

**#9430** Store at -20°C

# PhosphoPlus® p70 S6 Kinase (Thr389, Thr421/Ser424) Antibody Kit

✓ 10 western blots



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

rev. 02/06/12

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.

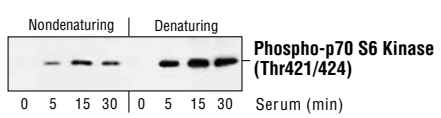
Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb	9234	100 µl	70, 85 kDa	Rabbit IgG
Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody	9204	100 µl	70, 85 kDa	Rabbit IgG
p70 S6 Kinase (49D7) Rabbit mAb	2708	100 µl	70, 85 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked (D5A7) Rabbit mAb	5571	100 µl		Rabbit IgG
Biotinylated Protein Ladder Detection Pack	7727	100 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		
p70 S6 Kinase Cell Extracts	9203	40 µl		

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The PhosphoPlus® p70 S6 Kinase (Thr389, Thr421/Ser424) Antibody Kit provides reagents and protocols for the rapid analysis of p70 S6 kinase phosphorylation at Thr389 and Thr421/Ser424.

**p70 S6 Kinase Control Cell Extracts:** Total cell extracts from MCF7 cells, prepared with or without insulin treatment to serve as positive and negative controls.

**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



Immunoprecipitation of extracts from serum-treated 293 cells under nondenaturing or denaturing conditions, using **Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody #9204**. Western blot was then performed using the same antibody.

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).

**Specificity/Sensitivity:** p70 S6 Kinase (49D7) Rabbit mAb #2708 detects endogenous levels of total p70 S6 Kinase. It also recognizes p85 S6 Kinase. Phospho-p70 S6 Kinase (Thr389) Antibody and Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody detect endogenous levels of p70 S6 Kinase only when phosphorylated at the indicated sites. These antibodies also recognize p85 S6 Kinase when phosphorylated at the corresponding sites, Thr412 or Thr444/Ser447, respectively. Phospho-p70 S6 Kinase (Thr389) Antibody may also detect S6KII phosphorylated at Thr401.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr421/Ser424 of human p70 S6 kinase. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr389 of human p70 S6 Kinase, or corresponding to the sequence of human p70 S6 kinase. Antibodies are purified by Protein A and peptide affinity chromatography.

**Entrez-Gene ID** #6198  
**Swiss-Prot Acc.** #P23443

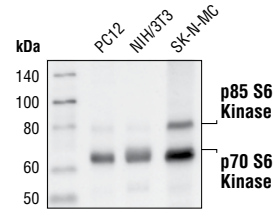
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

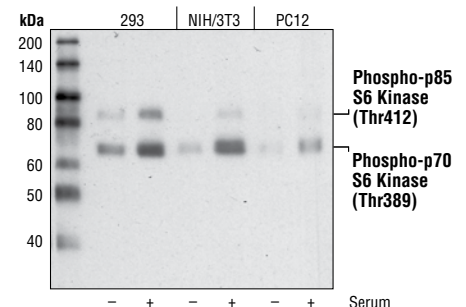
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**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, 100–109.
- (3) Weng, Q.P. et al. (1998) *J. Biol. Chem.* 273, 16621–16629.
- (4) Pullen, N. et al. (1998) *Science* 279, 707–710.
- (5) Alessi, D.R. et al. (1998) *Curr. Biol.* 8, 69–81.
- (6) Polakiewicz, R.D. et al. (1998) *J. Biol. Chem.* 273, 23534–23541.
- (7) Fingar, D.C. et al. (2002) *Genes Dev.* 16, 1472–1487.
- (8) Saitoh, M. et al. (2002) *J. Biol. Chem.* 277, 20104–20112.



Western blot analysis of extracts from PC12, NIH/3T3, and SK-N-MC cells, using **p70 S6 Kinase (49D7) Rabbit mAb #2708**.



Western blot analysis of extracts from serum starved or serum-treated (20%) 293, NIH/3T3 and PC12 cells using **Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb #9234**.

Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

**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.

© 2012 Cell Signaling Technology, Inc. PhosphoPlus® and Cell Signaling Technology® are trademarks of Cell Signaling Technology, Inc.

## Western Immunoblotting Protocol (Primary Antibody Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.