

#5106 Store at -20°C

Phospho-Akt (Thr308) (L32A4) Mouse mAb

100 µl
 (10 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.

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W Endogenous	H, M, R, Mk	60 kDa	Mouse IgG1**

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).

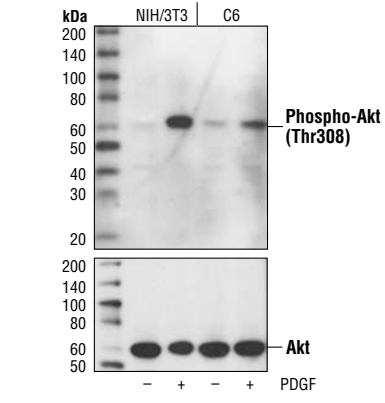
Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

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 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) (L32A4) Mouse mAb detects endogenous levels of Akt only when phosphorylated at Thr308.

Source/Purification: Monoclonal antibodies are produced by immunizing mice with a synthetic phosphopeptide (KLH-coupled) corresponding to residues around Thr308 of mouse Akt.

Background References:
 (1) Franke, T.F. et al. (1997) *Cell* 88, 435-7.



Western blot analysis of extracts from NIH/3T3 and C6 cells, untreated or PDGF-treated, using Phospho-Akt (Thr308) (L32A4) Mouse mAb (upper) or Akt (pan) (11E7) Rabbit mAb #4685 (lower).

(2) Burgering, B.M. and Coffey, 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.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by Western blot.**
****Anti-mouse secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:
 Western blotting 1:1000

Companion Products:
 Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060
 Phospho-Akt (Thr308) (C31E5E) Rabbit mAb #2965
 Akt (pan) (C67E7) Rabbit mAb #4691
 Phospho-Akt (Ser473) (587F11) Mouse mAb #4051
 Akt (5G3) Mouse mAb #2966
 Akt (pan) (11E7) Rabbit mAb #4685
 Akt2 (5B5) Rabbit mAb #2964
 Akt3 (62A8) Rabbit mAb #3788
 Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072
 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

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.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

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

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.