

#4059 Store at -20°C

# Akt3 Antibody



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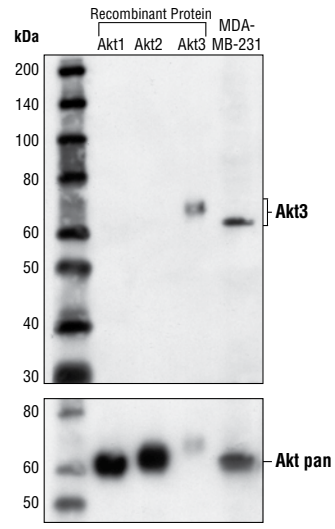
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H, M, R	60 kDa	Rabbit**

**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 $\alpha$  and  $\beta$  (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 $\beta$  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).

**Specificity/Sensitivity:** Akt3 Antibody detects endogenous levels of total Akt3, but does not recognize the truncated form of rat Akt3. The antibody does not cross-react with recombinant Akt1 or Akt2.



Western blot analysis of recombinant Akt1, Akt2 and Akt3 proteins, and extracts from MDA-MB-231 cells, using Akt3 Antibody (upper) or Akt Antibody #9272 (lower). Recombinant Akt3 is a 6His-fusion protein, MW=66 kDa.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues of human Akt3. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID # 10000  
Swiss-Prot Acc. # Q9Y243

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. 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.

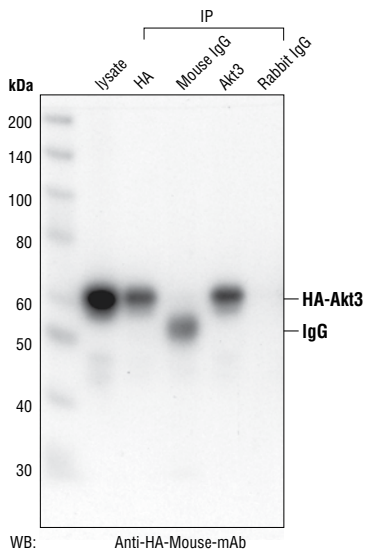
**Recommended Antibody Dilutions:**  
Western blotting 1:1000  
Immunoprecipitation 1:25

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).  
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

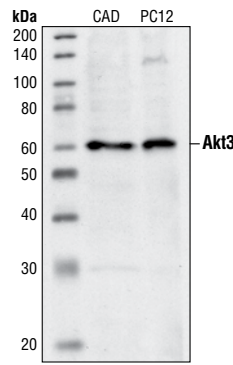
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

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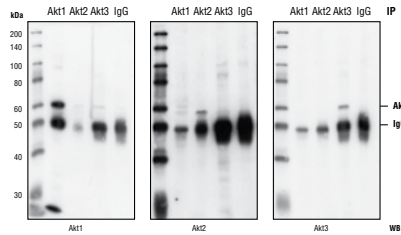
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish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



Western blot analysis (lane 1) and Immunoprecipitation (lanes 2-5) of extracts from HA-Akt3-transfected HeLa cells. HA-Akt3 was immunoprecipitated with HA-Tag (6E2) Mouse mAb #2367 (lane 2) or with Akt3 Antibody (lane 4). Immunoprecipitation with mouse IgG (lane 3) and rabbit IgG (lane 5) confirm specificity. Immunoblot was performed using HA-Tag (6E2) Mouse mAb #2367.



Western blot analysis of extracts from CAD and PC12 cells, using Akt3 Antibody.



Western blot analysis of immunoprecipitates from MDA-MB-231 cell lysates. Akt1 was immunoprecipitated with Akt1 (2H10) mAb #2967, Akt 2 was immunoprecipitated with Akt2 Antibody #2962, Akt 3 was immunoprecipitated with Akt3 Antibody, and specificity was confirmed by immunoprecipitation with rabbit IgG. Immunoblot was performed using Akt1 Antibody #2967 (left), Akt2 Antibody #2962 (middle) and Akt3 Antibody #4059 (right).

#### Background References:

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