

PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit

✓ 1 Kit
(96 assays)



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

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.

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).

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).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
S6 Ribosomal Protein Mouse mAb Coated Microwells*	39730	96 tests		4°C
S6 Ribosomal Protein Rabbit Detection mAb	14019	1 each	Green (Lyophilized)	4°C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

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

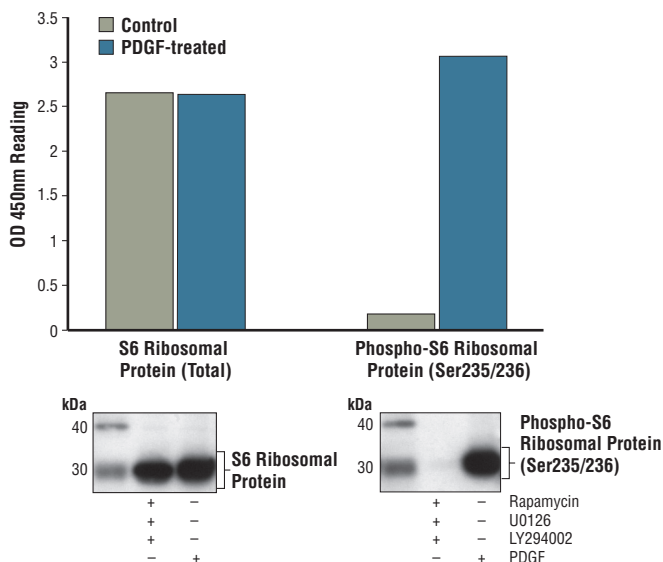


Figure 1: Treatment of NIH/3T3 cells with PDGF stimulates phosphorylation of S6 ribosomal protein at Ser235/236, detected by PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Kit #7205, but does not affect the level of total S6 ribosomal protein detected by PathScan® Total S6 Ribosomal 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.

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

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 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.

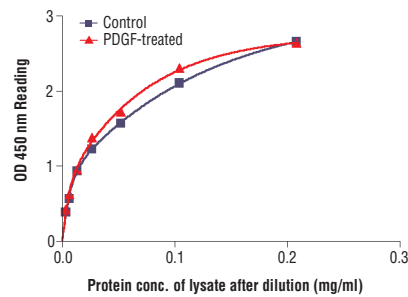


Figure 2: The relationship between protein concentration of lysates from triple inhibitor-treated (as control) and PDGF-treated NIH/3T3 cells and kit assay optical density readings is shown. NIH/3T3 cells (70–85% confluence) were treated with PDGF (50 ng/ml), and lysed after incubation at 37°C for 30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

PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- TMB Substrate** (#7004).
- STOP Solution** (#7002).

*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

B Preparing Cell Lysates

For adherent cells.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 rpm) 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.

For suspension cells

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 rpm) 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

- 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.
- Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
- Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X 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.
- Add 100 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
- 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.

- Read results.
 - Visual Determination:** Read within 30 min after adding STOP Solution.
 - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.