SignalSilence® p70/85 S6 Kinase siRNA II

#6572 Store at -20°C

☑ 10 µM in 300 µl
(100 transfections)

Cell Signaling TECHNOLOGY®

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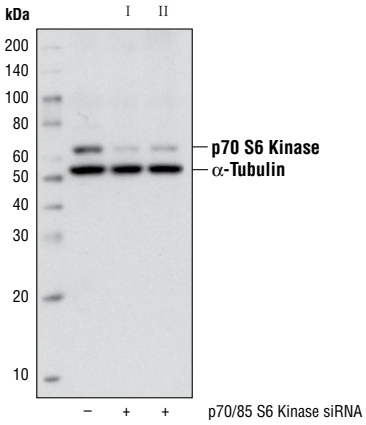
For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, (R, Mk)

Description: SignalSilence® p70/85 S6 Kinase siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit p70/85 S6 kinase expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

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: CST recommends transfection with 100 nM p70/85 S6 Kinase siRNA II 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® p70/85 S6 Kinase siRNA I #6566 (+) or SignalSilence® p70/85 S6 Kinase siRNA II (+), using p70 S6 Kinase (49D7) Rabbit mAb #2708 and α-Tubulin (11H10) Rabbit mAb #2125. The p70 S6 Kinase (49D7) Rabbit mAb confirms silencing of p70/85 S6 kinase expression, while the α-Tubulin (11H10) Rabbit mAb is used to control for loading and specificity of p70/85 S6 kinase siRNA.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Specificity/ Sensitivity: p70/85 S6 Kinase siRNA II will inhibit human, rat and monkey p70/85 S6 Kinase expression.

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

Storage: p70/85 S6 Kinase siRNA II is supplied in RNAse-free water. Aliquot and store at -20°C.

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

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.

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.