#13038 Store at -20°C

Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb

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Entrez Gene ID #207, 208, 1000
UniProt ID #P31749, P31751, Q94243

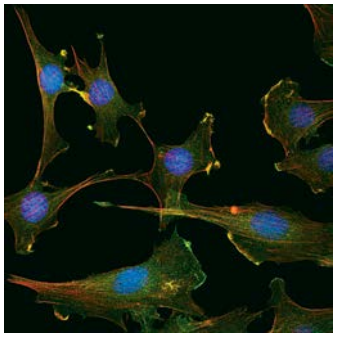
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F Endogenous	H, M, R, Mk	60 kDa	Rabbit IgG**

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 through phosphorylation and inactivation of 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 Kip1 (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 (18,19).

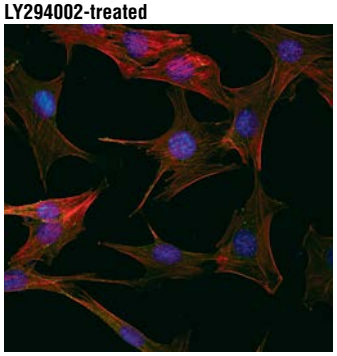
Specificity/Sensitivity: Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb recognizes endogenous levels of Akt1 protein only when phosphorylated at Thr308. This antibody also recognizes endogenous levels of Akt2 protein when phosphorylated at Thr309 or Akt3 protein when phosphorylated at Thr305.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr308 of human Akt1 protein.

Insulin-treated



LY294002-treated



Confocal immunofluorescent analysis of C2C12 cells, insulin-treated (100 nM, 15 min; upper) or treated with LY294002 #9901 (50 µM, 2 hr; lower), using Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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	1:50
Immunofluorescence (IF-IC)	1:800
Flow Cytometry	1:6400

For product 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:

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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 nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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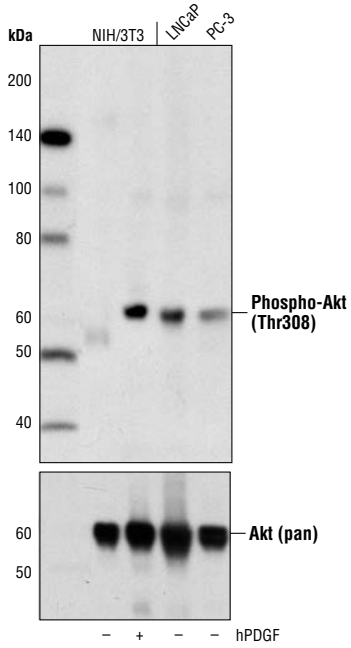
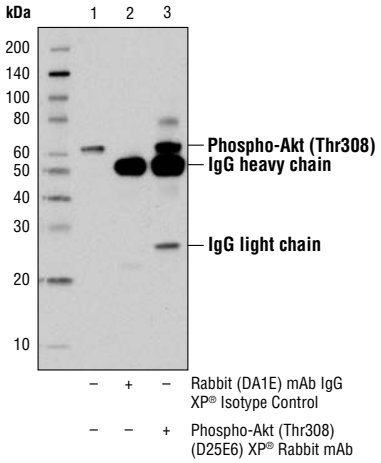
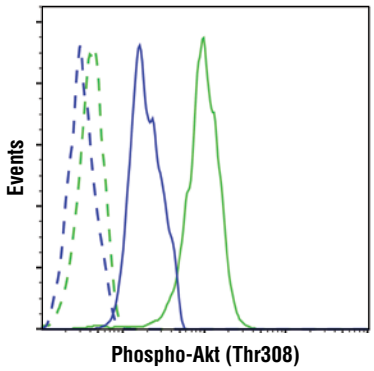
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



Western blot analysis of extracts from NIH/3T3 cells, untreated (-) or treated with Human Platelet-Derived Growth Factor AA (hPDGF-AA) #8913 (100 ng/ml, 5 min; +), and untreated (-) LNCaP and PC-3 cells, using Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb (upper) or Akt (pan) (C67E7) Rabbit mAb #4691 (lower).



Immunoprecipitation of phospho-Akt (Thr308) from Jurkat cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb.



Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002 #9901, wortmannin #9951 and U0126 #9903 (blue), using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (solid line) compared to a concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed line). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.