

p70 S6 Kinase Control Cell Extracts

✓ Controls for 10 Western mini-blot

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

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 Thr229 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).

Description: Nonphosphorylated p70 S6 Kinase Control Cell Extracts: Total cell extracts from NIH/3T3 cells, prepared without treatment, serve as a negative control. Supplied in SDS Sample Buffer. Phosphorylated p70 S6 Kinase Control Cell Extracts: Total cell extracts from NIH/3T3 cells, prepared with serum treatment, serve as a positive control. Supplied in SDS Sample Buffer.

Applications: As controls, CST recommends using 20 µl of phosphorylated and nonphosphorylated p70 S6 kinase cell extracts. Boil sample before use.

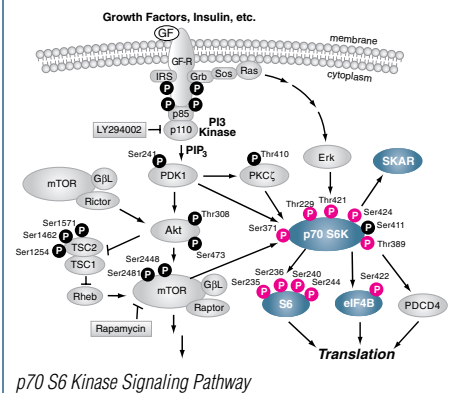
Background References:

- (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.

Storage: Supplied in 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.

Companion Products:

- p70 S6 Kinase Antibody #9202
Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody #9204
Phospho-p70 S6 Kinase (Thr389) Antibody #9205
PhosphoPlus® p70 S6 Kinase (Thr389, Thr421/Ser424) Antibody Kit #9430
Anti-rabbit IgG, HRP-linked Antibody #7074
Anti-mouse IgG, HRP-linked Antibody #7076
Prestained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder Detection Pack #7727
20X LumiGLO® Reagent and 20X Peroxide #7003
Phospho-p70 S6 Kinase (Thr389) (1A5) Mouse mAb #9206
Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb #9234
Phospho-p70 S6 Kinase (Ser371) Antibody #9208
p70 S6 Kinase (49D7) Rabbit mAb #2708



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 IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFI[®]
Z—zebra fish B—bovine All—all species expected