

#7966 Store at 4°C and -20°C

PathScan® Total RSK1 Sandwich ELISA Kit

✓ 1 Kit (96 assays)



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New 09/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #6195
Swiss-Prot Acc. #Q15418

Species Cross-Reactivity: H

Description: PathScan® Total RSK1 Sandwich ELISA Kit from Cell Signaling Technology is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total RSK1 protein. A RSK1 Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, both phospho- and nonphospho-RSK1 proteins are captured by the coated antibody. Following extensive washing, a RSK1 Mouse Antibody is added to detect both the captured phospho- and nonphospho-RSK1 protein. Anti-mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total RSK1 protein.

Specificity/Sensitivity: CST's PathScan® Total RSK1 Sandwich ELISA Kit #7966 detects endogenous levels of total RSK1 protein. As shown in Figure 1, a significant induction of RSK1 phosphorylation at Ser380 can be detected in HeLa cells following treatment with TPA using the Phospho-RSK1 (Ser380) Sandwich ELISA Kit #7965. The levels of total RSK1 (phospho and nonphospho) remain unchanged, as shown by Western analysis and by PathScan® Total RSK1 Sandwich ELISA Kit #7966 (Figure 1).

Background: The 90 kDa ribosomal S6 kinases (RSK1-4) are a family of widely expressed serine/threonine kinases characterized by two nonidentical, functional kinase domains (1) and a carboxy-terminal docking site for extracellular signal-regulated kinases (ERKs) (2). Several sites both within and outside of the RSK kinase domain, including Ser380, Thr359, Ser363 and Thr573, are important for kinase activation (3). RSK1-3 are activated via coordinated phosphorylation by MAPKs, by autophosphorylation, and by phosphoinositide-3-OH kinase (PI3K) in response to many growth factors, polypeptide hormones and neurotransmitters (3).

Background References:

- (1) Fisher, T.L. and Blenis, J. (1996) *Mol Cell Biol* 16, 1212-9.
- (2) Smith, J.A. et al. (1999) *J Biol Chem* 274, 2893-8.
- (3) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496-505.

Products Included	Volume	Solution Color
RSK1 Antibody Coated Microwells	96 tests	
RSK1 Detection Antibody	11 ml	green
Anti-mouse 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
1X PathScan® Sandwich ELISA Lysis Buffer #7018**	30 ml	colorless

* 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 1X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

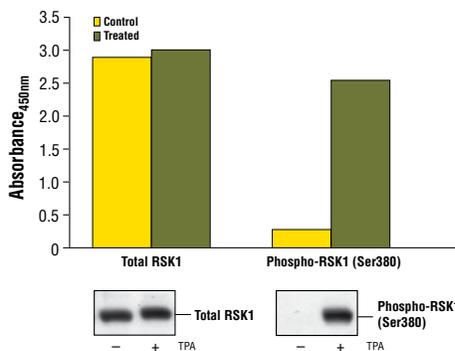


Figure 1. Treatment of HeLa cells with TPA stimulates phosphorylation of RSK1 at Ser380, detected by the PathScan® Phospho-RSK1 (Ser380) Sandwich ELISA Kit #7965, but does not affect the levels of total RSK1 detected by PathScan® Total RSK1 Sandwich ELISA Kit #7966. HeLa cells (80-90% confluent) were treated with 200 nM TPA #4174 for 30 minutes. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using RSK1 Antibody #9333 (left panel) or Phospho-p90RSK (Ser380) (9D9) Rabbit mAb #9335 (right panel) are shown in the bottom figure.

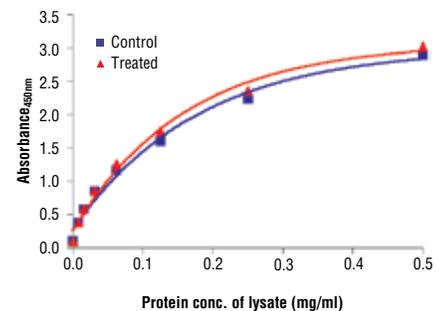


Figure 2. The relationship between the protein concentration of lysates from HeLa cells, untreated or treated with TPA #4174 and the absorbance at 450 nm using the PathScan® Total RSK1 Sandwich ELISA Kit is shown.

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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.

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:**
 - 20 mM Tris-HCl (pH 7.5)
 - 150 mM NaCl
 - 1 mM Na₂ EDTA
 - 1 mM EGTA
 - 1% Triton
 - 20 mM sodium pyrophosphate
 - 25 mM sodium fluoride
 - 1 mM β-glycerophosphate
 - 1 mM Na₂VO₄
 - 1 μg/ml leupeptin

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 0.5–1.0 ml ice-cold 1X PathScan® Sandwich ELISA Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 2 minutes.
4. Collect cell lysate into new tubes. This cell lysate solution can be used directly in this ELISA Kit, or stored 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.