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#58295

Akt (pan) (E7J2C) Mouse mAb

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Entrez-Gene ID #207 , 208, 10000
UniProt ID #P31749 , P31751, Q9Y243

rev. 03/08/19

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC	H, M, R, Mk	60 kDa	Mouse IgG2a**
Endogenous			

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).

Background References:

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(6) Jacinto, E. et al. (2006) *Cell* 127, 125-37.

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(8) Brunet, A. et al. (1999) *Cell* 96, 857-68.

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(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.

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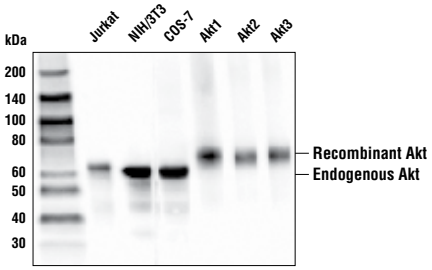
(17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.

(18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.

(19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151-62.

Specificity/Sensitivity: Akt (pan) (E7J2C) Mouse mAb recognizes endogenous levels of total Akt protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human Akt.



Western blot analysis of various cell lines and recombinant Akt1, Akt2 and Akt3 proteins using Akt (pan) (E7J2C) Mouse mAb. Recombinant Akt1, Akt2 and Akt3 each contain an N-terminal 6XHis and FLAG-Tag, MW=66kDa.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-mouse secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:200
Fixative:	4% Formaldehyde
Permeabilization:	0.3% Triton X-100

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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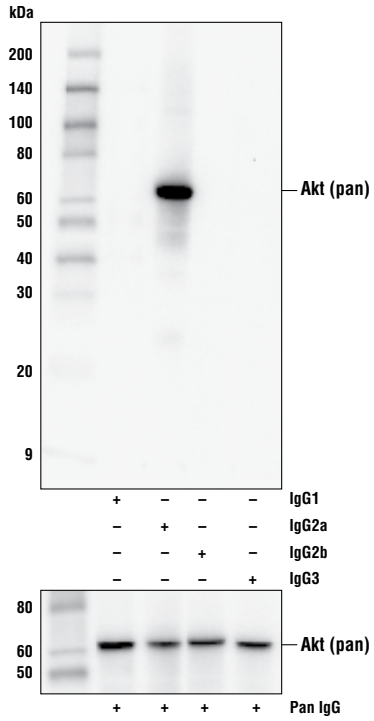
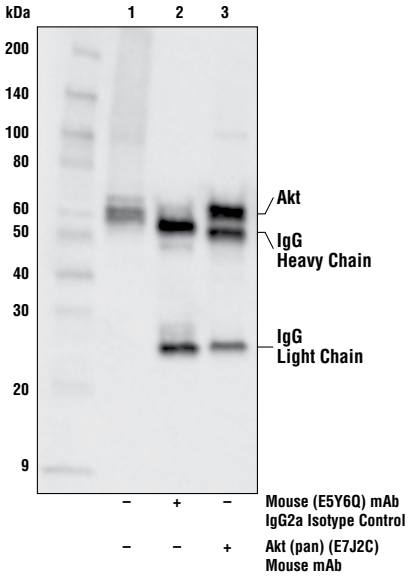
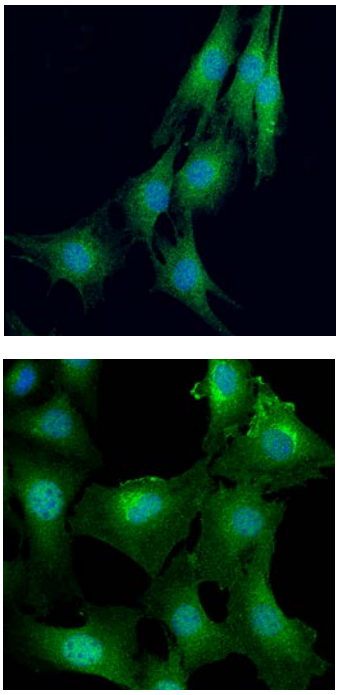
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



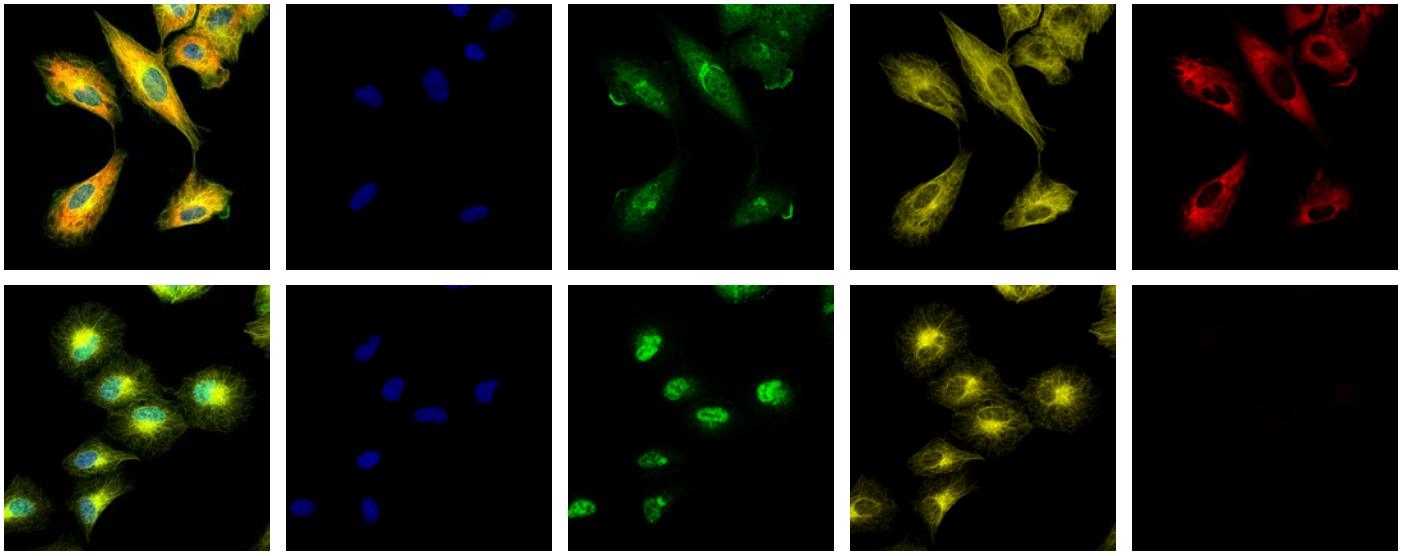
Western blot analysis of C2C12 cell extracts using Akt (pan) (E7J2C) Mouse mAb as the primary antibody. Various anti-mouse isotype specific antibodies (upper) and Anti-mouse IgG, HRP-linked Antibody #7076 (lower) were used as secondary antibodies.



Immunoprecipitation of Akt protein from Jurkat cell extracts. Lane 1 is 10% input, lane 2 is Mouse (E5Y6Q) mAb IgG2a Isotype Control #61656, and lane 3 is Akt (pan) (E7J2C) Mouse mAb. Western blot analysis was performed using Akt (pan) (E7J2C) Mouse mAb.



Confocal immunofluorescent analysis of C2C12 cells, treated with LY294002 #9901 (50 µM, 2 hr; upper) or insulin (100 nM, 20 min; lower), using Akt (pan) (E7J2C) Mouse mAb (green). Blue = DAPI #4083 (fluorescent DNA dye). Note the translocation of Akt to ruffling membranes following stimulation.



Confocal immunofluorescent analysis of A549 cells, treated with insulin (100 nM, 20 min; top) or LY294002 #9901 (50 µM, 2 hr; bottom) using Akt (pan) (E712C) Mouse mAb (green; mouse IgG2a), α-Tubulin (DM1A) Mouse mAb #3873 (yellow; mouse IgG1), and Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb #5364 (red; rabbit IgG). Samples were mounted in Prolong Gold Antifade Reagent® with DAPI #8961 (blue). Note the translocation of Akt to ruffling membranes following stimulation with insulin (arrow heads).