#7316C Store at 4°C

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

1 Kit
(96 assays)
Low volume microplate

Cell Signaling TECHNOLOGY®

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

**Species Cross-Reactivity: H, M**

**Description:** The PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-S6 ribosomal protein (Ser235/236) with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using smaller samples. A Phospho-S6 Ribosomal Protein (Ser235/236) Rabbit mAb has been coated on the microwells. After incubation with cell lysates, phospho-S6 ribosomal protein is captured by the coated antibody. Following extensive washing, a total S6 Ribosomal Protein Mouse mAb is added to detect the captured phospho-S6 ribosomal protein (Ser235/236). Anti-mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of phospho-S6 ribosomal protein (Ser235/236).

*Antibodies in kit are custom formulations specific to kit.

**Specificity/Sensitivity:** The PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of phospho-S6 ribosomal protein phosphorylated on serines 235/236. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

**Source/Purification:** PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of phospho-S6 ribosomal protein (Ser235/236) in human and mouse cells.

**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 |
|---|---|---|---|---|
| P-S6 RiboProt (S235/236) RmAb Coated* | 93314 | 96 tests | | 4°C |
| S6 Ribosomal Protein Mouse Detection smAb | 2355 | 1 each | Green (Lyophilized) | 4°C |
| Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated) | 13304 | 1 each | Red (Lyophilized) | 4°C |
| Detection Antibody Diluent | 13339 | 5.5 ml | Green | 4°C |
| HRP Diluent | 13515 | 5.5 ml | Red | 4°C |
| Luminol/Enhancer Solution | 84850 | 3 ml | | RT |
| Stable Peroxide Buffer | 42552 | 3 ml | | RT |
| 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 |

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

Note: This kit contains components with mixed storage temperatures. Please store this entire kit at 4°C for long term storage. Upon first use, please store each component as indicated in the chart above and on individual component labels.

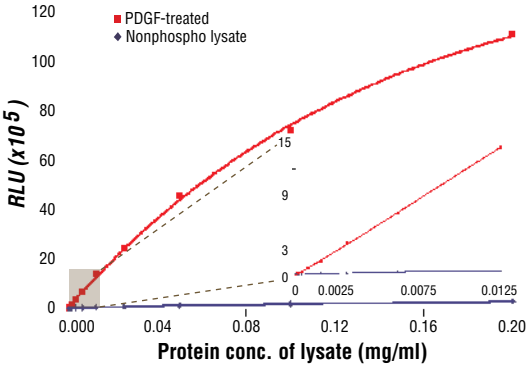


*Figure 1. Relationship between protein concentration from nonphospho cell lysate and PDGF-treated NIH/3T3 cell lysate and immediate light generation with chemiluminescent substrate is shown. Cells (80% confluence) lysed without the addition of phosphatase inhibitor to the lysis buffer (nonphospho lysate) or treated with PDGF (50 ng/ml) and lysed with Cell Lysis Buffer #9803 after incubation at 37°C for 30 minutes. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.*



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

**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.

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

**NOTE:** Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

## A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 0.5 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 0.5 ml volume of reconstituted Detection Antibody to 5.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 0.5 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 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (5.5 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
6. **Sample Diluent:** Blue colored diluent for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **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 phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **Luminol/Enhancer Solution** and **Stable Peroxide Buffer**

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

## B Preparing Cell Lysates

**For adherent cells.**

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. 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.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. 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**

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x $10^6$ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. 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

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. 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.
3. Add 50 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 room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
   **a.** Discard plate contents into a receptacle.
   **b.** Wash 4 times with 1X Wash Buffer, 150 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 50 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 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 at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the Detection Reagent Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. *Optimal signal intensity is achieved when read within 10 min.*

**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.