Store at -20°C

#2920 Akt (pan) (40D4) Mouse mAb

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

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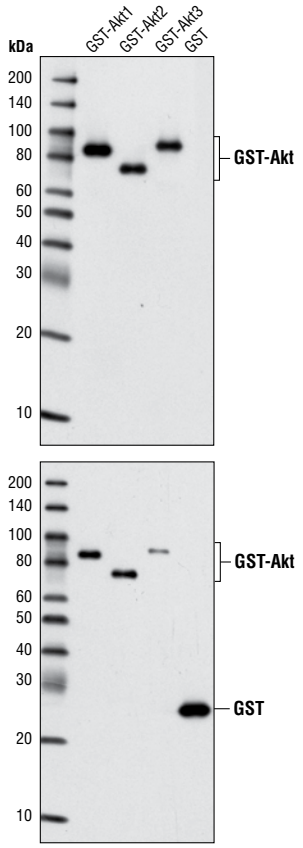
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H, M, R, Mk	60 kDa	Mouse IgG1κ**

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: Akt (pan) (40D4) Mouse mAb detects endogenous levels of total Akt protein. This antibody does not cross-react with other related proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide at the carboxy-terminal sequence of human Akt.



Western blot analysis of recombinant Akt1, Akt2, Akt3 and GST proteins using Akt (pan) (40D4) Mouse mAb (upper) and GST (91G1) Rabbit mAb #2625 (lower).

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	1:2000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:250
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:50

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

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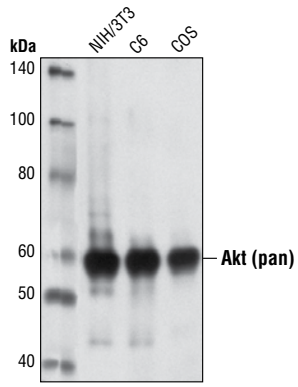
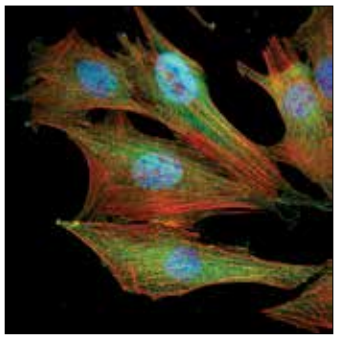
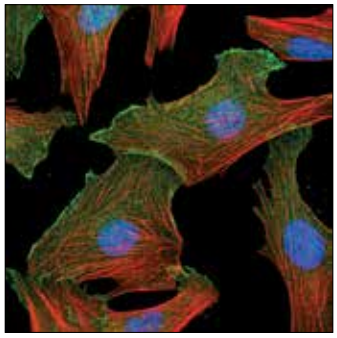
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken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

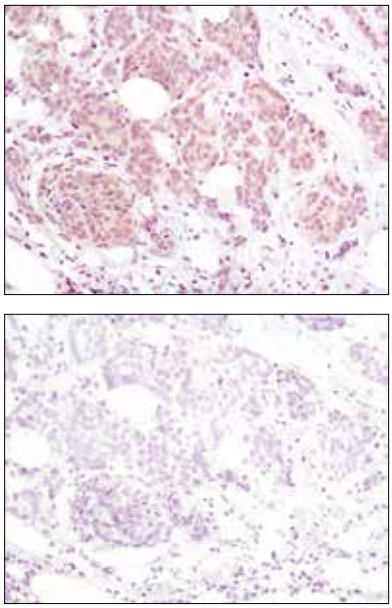


Western blot analysis of extracts from NIH/3T3, C6 and COS cells using Akt (pan) (40D4) Mouse mAb.

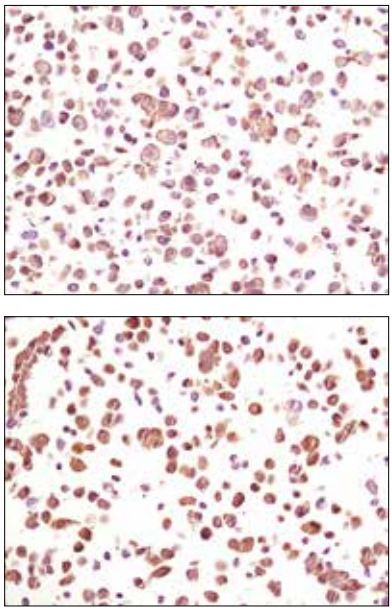




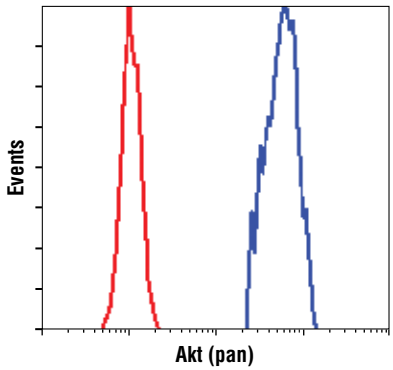
Confocal immunofluorescent analysis of C2C12 cells, either LY294002-treated (upper) or insulin-treated (lower), using pan-Akt (40D4) Mouse mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudo-color = DRAQ5® #4084 (fluorescent DNA dye).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Akt (pan) (40D4) Mouse mAb in the presence of control peptide (upper) or Akt (pan) Blocking Peptide #1085 (lower).



Immunohistochemical analysis using Akt (pan) (40D4) Mouse mAb on SignalSlide® Phospho-Akt (Ser473) IHC Controls #8101 (paraffin-embedded LNCaP cells, untreated (upper) or LY294002-treated (lower)).



Flow cytometric analysis of untreated Jurkat cells using Akt (pan) (40D4) Mouse mAb (blue) compared to a nonspecific negative control antibody (red).

Background References:

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