Store at 4°C and –20°C

#7201

PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Antibody Pair

☑ 1 Kit
(4 X 96 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 02/18/16

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

Entrez-Gene ID #6194
UniProt ID #P62753

Species Cross-Reactivity: H, M

Description: CST's PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Antibody Pair is being offered as an alternative to our PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Kit #7205. Capture and Detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are provided for performing 4 x 96 well ELISAs. The Phospho-S6 Ribosomal Protein (Ser235/236) Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added, followed by S6 Ribosomal Protein Detection Antibody and HRP-conjugated secondary antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance at 450 nm is proportional to the quantity of phospho-S6 Ribosomal protein (Ser235/236) protein.

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

Reagents not supplied:
Phosphate Buffered Saline (PBS-20X) #9808

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

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer- PBS+0.05% Tween®20, 1% BSA

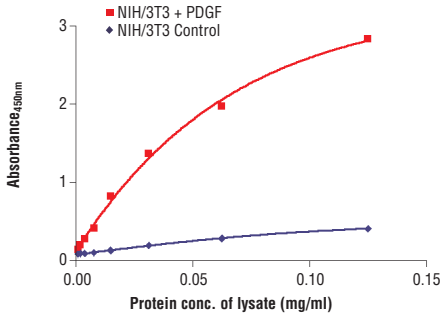
96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and protocol. Solutions should be made fresh daily.

Products Included	Volume	Cap Color	Storage
Phospho-S6 Ribosomal Protein (Ser235/236) Capture Antibody (100X)			
S6 Ribosomal Protein Capture Antibody (100X)	0.4 ml	Pink	4°C
S6 Ribosomal Protein Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Mouse IgG HRP-Linked Antibody (1000X)	0.04 ml	Red	–20°C



The relationship between lysate protein concentration from untreated and PDGF treated NIH/3T3 cells and the absorbance at 450 nm using PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Antibody Pair #7201 is shown. NIH/3T3 cells were starved overnight, treated with PDGF for 20 minutes at 37°C and then lysed.

Background: To effectively promote growth and cell division in a sustained manner, growth factors and mitogens must upregulate translation (1,2). 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 (2). 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 (2,3). 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 (4,5).

Storage: S6 Ribosomal Protein Capture Antibody and Phospho-S6 Ribosomal Protein (Ser 235/236) Detection Antibody are stored at 4°C.

Anti-Rabbit IgG-HRP Linked Antibody is stored at -20°C.

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.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween®20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween®20, 1% BSA
4. **1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #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 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 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.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C 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 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.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.