

PathScan® Phospho-Akt2 (Ser474) Sandwich ELISA Kit (Mouse Preferred)

✓ 1 Kit
(96 assays)

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

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

rev. 08/02/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #208
Swiss-Prot Acc. #P31751

Species Cross-Reactivity: M

Description: CST's PathScan® Phospho-Akt2 (Ser474) Sandwich ELISA Kit (Mouse Preferred) is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Akt2 protein phosphorylated at Ser474. A phospho-Akt rabbit antibody has been coated onto the microwells. After incubation with cell lysates, phospho-Akt protein is captured by coated antibody. Following extensive washing, Akt2 mouse antibody is added to detect the captured phospho-Akt2 protein. Anti-mouse IgG, HRP-linked antibody* is then used to recognize the bound detection antibody. HRP substrate TMB is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of Akt2 phosphorylated at Ser474.

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

Specificity/Sensitivity: CST's PathScan® Phospho-Akt2 (Ser474) Sandwich ELISA Kit (Mouse Preferred) detects endogenous levels of Akt2 protein phosphorylated at Ser474 in mouse (NIH/3T3) cells, as shown in Figure 1. Phospho-Akt protein specificity is demonstrated in Figure 2, while Akt2 kit sensitivity is shown in figure 3.

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/CIP1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive

Products Included	Volume	Solution Color
Phospho-Akt Rabbit Antibody Coated Microwells*	96 tests	
Akt2 Mouse Detection Antibody	11 ml	green
Anti-Mouse IgG, HRP-Linked Antibody	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

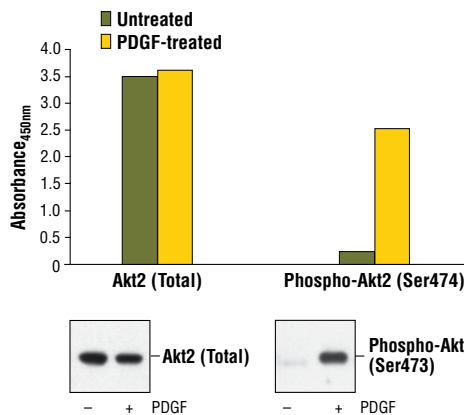


Figure 1: Treatment of NIH/3T3 cells with PDGF #9909 stimulates phosphorylation of Akt2 at Ser474, detected by PathScan® Phospho-Akt2 (Ser474) Sandwich ELISA Kit (Mouse Preferred) #7932, but does not affect levels of total Akt2 protein detected by PathScan® Total Akt2 Sandwich ELISA kit #7930. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using Akt2 (5B5) Rabbit mAb #2964 (left panel) and Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058 (right panel) are shown in the bottom figure.

complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery by inactivating p70 S6 kinase and activating the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation (18,19).

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Background References:

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

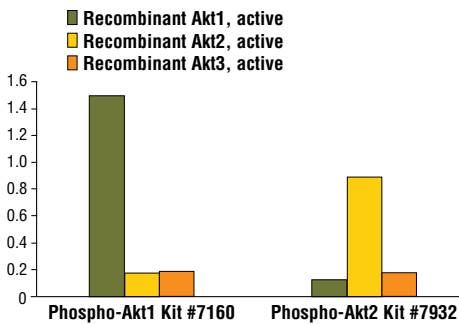


Figure 2: Comparison of specificity between two sandwich ELISA kits, #7160 for phospho-Akt1 (Ser473) and #7932 for phospho-Akt2 (Ser474), in the detection of recombinant Akt1 protein, recombinant Akt2 protein or recombinant Akt3 protein, which are active phosphorylated forms. 1.0 ng of recombinant protein (0.25 ng for Akt1) added in each microwell is assayed by using these two ELISA kits.

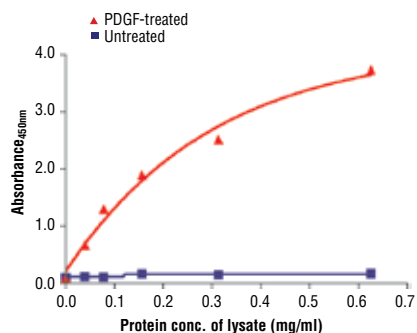


Figure 3: The relationship between protein concentration of lysates from untreated or PDGF-treated NIH/3T3 cells and the absorbance at 450 nm is shown. After starvation, NIH/3T3 cells (85% confluence) were treated with PDGF #9909 (50 ng/ml) for 10 min at 37°C and then lysed.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #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_2VO_4 , 1 $\mu\text{g}/\text{ml}$ leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. 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 in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. 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.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.

3. Add 100 μl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X Wash Buffer, 200 μl each time for each well.
 - c. 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.
 - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 μl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 μl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
 - a. Visual Determination — Read within 30 minutes after adding STOP Solution.
 - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.