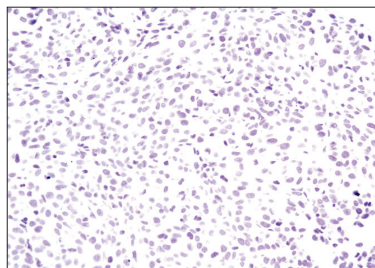
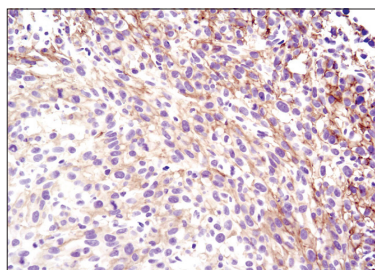
**IMPORTANT:** For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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

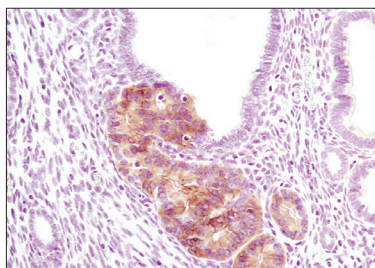
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



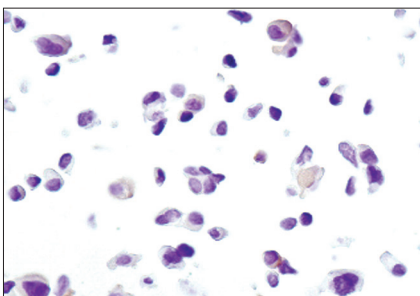
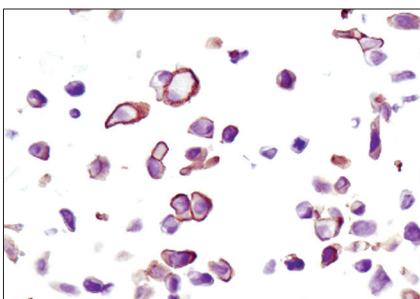
Confocal immunofluorescent analysis of C2C12 cells, LY294002-treated (upper) or insulin-treated (lower), using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



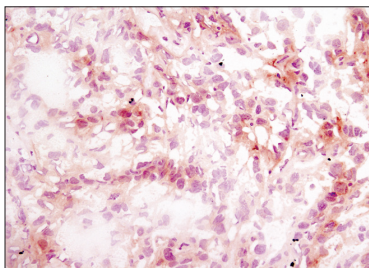
Immunohistochemical analysis of paraffin-embedded U-87MG xenograft, untreated (upper) or  $\lambda$  phosphatase-treated (lower), using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb.



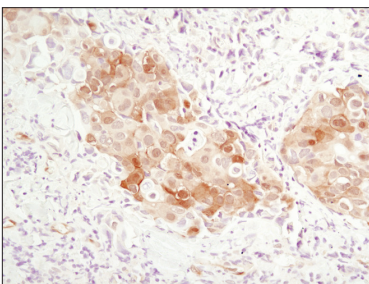
Immunohistochemical analysis of paraffin-embedded PTEN heterozygous mutant mouse endometrium using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb. (Tissue section courtesy of Dr. Sabina Signoretti, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.)



Immunohistochemical analysis using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb on SignalSlide® Phospho-Akt (Ser473) IHC Controls #8101 (paraffin-embedded LNCaP cells, untreated (upper) or LY294002-treated (lower).



Immunohistochemical analysis of frozen SKOV3 xenograft using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma comparing SignalStain® Antibody Diluent #8112 (upper) to TBST/5% normal goat serum (lower) using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060.

## Background References:

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