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Akt (pan) (C67E7) Rabbit mAb

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Entrez-Gene ID #207
UniProt ID #P31749

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H, M, R, Mk, Dm, (Pg)	60 kDa	Rabbit IgG**

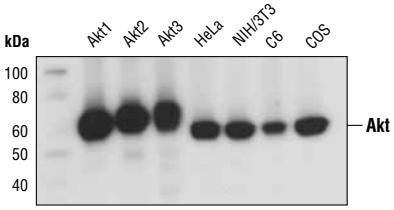
Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid 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).

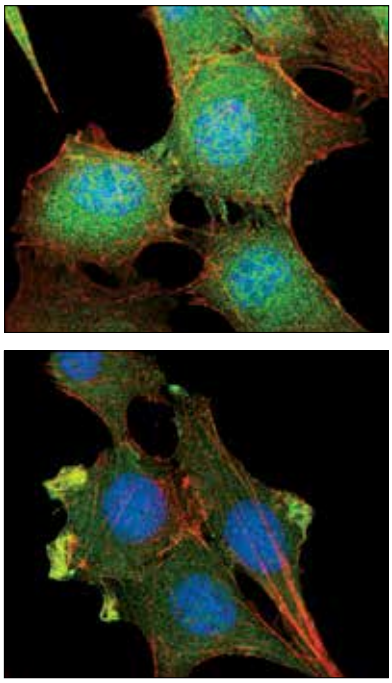
In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (16). Akt also plays a critical 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-EP1), an inhibitor of translation (18,19).

Specificity/Sensitivity: Akt (pan) (C67E7) Rabbit 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 mouse Akt.



Western blot analysis of recombinant Akt1, Akt2 and Akt3 proteins, and extracts from various cell lines, using Akt (pan) (C67E7) Rabbit mAb.



Confocal immunofluorescent analysis of C2C12 cells, LY294002-treated (upper) or insulin-treated (lower), using Akt (pan) (C67E7) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudo-color = DRAQ5® #4084 (fluorescent DNA dye).

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

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:300†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:400
Flow Cytometry	1:100

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.

U.S. Patent No. 5,675,063

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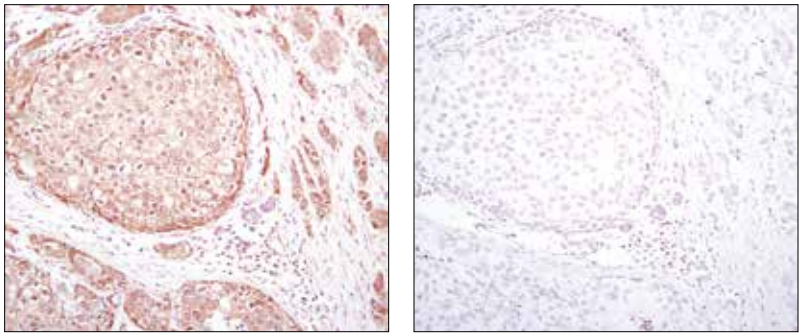
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

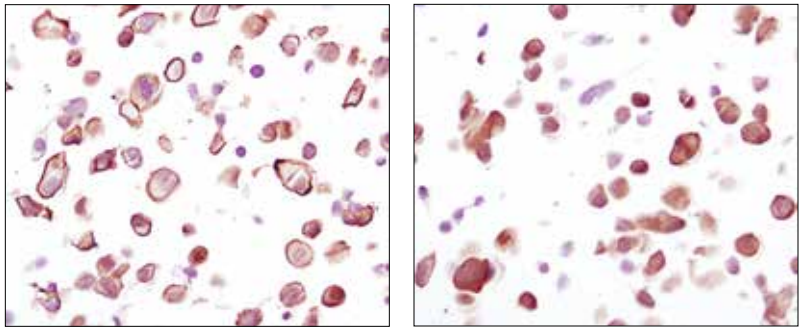
Tween®20 is a registered trademark of ICI Americas, Inc.

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

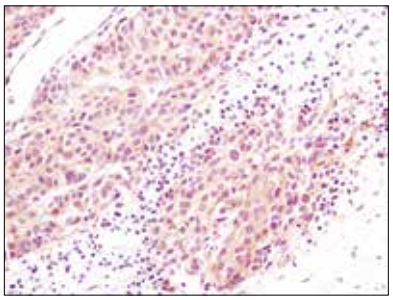
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



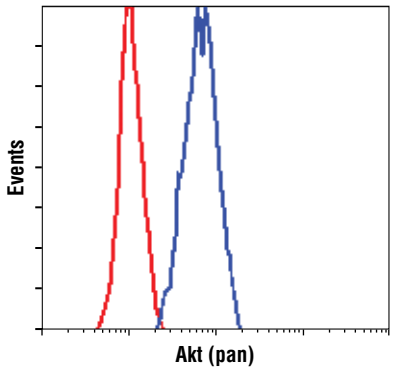
Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Akt (pan) (C67E7) Rabbit mAb in the presence of control peptide (left) or Akt (pan) Blocking Peptide #1085 (right).



Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (left) or LY294002-treated (right), using Akt (pan) (C67E7) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human melanoma using Akt (pan) (C67E7) Rabbit mAb.



Flow cytometric analysis of Jurkat cells using Akt (pan) (C67E7) Rabbit mAb (blue) compared to a nonspecific negative control antibody (red).

Background References:

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