

PathScan® Cell Growth Multi-Target Sandwich ELISA Kit



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For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, M

Introduction: CST's PathScan® Cell Growth Multi-Target Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that combines the reagents necessary to detect endogenous levels of S6 ribosomal protein, phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204). These molecules represent key signaling proteins in pathways controlling growth and differentiation. Sixteen assays are provided for each target protein. Specific assay formulations for the indicated target proteins can be found in the datasheets associated with the individual sandwich ELISA kits*. Briefly, a capture antibody** has been coated onto the microwells. After incubation with cell lysates, the target protein is captured by the coated antibody. Following extensive washing, a detection antibody** is added to detect the captured target protein. An HRP-linked secondary antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of bound target protein.

* See companion products.

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

Specificity/Sensitivity: CST's PathScan® Cell Growth Multi-Target Sandwich ELISA Kit #7239 detects endogenous levels of six proteins: S6 ribosomal protein, phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204). Activation of these proteins can be observed over time in response to PDGF. As shown in Figure 1, stimulation of serum-starved NIH/3T3 cells with PDGF promotes phosphorylation of Akt1 at Thr308 and Ser473, S6 ribosomal protein at Ser235/236 and p44/42 MAPK at Thr202/Tyr204. The level of each target protein (phospho and nonphospho) remains unchanged throughout the 80 minute time course as demonstrated by Western analysis.

The relationship between the protein concentration of the lysate and the absorbance at 450 nm can be found in the datasheets associated with the individual PathScan® Sandwich ELISA Kits*.

* See companion products.

Companion Products:

PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit #7225

PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Kit #7205

PathScan® Total Akt1 Sandwich ELISA Kit #7170

Products Included	Quantity	Solution Color	Cap Color
Akt Rabbit Antibody Coated Microwells	16 tests		
Akt1 Mouse Detection Antibody	1.8 ml	Green	Red
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Red
Phospho-Akt (Ser473) Rabbit Antibody Coated Microwells	16 tests		
Akt1 Mouse Detection Antibody	1.8 ml	Green	Tan
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Tan
Akt Rabbit Antibody Coated Microwells	16 tests		
Phospho-Akt (Thr308) Mouse Detection Antibody	1.8 ml	Green	Blue
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Blue
Phospho-p44/42 MAPK (Thr202/Tyr204) Rabbit Antibody Coated Microwells	16 tests		
p44/42 MAPK Mouse Detection Antibody	1.8 ml	Green	Light Pink
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Light Pink
S6 Ribosomal Protein Mouse Antibody Coated Microwells	16 tests		
S6 Ribosomal Protein Rabbit Detection Antibody	1.8 ml	Green	Purple
Anti-Rabbit IgG HRP-Linked Antibody	1.8 ml	Red	Purple
Phospho-S6 Ribosomal Protein (Ser235/236) Rabbit Antibody Coated Microwells	16 tests		
S6 Ribosomal Protein Mouse Detection Antibody	1.8 ml	Green	Green
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Green
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	
Cell Lysis Buffer (10X)	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).

PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160

PathScan® Phospho-Akt (Thr308) Sandwich ELISA Kit #7252

PathScan® Phospho-p44/p42 MAPK (Thr202/Tyr204) Sandwich ELISA Kit #7177

Anti-rabbit IgG, HRP-linked Antibody #7074

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

STOP Solution #7002

TMB Substrate #7004

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

U.S. Patent No. 5,675,063

Tween®20 is a registered trademark of ICI Americas, Inc.

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

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.

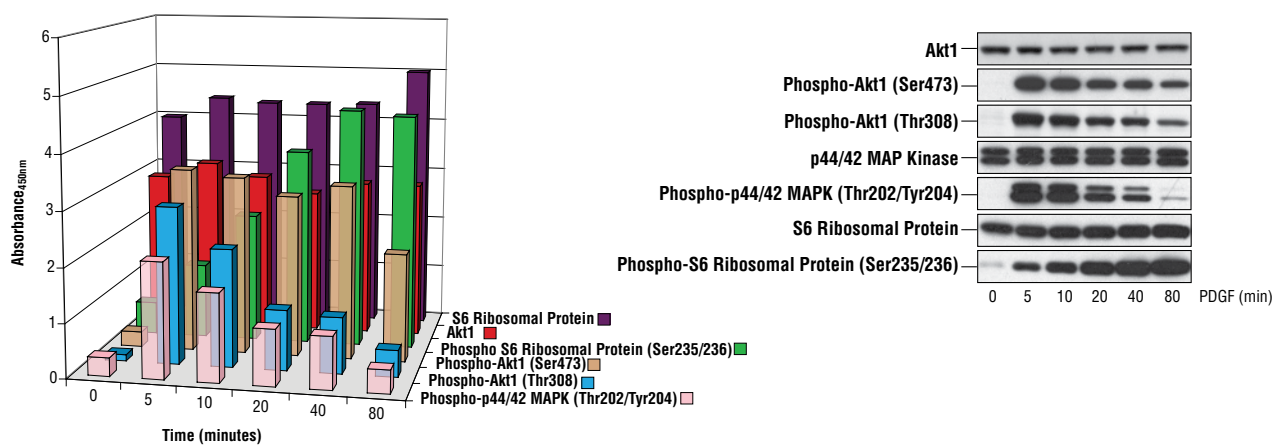


Figure 1. Treatment of NIH/3T3 cells with PDGF induces phosphorylation of Akt1 at Thr308 and Ser473, S6 Ribosomal Protein at Ser235/236 and p44/42 MAPK at Thr202/Tyr204 as detected by the PathScan® Cell Growth Multi-Target Sandwich ELISA Kit #7239. While dynamic phosphorylation is observed throughout the time course, the level of total p44/42 MAPK, Akt1 and S6 ribosomal protein remains unchanged as demonstrated by sandwich ELISA and Western analysis. NIH/3T3 cells (80-90% confluent) were starved overnight and stimulated with PDGF (100 ng/mL) for 5, 10, 20, 40 and 80 minutes at 37°C. Lysates were assayed at a protein concentration of 0.45 mg/mL. The absorbance readings at 450 nm are shown as a 3-dimensional representation in the left figure, while the corresponding Western blots are shown in the right figure. The antibodies used for the Western analyses include S6 Ribosomal Protein Rabbit mAb #2217, Phospho-S6 Ribosomal Protein (Ser235/236) Antibody #2211, Akt Antibody #9272, Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058, Phospho-Akt (Thr308) Antibody #9275, Phospho-p44/42 MAPK (Thr202/Tyr204) (E10) Mouse mAb #9106, p44/42 MAP Kinase Antibody #9102.

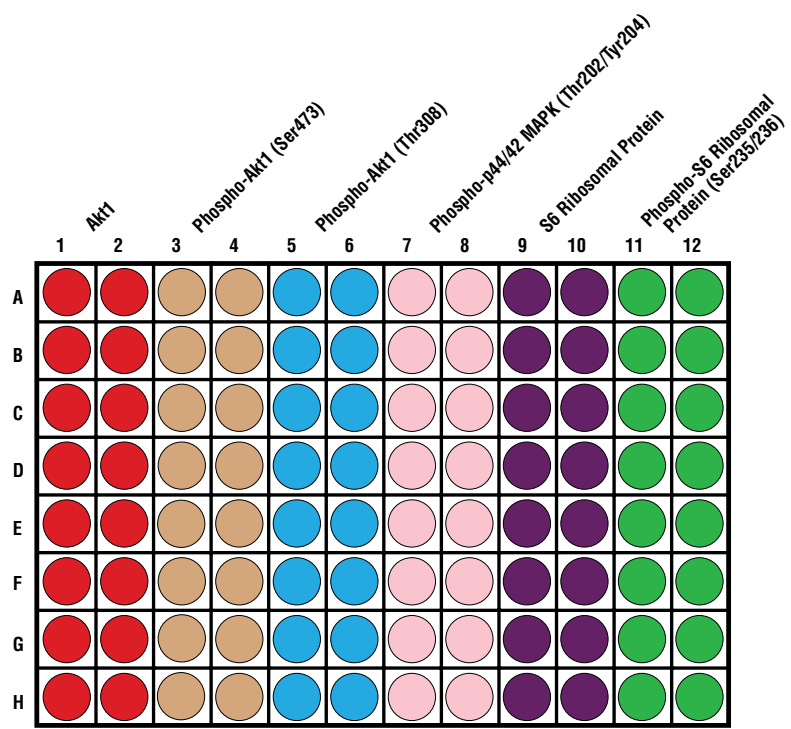


Figure 2. Schematic representation of a 96-well plate depicting the color-code of the reagents used to detect endogenous levels of Akt1 (red; 1 & 2), phospho-Akt1 (Ser473) (tan; 3 & 4), phospho-Akt1 (Thr308) (blue; 5 & 6), phospho-p44/42 MAPK (Thr202/Tyr204) (light pink; 7 & 8), S6 ribosomal protein (purple; 9 & 10) and phospho-S6 ribosomal protein (Ser235/236) (green; 11 & 12).

Background: Akt is a protooncogene with a critical regulatory role in diverse cellular processes including growth, survival and the cell cycle. Akt is also a major regulator of insulin signaling and glucose metabolism (1-4). Akt is activated by PI3 kinase signaling and activation loop phosphorylation at Thr308 by PDK1 and by phosphorylation within the carboxy terminus at Ser473 by the mTOR-riCTOR complex (TORC1) (5-7).

Both p44 and p42 MAP kinases (Erk1 and Erk2) function in a protein kinase cascade that plays a critical role in the regulation of cell growth and differentiation (8-13). MAP kinases are activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones and neurotransmitters. Activation of MAP kinases occurs through phosphorylation of threonine and tyrosine (202 and 204 of human MAP kinase or 183 and 185 of rat MAP kinase) at the sequence T*EY* by a single upstream MAP kinase kinase (MEK) (14,15).

To effectively promote growth and cell division in a sustained manner, growth factors and mitogens must upregulate translation (16,17). Growth factors and mitogens induce the activation of p70 S6 kinase, which in turn phosphorylates the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation, particularly of mRNAs with an oligopyrimidine tract in their 5' untranslated regions (17). This group of mRNAs (5'TOP) encodes proteins involved in cell cycle progression and proteins that are part of the translational machinery, such as ribosomal proteins and elongation factors (17,18). The main *in vivo* S6 ribosomal protein phosphorylation sites, including Ser235, Ser236, Ser240 and Ser244, are located within a small 19 amino acid region in the S6 carboxy terminus (19,20).

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_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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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.