

# PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Antibody Pair

✓ 1 Kit  
(4 X 96 assays)

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

New 05/07

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

## Species Cross-Reactivity: H, M, R

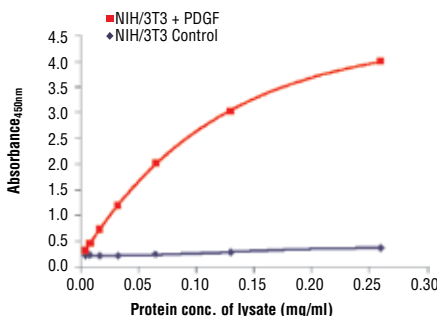
**Description:** CST's PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Phospho-Akt (Ser473) Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by Akt1 Detection Antibody and HRP-conjugated Anti-Mouse IgG. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-Akt1 (Ser473) protein.

\*Antibodies in this kit are custom formulations specific to the kit.

### Reagents not supplied:

- Phosphate Buffered Saline (PBS-20X) #9808
  - Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809
  - Cell Lysis Buffer (10X) #9803
  - TMB Substrate #7004
  - STOP Solution #7002
  - Blocking Buffer- PBS+0.05% Tween-20, 1% BSA
  - 96 Well Microplates\*\*
  - Microplate Reader
- \*\* Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Products Included	Volume	Cap Color	Storage
Phospho-Akt (Ser473) Capture Antibody (100X)	0.4 ml	Pink	4°C
Akt1 Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Mouse IgG, HRP-Linked Antibody (1000X)	0.04 ml	Yellow	-20°C



The relationship between lysate protein concentration from untreated and PDGF-treated NIH/3T3 cells and the absorbance at 450 nm using PathScan Phospho-Akt1 (Ser473) Sandwich ELISA Antibody Pair #7143 is shown. After overnight starvation, NIH/3T3 cells were treated with PDGF (50 ng/ml) for 20 minutes at 37°C and then lysed.

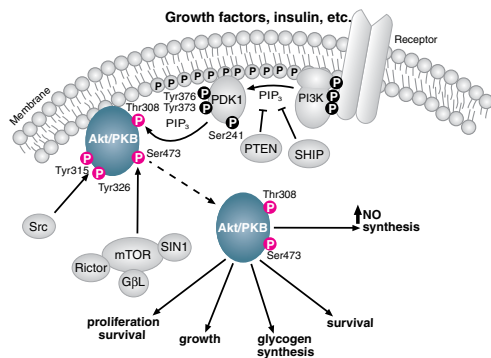
**Note:** Antibody pairs have been optimized using recommended buffers, reagents, plates and protocol. Solutions should be made fresh daily.

**Storage:** Phospho-Akt (Ser 473) Capture and Akt1 Detection Antibodies are stored at 4°C.

Anti-Mouse IgG-HRP Linked Antibody is stored at -20°C.

### Companion Products:

- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809
- BSA #9998
- TMB Substrate #7004
- STOP Solution #7002
- Anti-mouse IgG, HRP-linked Antibody #7076
- PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160
- PathScan® Total Akt1 Sandwich ELISA Kit #7170
- PathScan® Phospho-Akt (Thr308) Sandwich ELISA Kit #7252
- PathScan® Total Akt1 Sandwich ELISA Antibody Pair #7142
- PathScan® Phospho Akt (Thr 308) Sandwich ELISA Antibody Pair #7144
- Akt (pan) (11E7) Rabbit mAb #4685
- Akt Antibody #9272
- Akt1 (2H10) Mouse mAb #2967
- Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058
- Phospho-Akt (Ser473) (587F11) Mouse mAb #4051
- Phospho-Akt (Ser473) Antibody #9271
- Phospho-Akt (Thr308) (244F9) Rabbit mAb #4056
- Phospho-Akt (Thr308) Antibody #9275
- Phospho-Akt (Thr308) Antibody #5110



**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.



**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 and functions 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 its ability to phosphorylate and inactivate 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).

One of the essential functions of Akt is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 $\alpha$  and  $\beta$  (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 $\beta$  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 (19,20).

#### Background References:

- (1) Franke, T.F. (1997) *Cell* 88, 435–437.
- (2) Burgering, B.T. 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) Zimmerman, S. et al. (1999) *Science* 286, 1741–1744.
- (10) Cantley, L.C. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 4240–4245.
- (11) Vlahos, C. et al. (1994) *J. Biol. Chem.* 269, 5241–5248.
- (12) Hajdich, E. et al. (2000) *FEBS Lett.* 492, 199–203.
- (13) Cross, D.A. et al. (1995) *Nature* 373, 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) Nave, B.T. et al. (1999) *Biochem. J.* 344, 427–431.
- (18) Manning, B.D. et al. (2000) *Mol. Cell* 4, 648–657.
- (19) Manning, B.D. et al. (2002) *Mol. Cell* 10, 151–162.
- (20) Inoki, K. et al. (2002) *Nat. Cell Biol.* 4, 648–657.

## PathScan® Sandwich ELISA Antibody Pair Protocol

### A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)  
3.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)  
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA),  
1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA),  
1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,  
1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

**NOTE:** Reagents should be made fresh daily

### B Coating Procedure

- Rinse microplate with dH<sub>2</sub>O. Add 200 μl of dH<sub>2</sub>O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
  - Discard plate contents into a receptacle.
  - Wash 4 times with Wash Buffer, 200 μl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

### C 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 plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm<sup>2</sup>) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

### D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.