Store at 4°C

#12046

Akt Alexa Fluor® 488 Conjugated Antibody Sampler Kit

☑ 1 Kit
(4 x 40 µl)

Cell Signaling TECHNOLOGY®

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rev. 01/05/15

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

Products Included	Product #	Quantity	Isotype	Flow Cytometry Dilution	IF-IC Dilution
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	4071	40 µl	Rabbit IgG	1:50	N/A
Phospho-Akt (Thr308) (C31E5E) Rabbit mAb (Alexa Fluor® 488 Conjugate)	2918	40 µl	Rabbit IgG	1:50	N/A
Akt (pan) (C67E7) Rabbit mAb (Alexa Fluor® 488 Conjugate)	5084	40 µl	Rabbit IgG	1:50	1:50
Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate)	2975	40 µl	Rabbit IgG	N/A	N/A

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Akt Alexa Fluor® 488 Conjugated Antibody Sampler Kit provides an economical means to study the activation state of Akt without the need for a fluorescent secondary antibody.

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-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).

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of Akt only when phosphorylated at Ser473. Phospho-Akt (Thr308) (C31E5E) Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of Akt only when phosphorylated at Thr308. Akt (pan) (C67E7) Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of total Akt protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of human Akt protein, a synthetic phosphopeptide corresponding to residues around Thr308 of mouse Akt protein, or a synthetic peptide corresponding to residues near the carboxy terminus of mouse Akt protein.

Background References:

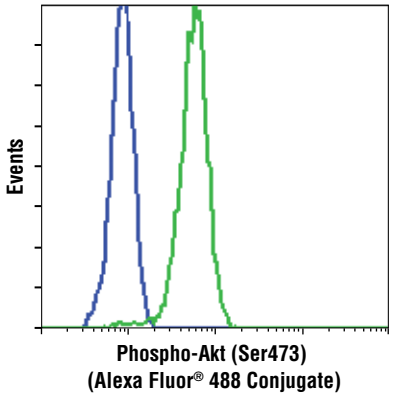
(1) Franke, T.F. et al. (1997) *Cell* 88, 435-7.
(2) Burgering, B.M. and Coffer, P.J. (1995) *Nature* 376, 599-602.
(3) Franke, T.F. et al. (1995) *Cell* 81, 727-36.
(4) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-51.
(5) Sarbassov, D.D. et al. (2005) *Science* 307, 1098-101.
(6) Jacinto, E. et al. (2006) *Cell* 127, 125-37.
(7) Cardone, M.H. et al. (1998) *Science* 282, 1318-21.
(8) Brunet, A. et al. (1999) *Cell* 96, 857-68.
(9) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-4.
(10) Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240-5.
(11) Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241-8.
(12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199-203.
(13) Cross, D.A. et al. (1995) *Nature* 378, 785-9.
(14) Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499-511.
(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.
(19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151-62.

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 antibodies. Protect from light. Do not freeze.*

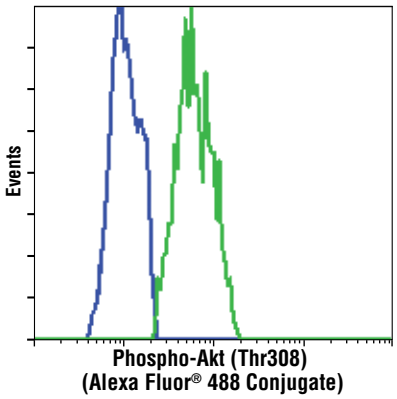
For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

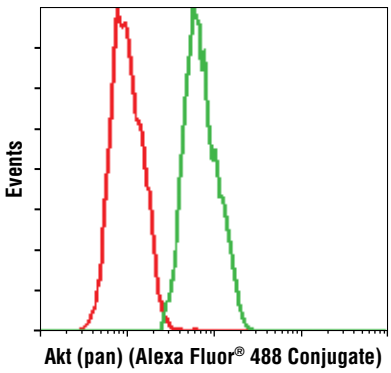
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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 Species enclosed in parentheses are predicted to react based on 100% homology.



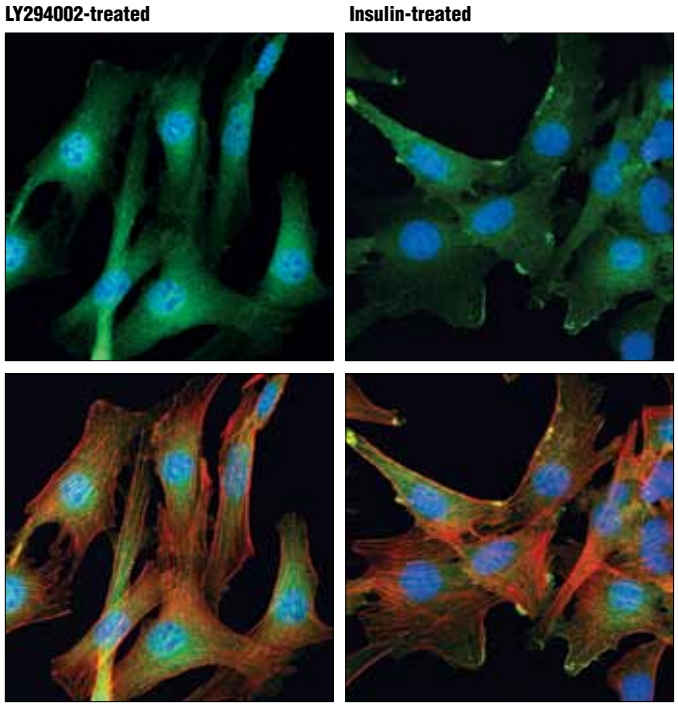
Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002 #9901, Wortmannin #9951, and U0126 #9903 (blue), using ***Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #4071****.*



Flow cytometric analysis of NIH/3T3 cells, untreated (blue) or PDGF-treated (green) using ***Phospho-Akt (Thr308) (C31E5E) Rabbit mAb (Alexa Fluor® 488 Conjugate) #2918****.*



Flow cytometric analysis of Jurkat cells using ***Akt (pan) (C67E7) Rabbit mAb (Alexa Fluor® 488 Conjugate) #5084*** *(green) compared to Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (red).*



Confocal immunofluorescent analysis of C2C12 cells, treated with LY294002 #9901 (left) or insulin (right), using ***Akt (pan) (C67E7) Rabbit mAb (Alexa Fluor® 488 Conjugate) #5084*** *(green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary 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 Unlabeled Primary and Conjugated Secondary 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 0.5-1x10^6 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from CST.
#4412 Alexa Fluor® 488 F(ab')2 fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
#4408 Alexa Fluor® 488 F(ab')2 fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)