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#7153

## PathScan® Phospho-p70 S6 Kinase (Thr389) Chemiluminescent Sandwich ELISA Kit

Species Cross Reactivity: H M    UniProt ID: #P23443    Entrez-Gene Id: #6198

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity	Color	Storage Temp
p70 S6 Kinase Rabbit mAb Coated Microwells	87842	96 tests		+4C
Phospho-p70 S6 Kinase (Thr389) Mouse Detection mAb	13960	1 ea	Green (Lyophilized)	+4C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	5.5 ml	Green	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
Luminol/Enhancer Solution	84850	3 ml	Colorless	RT
Stable Peroxide Buffer	42552	3 ml	Colorless	RT
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

### Description

The PathScan® Phospho-p70 S6 Kinase (Thr389) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-p70 S6 kinase (Thr389) protein with a chemiluminescent readout. Chemiluminescence 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 a smaller sample size. A p70 S6 kinase rabbit antibody has been coated on the microwells. After incubation with cell lysates, both phospho- and nonphospho-p70 S6 kinase proteins are captured by the coated antibody. Following extensive washing, phospho-p70 S6 kinase (Thr389) mouse antibody is added to detect the captured phospho-p70 S6 kinase (Thr389) protein. 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-p70 S6 kinase (Thr389) protein. Antibodies in kit are custom formulations specific to kit.

### Specificity/Sensitivity

PathScan® Phospho-p70 S6 Kinase (Thr389) Chemiluminescent Sandwich ELISA Kit #7153 detects endogenous levels of phospho-p70 S6 kinase (Thr389) in human and mouse cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

### Background

p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression (1,2). p70 S6 kinase phosphorylates the S6 protein of the 40S ribosomal subunit and is involved in translational control of 5' oligopyrimidine tract mRNAs (1). A second isoform, p85 S6 kinase, is derived from the same gene and is identical to p70 S6 kinase except for 23 extra residues at the amino terminus, which encode a nuclear localizing signal (1). Both isoforms lie on a mitogen activated signaling pathway downstream of phosphoinositide-3 kinase (PI-3K) and the target of rapamycin, FRAP/mTOR, a pathway distinct from the Ras/MAP kinase cascade (1). The activity of p70 S6 kinase is controlled by multiple phosphorylation events located within the catalytic, linker and pseudosubstrate domains (1). Phosphorylation of Thr229 in the catalytic domain and Thr389 in the linker domain are most critical for kinase function (1). Phosphorylation of Thr389, however, most closely correlates with p70 kinase activity *in vivo* (3). Prior phosphorylation of Thr389 is required for the action of phosphoinositide 3-dependent protein kinase 1 (PDK1) on Thr229 (4,5). Phosphorylation of this site is stimulated by growth factors such as insulin, EGF and FGF, as well as by serum and some G-protein-

coupled receptor ligands, and is blocked by wortmannin, LY294002 (PI-3K inhibitor) and rapamycin (FRAP/mTOR inhibitor) (1,6,7). Ser411, Thr421 and Ser424 lie within a Ser-Pro-rich region located in the pseudosubstrate region (1). Phosphorylation at these sites is thought to activate p70 S6 kinase via relief of pseudosubstrate suppression (1,2). Another LY294002 and rapamycin sensitive phosphorylation site, Ser371, is an *in vitro* substrate for mTOR and correlates well with the activity of a partially rapamycin resistant mutant p70 S6 kinase (8).

## Background References

1. Pullen, N. and Thomas, G. (1997) *FEBS Lett* 410, 78-82.
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  3. Weng, Q.P. et al. (1998) *J Biol Chem* 273, 16621-9.
  4. Pullen, N. et al. (1998) *Science* 279, 707-10.
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# #7153

## PathScan<sup>®</sup> Phospho-p70 S6 Kinase (Thr389) Chemiluminescent Sandwich ELISA Kit

### ELISA Chemiluminescent

**NOTE:** Refer to product-specific datasheets or product webpage for assay incubation temperature. This /product/productDetail.jsp?productId=luminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 µl per microwell.

### A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 10X PBS to 950 ml dH<sub>2</sub>O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X wash buffer by diluting 20X Wash Buffer (included in each PathScan<sup>®</sup> Sandwich ELISA Kit) in dH<sub>2</sub>O.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH<sub>2</sub>O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

**Recommended:** Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **20X LumiGLO<sup>®</sup> Reagent and 20X Peroxide:** (#7003).

### 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 (x14,000 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 (~1,200 rpm) when the culture reaches 0.5-1.0 x 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5-10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth medium 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 (x14,000 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 in the storage bag and stored at 4°C immediately.
2. Cell lysates can be used undiluted or diluted with sample diluent (supplied in each PathScan<sup>®</sup> Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
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:**
  1. Discard plate contents into a receptacle.
  2. Wash 4 times with 1X Wash Buffer, 150 µl each time per well.
  3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to dry completely at any time.

4. Clean the underside of all wells with a lint-free tissue.
5. Add 50  $\mu$ l of detection antibody (green color) to each well. Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50  $\mu$ l of HRP-linked secondary antibody (red color) to each well. 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 2X LumiGLO<sup>®</sup> Reagent and 2X Peroxide.
10. Add 50  $\mu$ l of the detection reagent working solution to each well.
11. Use a plate-based luminometer set at 425 nm to measure Relative Light Units (RLU) within 1–10 min following addition of the substrate.
  1. Optimal signal intensity is achieved when read within 10 min.

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