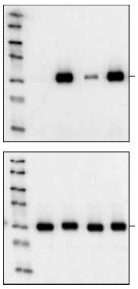
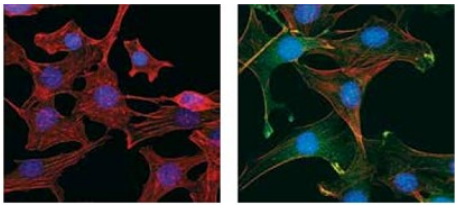
Product Data Sheet: Phospho-AKT1 (S473)

Cat. No:	MAB-94266
Conjugate:	Unconjugated
Size:	100 ul
Clone:	193H12
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	Peptide surrounding pSer-473 at the C-terminal sequence of human AKT protein
Reactivity:	Hu, Ms, Rt
Applications:	Western blotting 1:1000 Immunoprecipitation 1:200 Immunofluorescence (IF-IC) 1:200 Immuncytochemistry 1:200
Molecular Weight:	60 kDa
Purification:	Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of mouse Akt.
Synonyms:	AKT;CWS6;PKB;PKB-ALPHA;PRKBA;RAC;RAC-ALPHA;AKT1
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 acritical role in cell growth by directlyphosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). Moreimportantly, Akt phosphorylates and inactivatestuberin (TSC2), an inhibitor of mTOR within themTOR-raptor complex (18,19).Phospho-Akt (Ser473) (193H12) Rabbit mAb detects endogenous levels of Akt only when phosphorylated at Ser473.
Form:	liquid
Buffer:	Supplied in 20 mM Tris-HCl, pH 8.0 - 100 mg/ml BSA, 0.05% sodium azide.
Storage:	Store: At +4°C for short term, at-20°C for longer term Avoid freezing and thawing cycles



Western blot analysis of extracts from untreated or PDGF¬treated NIH/3T3 cells, pretreated with wortmannin and/ or rapamycin as indicated, using Phospho-Akt (Ser473) (193H12) Rabbit mAb (upper) or Akt Antibody (lower).



Confocal immunofluorescent analysis of C2C12 cells, U0126/LY294002/Rapamycin-treated (left) or insulin¬treated (right) using Phospho-Akt (Ser473) (193H12) Rabbit mAb (green).

IMMUNOCYTOCHEMISTRY (ICC) PROTOCOL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS.
2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT.
3. Wash 2 x 3 min with PBS.
4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice.
5. Wash 2 x 3 min with PBS.
6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT.
7. Incubate the cells with primary antibody: anti-Akt1, pSer-473 Monoclonal antibody at the dilution of 1:200 – 1:500 in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber.
8. Wash 2 x 3 min with PBS.
9. Apply the secondary antibody (in this case, the goat anti-rabbit IgG-FITC , was used at 1:300 in antibody dilution buffer,and cells were incubated for 1 hour at RT in dark).
10. Wash 3 x 3 min with PBS.
11. Rinse once with distilled water.
12. Mount the slide for observation, with a drop of anti-fade mounting medium

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.