

Store at 4°C

#2337

Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 647 Conjugate)

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UniProt ID #P31749,

rev. 12/21/15

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

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 Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058 reacts with Phospho-Akt (Ser473) from human, mouse and rat. CST expects that Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 647 Conjugate) 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 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 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 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).

Specificity/Sensitivity: Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 647 Conjugate) 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 mouse Akt. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-6. 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.

Background References:

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- (7) Cardone, M.H. et al. (1998) *Science* 282, 1318-21.
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- (12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199-203.
- (13) Cross, D.A. et al. (1995) *Nature* 378, 785-9.
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- (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.
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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.

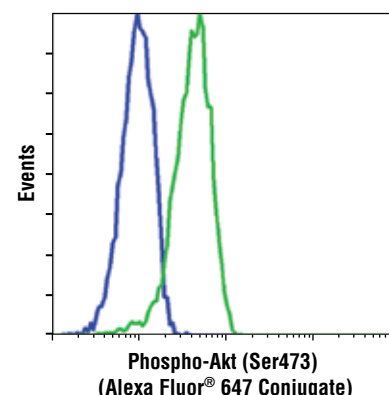
*Species cross-reactivity other than human is determined by western blot using the unconjugated antibody.

Recommended Antibody Dilutions:

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

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Flow cytometric analysis of Jurkat cells, untreated (green) or LY294002 and wortmannin treated (blue), using Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 647 Conjugate).

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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.