

Store at  
4°C  
**#43506**

# Phospho-Akt (Thr308) (D25E6) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate)



**Support:** +1-978-867-2388 (U.S.)  
www.cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)  
orders@cellsignal.com

**Entrez-Gene ID** #207, 208, 10000  
**UniProt ID** #P31749, P31751, Q9Y243

New 07/16

**For Research Use Only. Not For Use In Diagnostic Procedures.**

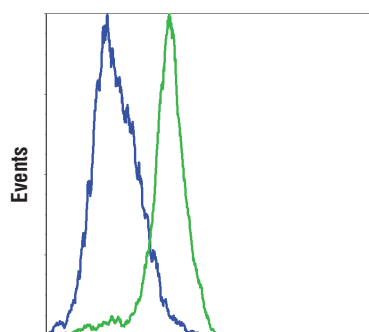
**Applications**  
IF-IC, F  
Endogenous

**Species Cross-Reactivity\***  
H, M, R, Mk

**Isotype**  
Rabbit IgG

**Description:** This Cell Signaling Technology antibody is conjugated to Alexa Fluor<sup>®</sup> 488 fluorescent dye and tested in-house for direct flow cytometric (mouse cells) and immunofluorescence (human cells) analysis. This antibody is expected to exhibit the same species cross-reactivity as the unconjugated Phospho-Akt (Thr308) (D25E6) XP<sup>®</sup> Rabbit mAb #13038.

**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-3 $\alpha$  and  $\beta$  (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-3 $\beta$ -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 tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).



**Phospho-Akt (Alexa Fluor<sup>®</sup> 488 Conjugate)**

Flow cytometric analysis of serum-starved NIH/3T3 cells, untreated (blue) or treated with mouse platelet-derived growth factor BB (200 ng/ml, 15 min; green), using Phospho-Akt (Thr308) (D25E6) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate).

**Specificity/Sensitivity:** Phospho-Akt (Thr308) (D25E6) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) 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.

**Storage:** Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. Do not aliquot the antibody. Protect from light. Do not freeze.

**\*Species cross-reactivity is determined by western blot using the unconjugated antibody.**

**Recommended Antibody Dilutions:**

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

**For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com)**

**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) Hajdich, 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.

DRAQ5 is a registered trademark of Biostatus Limited.

Alexa Fluor is a registered trademark of Life Technologies Corporation.

The Alexa Fluor<sup>®</sup> dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor<sup>®</sup> dyes (except for Alexa Fluor<sup>®</sup> 430 dye) are covered by pending and issued patents.

Thank you for your recent purchase. If you would like to provide a review visit [www.cellsignal.com/comments](http://www.cellsignal.com/comments).

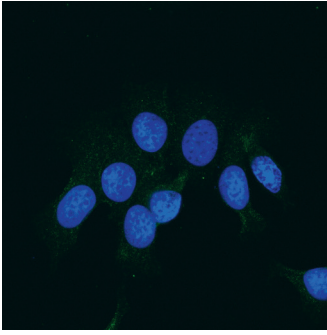
[www.cellsignal.com](http://www.cellsignal.com)

© 2016 Cell Signaling Technology, Inc.

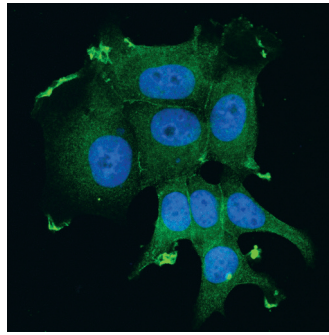
XP, and Cell Signaling Technology are trademarks of Cell Signaling Technology, Inc.

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

**LY294002-treated**



**Insulin-treated**



*Confocal immunofluorescent analysis of MCF7 cells, either treated with LY294002 #9901 (50  $\mu$ M, 2hrs; left) or treated with insulin (100 nM, 15 min; right), using Phospho-Akt (Thr308) (D25E7) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) (green). Blue pseudocolor = DRAQ5<sup>®</sup> #4084 (fluorescent DNA dye).*

Thank you for your recent purchase. If you would like to provide a review visit [cellsignal.com/comments](https://www.cellsignal.com/comments).

[www.cellsignal.com](https://www.cellsignal.com)