

PathScan® Total Akt1 Sandwich ELISA Kit



Cell Signaling
TECHNOLOGY®

✓ 1 Kit
(96 assays)

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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 #207
Swiss-Prot Acc. #P31749

Species Cross-Reactivity: H, M

Description: CST's PathScan® Total Akt1 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Akt1 protein. An Akt Antibody has been coated onto the microwells. After incubation with cell lysates, the Akt protein is captured by the coated antibody. Following extensive washing, Akt1 Mouse Monoclonal Antibody is added to detect the captured total Akt1 protein. HRP-linked anti-mouse antibody is then used to recognize the bound detection antibody. HRP substrate,

TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total Akt1 protein.

Specificity/Sensitivity: CST's PathScan® Total Akt1 Sandwich ELISA Kit detects endogenous levels of Akt1 including phosphorylated and nonphosphorylated. Inhibition of phosphorylation of Akt at serine 473 by LY294002 in NIH/3T3 cells is detected by PathScan Phospho-Akt1 (Ser473) Sandwich ELISA kit, #7160, while the level of total Akt1 protein detected by this ELISA kit #7170 is unchanged, as seen in Figure 1. Similar results have also observed in Jurkat cells (data not shown). This ELISA kit does not detect related kinases, such as PKC and p70 S6 kinase. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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-3 α and

| Products Included | Volume | Solution Color |
|--|----------|----------------|
| Akt Rabbit Antibody coated Microwells* | 96 tests | |
| Akt1 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).

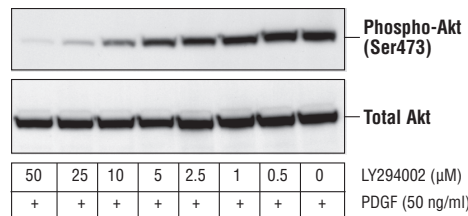
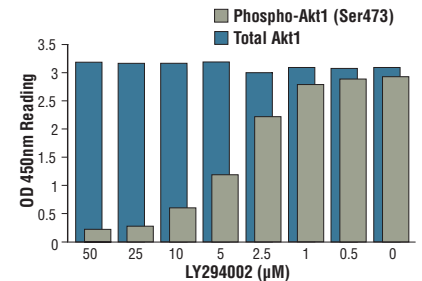


Figure 1: Treatment of NIH/3T3 cells with various concentrations of LY294002 blocks PDGF-stimulated phosphorylation of Akt1 at serine 473 detected by PathScan Phospho-Akt1 (Ser473) Sandwich ELISA kit, #7160, but does not affect the level of total Akt1 protein detected by PathScan Total Akt1 Sandwich ELISA kit #7170. OD450 readings are shown in the top figure, while the corresponding Western blot, using Phospho-Akt (Ser473) Antibody #9271 or Akt Antibody #9272, is shown in the bottom figure.



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 complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

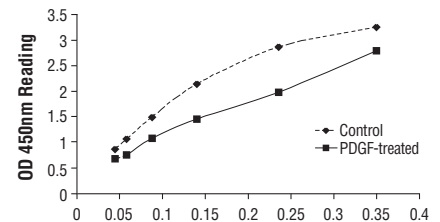


Figure 2: Linear relationship between protein concentration of lysates from either control NIH/3T3 cells (dotted line) or PDGF-treated NIH/3T3 cells (solid line) and kit assay optical density readings.

Background References:

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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_3VO_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 phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) 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.