Revision 4

Store at +4C

#7053

PathScan® Phospho-p70 S6 Kinase (Thr389) Sandwich ELISA Antibody Pair

Cell Signaling TECHNOLOGY®

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1 Kit (Reagents for 4 x 96 well plates)

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 #	Volume	Cap Color	Storage Temp
p70 S6 Kinase Capture Rabbit mAb (100X)	68680	400 µl	Pink	+4C
Phospho-p70 S6 Kinase (Thr389) Detection Mouse mAb (100X)	18249	400 µl	Blue	+4C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µl	Yellow	-20C

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

Description

Cell Signaling Technology's PathScan® Phospho-p70 S6 Kinase (Thr389) Sandwich ELISA Antibody Pair is offered as an economical alternative to our PathScan® Phospho-p70 S6 Kinase (Thr389) Sandwich ELISA Kit #7063. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The p70 S6 kinase rabbit capture antibody is coated onto a 96 well microplate overnight in PBS. After blocking, cell lysates are added followed by a phospho-p70 S6 kinase (Thr389) mouse detection antibody and anti-mouse IgG, HRP-linked antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-p70 S6 kinase (Thr389).
Antibodies in kit are custom formulations specific to 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: 1X PBS/0.5% 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).

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

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.
2. Dufner, A. and Thomas, G. (1999) *Exp Cell Res* 253, 100-9.
3. Weng, Q.P. et al. (1998) *J Biol Chem* 273, 16621-9.
4. Pullen, N. et al. (1998) *Science* 279, 707-10.
5. Alessi, D.R. et al. (1998) *Curr Biol* 8, 69-81.
6. Polakiewicz, R.D. et al. (1998) *J Biol Chem* 273, 23534-41.

7. Fingar, D.C. et al. (2002) *Genes Dev* 16, 1472-87.
8. Saitoh, M. et al. (2002) *J Biol Chem* 277, 20104-12.

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ELISA Antibody Pair

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 20X PBS to 950 ml dH_2O, mix.
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**: PathScan® Sandwich ELISA Lysis Buffer (#7018) 1X: This buffer is ready to use as is. 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. **Bovine Serum Albumin (BSA)**: (#9998).
6. **TMB Substrate**: (#7004).
7. **STOP Solution**: (#7002)

   NOTE: Reagents should be made fresh daily.

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 to 1 ml ice-cold PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 2 min.
4. Collect cell lysate in a clean tube.
5. Centrifuge for 10 min (14,000 x g) at 4°C and transfer the supernatant to a new tube. Store supernatant 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 10^6 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 media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Resuspend the cell pellet and incubate the tube on ice for 2 min.
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. Coating Procedure

1. Rinse microplate with 200 µl of dH_2O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hr).
3. **After overnight coating, gently uncover plate and wash wells**:
   1. Discard plate contents into a receptacle.
   2. Wash four times with wash buffer, 200 µl each time per well. 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 completely dry at any time.
   3. 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 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

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 hr.
2. Wash plate (Section C, Step 3).

3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 µl of detection 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 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin 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 min.
6. Wash plate (Section C, Step 3).
7. Add 100 µl of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 µl of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.

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