Revision 3

Store at -20C

#34499

p70 S6 Kinase MCF7 Control Cell Extracts

Controls for 10 western blots

Cell Signaling TECHNOLOGY®

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

Description

Nonphosphorylated p70 S6 Kinase Control Cell Extracts: Total cell extracts from MCF7 cells, serum-starved 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.

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

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).

Directions for Use

Boil for 3 minutes prior to use. Load 10 µl of phosphorylated and nonphosphorylated p70 S6 Kinase MCF7 Control Cell Extracts per lane.

Background References

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4. Pullen, N. et al. (1998) *Science* 279, 707-10.
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7. Fingar, D.C. et al. (2002) *Genes Dev* 16, 1472-87.
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