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p70 S6 Kinase MCF7 Control Cell Extracts

Cell Signaling
TECHNOLOGY®

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100 μl (10 western blots)

rev. 05/13/21

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

Product Includes	Product #	Quantity
p70 S6 Kinase MCF7 Control Cell Extracts (untreated)	39581	100 ul
p70 S6 Kinase MCF7 Control Cell Extracts (+hIGF-1)	50330	100 ul

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-proteincoupled 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 MCF7 cells, serumstarved overnight to serve as a negative control. Supplied in SDS Sample Buffer.

Phosphorylated p70 S6 Kinase Control Cell Extracts: Total cell extracts from MCF7 cells, serum-starved overnight and treated 100 ng/ml hIGF-1 #8917 for 10 min to serve as a positive control. Supplied in SDS Sample Buffer.

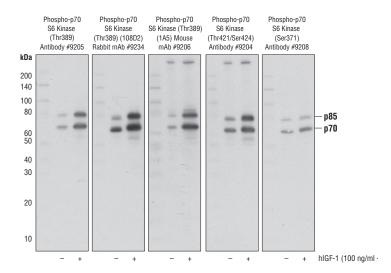
Directions for Use: Boil for 3 minutes prior to use. Load 10 µl of phosphorylated and nonphosphorylated p70 MCF7 Control Cell Extracts per lane.

Storage: Store at -20°C. 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.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

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 Non-phosphorylated p70 MCF7 Control Cell Extracts (-) and Phosphorylated p70 MCF7 Control Cell Extracts (+), using the indicated antibodies.

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