Revision 1

Store at +4C

#2337

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

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
FC-FP	H M R	Endogenous	Rabbit IgG	#P31751, #Q9Y243, #P31749	208, 10000, 207

Product Usage Information

Application	Dilution
Flow Cytometry (Fixed/Permeabilized)	1:50

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.

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.

Product 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 cell 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 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-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).

Background References

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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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Limited Uses