Store at 4°C

#2944

Akt (5G3) Mouse mAb (Alexa Fluor® 647 Conjugate)

Cell Signaling TECHNOLOGY®

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

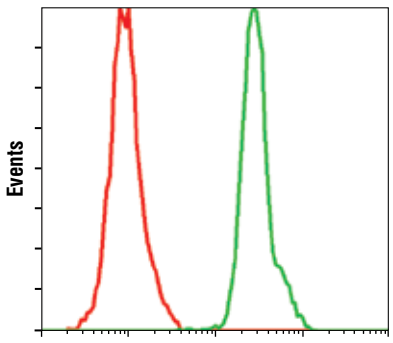
Applications	Species Cross-Reactivity	Isotype
F Endogenous	H, M, R, Hm	Mouse IgG1

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 #2966 reacts with human, mouse, rat and hamster Akt protein. CST expects that Akt (5G3) Mouse mAb (Alexa Fluor® 647 Conjugate) will also recognize 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-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 Kip (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). 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-BP1), an inhibitor of translation (18,19).



Flow cytometric analysis of untreated Jurkat cells (green) using Akt (5G3) Mouse mAb (Alexa Fluor® 647 Conjugate) compared to a nonspecific negative control antibody (red).

Specificity/Sensitivity: Akt (5G3) Mouse mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of Akt. This antibody does not cross-react with other related proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues 140-480 of human Akt1. 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.

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.*

Recommended Antibody Dilutions:

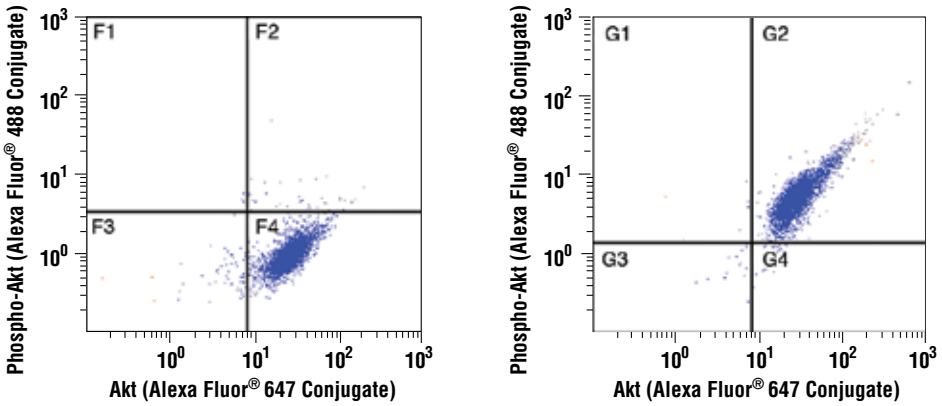
Flow Cytometry	1:50

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.

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

Species Cross-Reactivity Key: **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



Flow cytometric analysis of Jurkat cells, untreated (left) or or treated with LY294002, wortmannin and U0126 (right), using Akt (5G3) Mouse mAb (Alexa Fluor® 647 Conjugate) and Phospho-Akt (Ser473) (D9E) Rabbit mAb (Alexa Fluor® 488Conjugate) #4071.

Background References:

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