#12514 Store at -20°C

SignalSilence® Skp1 siRNA I

☑ 10 µM in 300 µl (3 nmol)

Cell Signaling TECHNOLOGY®

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New 01/13

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

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

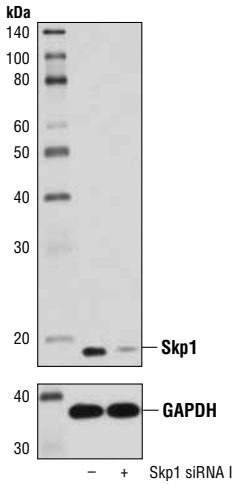
Description: SignalSilence® Skp1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Skp1 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: Ubiquitin can be covalently linked to many cellular proteins by the ubiquitination process, which targets proteins for degradation by the 26S proteasome. Three components are involved in the target protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thiolester complex with the activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2 and then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH_2 of the target protein lysine residue (1-3). Combinatorial interactions of different E2 and E3 proteins result in substrate specificity (4). Recent data suggests that activated E2 associates transiently with E3, and the dissociation is a critical step for ubiquitination (5). S phase kinase-associated protein 1 (Skp1) is a critical scaffold protein of the Skp1/CUL1/F-box (SCF) E3 ubiquitin ligase protein complex. Various F-box proteins (e.g., β-TrCP, Skp2) mediate an interaction with Skp1, via their defining and conserved domain of 40 amino acids, and with substrates to be ubiquitinated (e.g., β-catenin, p27) (4).

Specificity/Sensitivity: SignalSilence® Skp1 siRNA I inhibits human, mouse, rat, and monkey Skp1 expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Skp1 siRNA I 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.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Skp1 siRNA I (+), using Skp1 (D3J4N) Rabbit mAb #12248 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The Skp1 (D3J4N) Rabbit mAb confirms silencing of Skp1 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

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.

Entrez-Gene ID #6500
Swiss-Prot Acc. #P63208

Storage: Skp1 siRNA I 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) Ciechanover, A. (1998) *EMBO J.* 17, 7151-60.

(2) Hochstrasser, M. (2000) *Nat. Cell Biol.* 2, E153-7.

(3) Hochstrasser, M. (2000) *Science* 289, 563-4.

(4) DeSalle, L.M. and Pagano, M. (2001) *FEBS Lett.* 490, 179-89.

(5) Deffenbaugh, A.E. et al. (2003) *Cell* 114, 611-22.

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