

Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate)

✓ 100 µl
(50 tests)

New more concentrated formulation

rev. 03/02/10

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.

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Applications	Species Cross-Reactivity*	Isotype
F Endogenous	H, M, R	Rabbit IgG

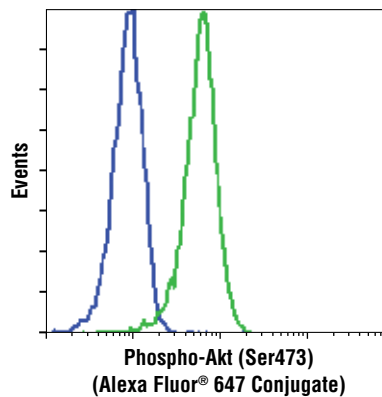
Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody #4060 reacts with human, mouse and rat Phospho-Akt protein. CST expects that Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Alexa Fluor® 647 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-3 α 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-3 β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (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 due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-EP1), an inhibitor of translation (18,19).

The Alexa Fluor® 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® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents. Alexa Fluor® is a registered trademark of Molecular Probes, Inc.



Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002, wortmannin and U0126 (blue), using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate).

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of Akt only when phosphorylated at Ser473.

The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-5. The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission, with a peak at 665 nm.

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 PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. Protect from light. *Do not freeze.*

***Species cross-reactivity is predicted by western analysis using the unconjugated antibody.**

Recommended Antibody Dilutions:

Flow Cytometry 1:50

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 Coffer, 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, 654-51.
- (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.