Revision 5

Store at -20C

#9271

Phospho-Akt (Ser473) Antibody

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IF-IC, FC-FP	H M R Hm Mk Dm B Dg	Endogenous	60	Rabbit	#P31751, #Q9Y243, #P31749	208, 10000, 207

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:50 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:100 - 1:400

Storage

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

Specificity/Sensitivity

Phospho-Akt (Ser473) Antibody detects endogenous levels of Akt1 only when phosphorylated at Ser473. This antibody also recognizes Akt2 and Akt3 when phosphorylated at the corresponding residues. It does not recognize Akt phosphorylated at other sites, nor does it recognize phosphorylated forms of related kinases such as PKC or p70 S6 kinase.

Species predicted to react based on 100% sequence homology

Chicken, Xenopus, Horse

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser473 of mouse Akt. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Akt, also referred to as PKB or Rac, plays a critical role in controlling cell 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 PI3K/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

1. Franke, T.F. et al. (1997) *Cell* 88, 435-7.
2. Burgering, B.M. and Coffer, P.J. (1995) *Nature* 376, 599-602.
3. Franke, T.F. et al. (1995) *Cell* 81, 727-36.
4. Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-51.
5. Sarbassov, D.D. et al. (2005) *Science* 307, 1098-101.
6. Jacinto, E. et al. (2006) *Cell* 127, 125-37.
7. Cardone, M.H. et al. (1998) *Science* 282, 1318-21.
8. Brunet, A. et al. (1999) *Cell* 96, 857-68.
9. Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-4.
10. Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240-5.
11. Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241-8.
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.

16. Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
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.
20. Devi, L. and Ohno, M. (2015) *Transl Psychiatry* 5, e562.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Hm:** Hamster **Mk:** Monkey **Dm:** D. melanogaster **B:** Bovine **Dg:** Dog

Trademarks and Patents

Limited Uses