#9204 Store at –20°C

Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody

Cell Signaling TECHNOLOGY®

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

rev. 09/27/17

For Research Use Only. Not For Use In Diagnostic Procedures.

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

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

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
Immunoprecipitation	1:50

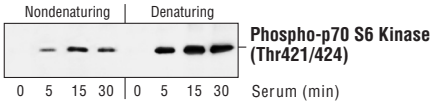
For application specific protocols please see the web page for this product at www.cellsignal.com.

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

Background: 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 (Thr421/Ser424) Antibody detects endogenous levels of p70 S6 kinase only when phosphorylated at Thr421/Ser424. This antibody also detects p85 S6 kinase when phosphorylated at the corresponding sites (Thr444/Ser447).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr421/Ser424 of human p70 S6 kinase. Antibodies are purified by protein A and peptide affinity chromatography.



Immunoprecipitation of phosphorylated p70 S6 Kinase from 293 cell extracts (cells were serum-stimulated as indicated) under nondenaturing or denaturing conditions, followed by Western blot analysis, using Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody.

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.

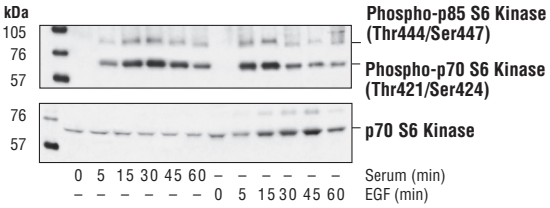
(4) Pullen, N. et al. (1998) *Science* 279, 707–710.

(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 extracts from 293 cells, untreated, serum-treated or EGF-treated (100 ng/ml), using Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody (upper) or p70 S6 Kinase Antibody #9202 (lower).

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.