Store at -20°C

#9203

p70 S6 Kinase Control Cell Extracts

☑ Controls for 10 western blots

Cell Signaling TECHNOLOGY®

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

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).

Description: Nonphosphorylated p70 S6 Kinase Control Cell Extracts: Total cell extracts from MCF-7 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 MCF-7 cells, prepared with insulin treatment, serve as a positive control. Supplied in SDS Sample Buffer.

Applications: As controls, CST recommends using 10 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.

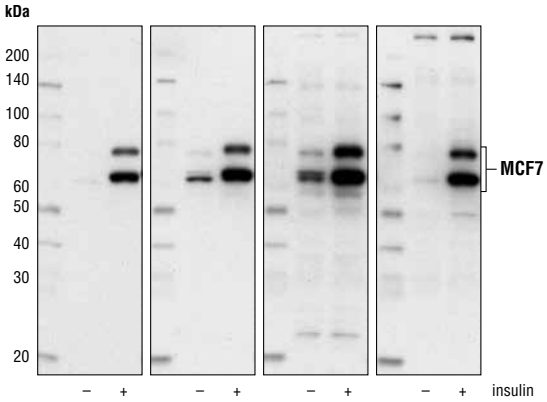
(4) Pullen, N. et al. (1998) *Science* 279, 707–710.

(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.



Western blot analysis of #9203 p70 Control Cell Extracts (MCF7-/+Insulin) using #9205 Phospho-p70 S6 Kinase (Thr389) Antibody, #9234 Phospho-p70 S6 Kinase (Thr389)(108D2) Rabbit mAb, #9204 Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody and #9206 Phospho-p70 S6 Kinase (Thr389)(1A5) Mouse mAb.

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

p70 S6 Kinase Signaling Pathway

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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.