

Phospho-p70 S6 Kinase (Thr389) Antibody



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, Mk	70, 85 kDa	Rabbit**

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).

Specificity/Sensitivity: Phospho-p70 S6 Kinase (Thr389) Antibody detects endogenous levels of p70 S6 kinase only when phosphorylated at threonine 389. This antibody also detects p85 S6 kinase when phosphorylated at the analogous site (Thr412), and possibly S6KII phosphorylated at Thr388.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Thr389 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.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

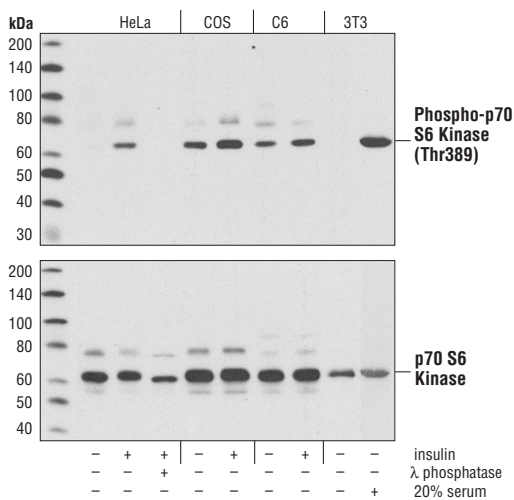
Western Blotting 1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit 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) Dunfer, A. et al. (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. et al. (1998) *Curr. Biol.* 8, 69–81.
- (6) Polakiewicz, R.D. et al. (1998) *J. Biol. Chem.* 273, 23534–23541.
- (7) Finger, 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 HeLa, COS, C6 and 3T3 cells, serum-starved overnight, then treated with insulin, λ-phosphatase or 20% serum as indicated. Upper panel probed with Phospho-p70 S6 Kinase (Thr389) Antibody #9205; lower panel probed with p70 S6 Kinase Antibody #9202.

IMPORTANT: 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.

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.