

PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit

1 Kit
(96 assays)



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

Entrez-Gene ID # 6194
Swiss-Prot Acc. # P62753

Species Cross-Reactivity: H, M

Description: The PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total S6 ribosomal protein. An S6 Ribosomal Protein Mouse mAb has been coated onto the microwells. After incubation with cell lysates, both phospho- and non-phospho-S6 ribosomal proteins are captured by the coated antibody. Following extensive washing, S6 Ribosomal Protein Antibody is added to detect phospho- and nonphospho-S6 ribosomal proteins. HRP-linked Anti-rabbit Antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density is proportional to the quantity of total ribosomal protein.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

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

S6 Ribosomal Protein Antibody #2212

Anti-rabbit IgG, HRP-linked Antibody #7074

Cell Lysis Buffer (10X) #9803

S6 Ribosomal Protein (5G10) Rabbit mAb #2217

S6 Ribosomal Protein (54D2) Mouse mAb #2317

PathScan® Total S6 Ribosomal Protein Sandwich ELISA Antibody Pair #7203

Specificity/Sensitivity: The PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit detects endogenous levels of S6 ribosomal protein. As shown in Figure 1, a significant induction of phospho-S6 ribosomal protein (Ser235/236) in PDGF treated NIH/3T3 cells is detected. However, the level of total S6 ribosomal protein (phospho and non-phospho) remains unchanged. NIH/3T3 or 293 cells treated with 20% FBS after starvation also show similar results (data not shown).

Products Included	Volume	Solution Color
S6 Ribosomal Protein Mouse Antibody Coated Microwells*	96 tests	
S6 Ribosomal Protein Rabbit Detection Antibody	11 ml	green
Anti-Rabbit 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).

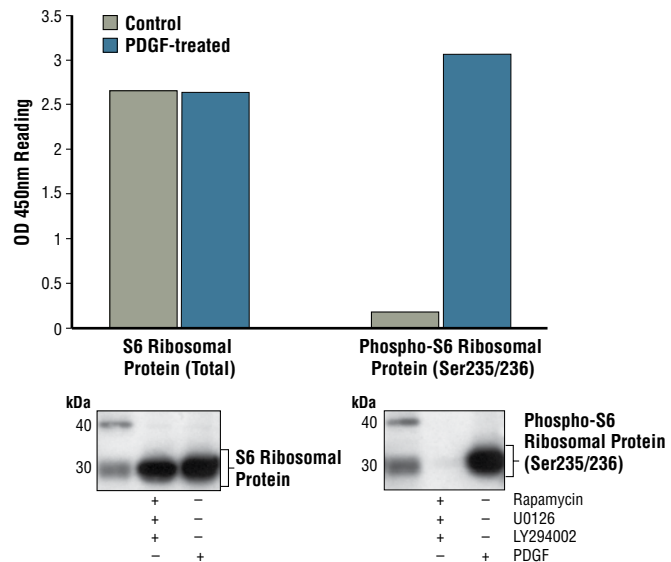


Figure 1: Treatment of NIH/3T3 cells with PDGF stimulates phosphorylation of S6 ribosomal protein at Ser235/236, detected by PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Kit #7205, but does not affect the level of total S6 ribosomal protein detected by PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit #7225. A combination of Rapamycin, a FRAP/mTOR inhibitor, U0126, a MEK1/2 inhibitor, and LY294002, a PI3 Kinase inhibitor, can totally suppress the phosphorylation of S6 ribosomal protein in cells. Triple inhibitor-treatment (37°C for 120 min after starvation) represents the nonphosphorylated form of S6 ribosomal protein whereas PDGF-treated cells represent the phosphorylated form, as shown in both Sandwich ELISA and Western analysis (upper/bottom right). OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-S6 Ribosomal Protein (Ser235/236) Ab #2211 (right panel) or S6 Ribosomal Protein Antibody #2212 (left panel), is shown in the bottom figure.



Background: One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' untranslated regions (2). These particular mRNA transcripts (5'TOP) encode proteins involved in cell cycle progression as well as ribosomal proteins and elongation factors necessary for translation (2,3). Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240 and Ser244) located within a small, carboxy-terminal region of the S6 protein (4,5).

Background References:

- (1) Dufner, A. and Thomas, G. (1999) *Exp. Cell Res.* 253, 100–109.
- (2) Peterson, R.T. and Schreiber, S.L. (1998) *Curr. Biol.* 8, R248–R250.
- (3) Jefferies, H.B. et al. (1997) *EMBO J.* 16, 3693–3704.
- (4) Ferrari, S. et al. (1991) *J. Biol. Chem.* 266, 22770–22775.
- (5) Flotow, H. and Thomas, G. (1992) *J. Biol. Chem.* 267, 3074–3078.

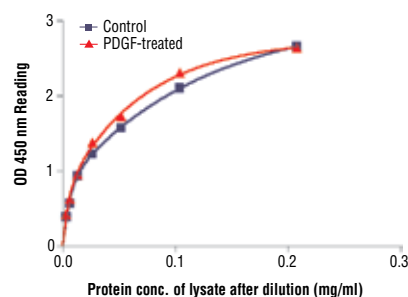


Figure 2: The relationship between protein concentration of lysates from triple inhibitor-treated (as control) and PDGF-treated NIH/3T3 cells and kit assay optical density readings is shown. NIH/3T3 cells (70–85% confluence) were treated with PDGF (50 ng/ml), and lysed after incubation at 37°C for 30 min. For the control, NIH/3T3 cells (70–85% confluence) were treated with rapamycin (100 nM), U0126 (10 µM), and LY294002 (50 µM), and lysed after incubation at 37°C for 120 min.

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 Na₃VO₄, 1 μg/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.