Cell Signaling Store at +4C Phospho-Akt (Ser473) (D9E) XP[®] Rabbit mAb (Alexa Fluor[®] 647 ΤΕСΗΝΟΙΟ<u>ΘΥ</u>® Conjugate) Orders: 877-616-CELL (2355) orders@cellsignal.com 877-678-TECH (8324) Support: Web: info@cellsignal.com cellsignal.com 3 Trask Lane | Danvers | Massachusetts | 01923 | USA For Research Use Only. Not for Use in Diagnostic Procedures. Source/Isotype: Applications: **Reactivity:** Sensitivity: UniProt ID: Entrez-Gene Id: #P31751, #Q9Y243, FC-FP H M R Hm Mk Endogenous Rabbit IgG 208, 10000, 207 Dm Z B #P31749 **Product Usage** Application Dilution Information Flow Cytometry (Fixed/Permeabilized) 1.20 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 Storage antibody. Protect from light. Do not freeze. Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of Specificity / Sensitivity Akt only when phosphorylated at Ser473. Chicken, Xenopus, Dog, Pig Species predicted to react based on 100% sequence homology: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to Source / Purification residues around Ser473 of human 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. **Product Description** This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested inhouse for direct flow cytometric analysis of human cells. The unconjugated antibody #4060 reacts with human, mouse, rat, hamster, bovine, D. melanogaster and zebra fish 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. Akt, also referred to as PKB or Rac, plays a critical role in controlling cell survival and apoptosis (1-3). This Background protein kinase is activated by insulin and various growth and survival factors to function in a wortmanninsensitive 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 PI3K/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-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). **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, 6541-51. 5. Sarbassov, D.D. et al. (2005) Science 307, 1098-101. 6. Jacinto, E. et al. (2006) Cell 127, 125-37.

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Species Reactivity	Species reactivity is determined by testing in at least one approved application (e.g., western blot).
Applications Key	FC-FP: Flow Cytometry (Fixed/Permeabilized)
Cross-Reactivity Key	 H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus 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 GP: Guinea Pig Rab: rabbit All: all species expected
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