

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

✓ 500 µl
(50 tests)

rev. 06/04/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

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Entrez-Gene ID # 207
Swiss-Prot Acc. # P31749

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

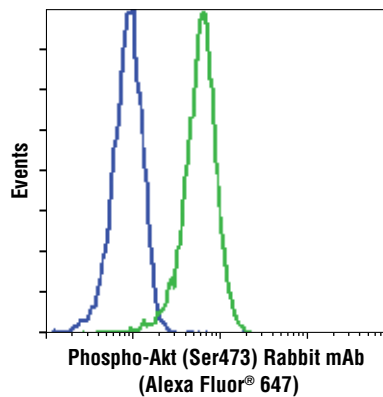
Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody #4060 reacts with human, mouse and rat Phospho-Akt protein. CST expects that Phospho-Akt (Ser473) (D9E) 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) Rabbit mAb (Alexa Fluor® 647 Conjugate).

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) 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 rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues around Ser473 of human Akt.

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.

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 1:10

Companion Products:

Rabbit IgG Isotype Control (Alexa Fluor® 647 Conjugate) #3452
Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb (Alexa Fluor® 488 Conjugate) #4803
Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060
Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4851
Akt (pan) (C67E7) Rabbit mAb #4691
Phospho-Akt (Thr308) (C31E5) Rabbit mAb #2965

Background References:

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- (17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.
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Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Conjugated Primary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.