

#9275 Store at **-20°C**

Phospho-Akt (Thr308) Antibody

- Small 100 μ l
(10 Western mini-blot)
- Large 300 μ l
(30 Western mini-blot)



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This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

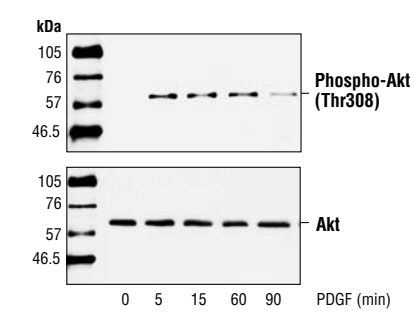
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, F Endogenous	H, M, R	60 kDa	Rabbit**

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).

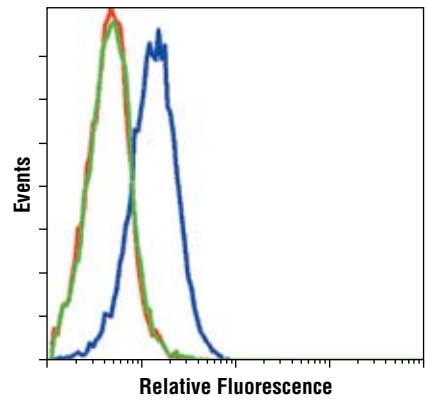
Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 β 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: Phospho-Akt (Thr308) Antibody detects endogenous levels of Akt only when phosphorylated at Thr308. This antibody does not detect Akt phosphorylated at other sites or related kinases such as PKC and p70 S6 kinase.



Western blot analysis of extracts from NIH/3T3 cells, untreated or PDGF treated (100 ng/ml), using Phospho-Akt (Thr308) Antibody (upper) or Akt Antibody #9272 (lower).



Flow cytometric analysis of Jurkat cells, untreated (blue) or LY294002-treated (green), using Phospho-Akt (Thr308) Antibody compared to a nonspecific negative control antibody (red).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues around Thr308 of mouse Akt. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID # 207
Swiss-Prot Acc. # P31749

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ 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	1:100
Flow Cytometry:	1:50

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.

Background References:

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- (17) Nave, B.T. et al. (1999) *Biochem. J.* 344, 427-431.
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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.