

Akt (5G3) Mouse mAb (Biotinylated)

✓ 50 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

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

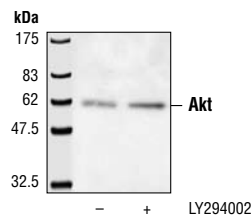
Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid 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: Akt (5G3) Mouse mAb (Biotinylated) detects endogenous levels of Akt. This antibody does not cross-react with other related proteins. The antibody is prepared by biotinylation via its primary amines. Biotinylated antibodies are useful for the detection or purification of Akt using avidin-biotin binding.

Source/Purification: Monoclonal antibody is produced by immunizing mice with an Akt1 recombinant protein containing human Akt1 residues 140-480.



Akt was immunoprecipitated from untreated or LY294002 treated Jurkat cell extracts using Akt (5G3) Mouse mAb (Biotinylated). This immunocomplex was then pulled down using streptavidin beads. Western blotting was performed with Akt Antibody #9272.

Background References:

- (1) Franke, T.F. et al. (1997) *Cell* 88, 435-437.
- (2) Burgering, B.M. and Coffey, P.J. (1995) *Nature* 376, 599-602.
- (3) Franke, T.F. et al. (1995) *Cell* 81, 727-736.
- (4) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-6551.
- (5) Sarbassov, D.D. et al. (2005) *Science* 307, 1098-1101.
- (6) Jacinto, E. et al. (2006) *Cell* 127, 125-137.
- (7) Cardone, M.H. et al. (1998) *Science* 282, 1318-1321.
- (8) Brunet, A. et al. (1999) *Cell* 96, 857-868.
- (9) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-1744.
- (10) Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240-4245.
- (11) Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241-5248.
- (12) Hajdich, E. et al. (2001) *FEBS Lett* 492, 199-203.
- (13) Cross, D.A. et al. (1995) *Nature* 378, 785-789.
- (14) Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499-3511.
- (15) Gesbert, F. et al. (2000) *J Biol Chem* 275, 39223-39230.
- (16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-252.
- (17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-431.
- (18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-657.
- (19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151-162.

Entrez-Gene ID #207

Swiss-Prot Acc. #P31749

Storage: Supplied in 58 mM Na₂HPO₄, 17 mM NaH₂PO₄ and 68 mM NaCl (pH 7.4). Store at 4°C. *Do not aliquot the antibody.*

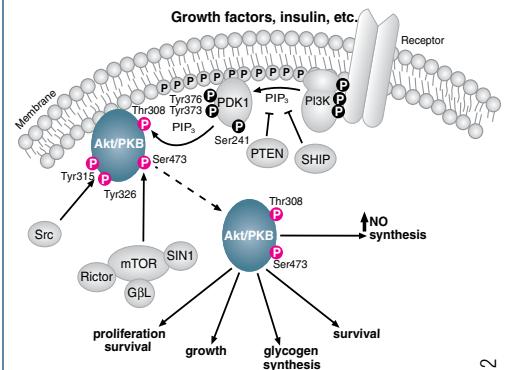
*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:

Immunoprecipitation 1:500

Companion Products:

- Akt (5G3) Mouse mAb #2966
- PhosphoPlus® Akt (Ser473) Antibody Kit #9270
- Phospho-Akt (Ser473) Antibody #9271
- Akt Antibody #9272
- Akt Control Cell Extracts #9273
- Phospho-Akt (Thr308) Antibody #9275
- Phospho-Akt (Ser473) (736E11) Rabbit mAb (IHC Specific) #3787
- Anti-mouse IgG, HRP-linked Antibody #7076
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003



Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g/ml}$ Leupeptin

NOTE: Add 1 mM PMSF immediately prior to use.

- Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
- Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
- 3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

B Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
- Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
- Sonicate samples on ice three times for 5 seconds each.
- Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

- Take 200 μl cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
- Add either protein A or G agarose beads (20 μl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 μl of 1X cell lysis buffer. Keep on ice during washes.
- Resuspend the pellet with 20 μl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
- Load the sample (15–30 μl) on SDS-PAGE gel (12–15%).
- Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Cell Lysate Pre-Clearing (Optional)

- Take 200 μl cell lysate and add to either Protein A or G agarose beads (20 μl of 50% bead slurry).
- Incubate at 4°C for 30 – 60 minutes.
- Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
- Proceed to step 1 of Immunoprecipitation.