Store at -20°C

#14183

SignalSilence® AUP1 siRNA II

www.cellsignal.com

Support: 877-678-TECH (8324)
info@cellsignal.com

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New 07/14

Entrez-Gene ID #550
UniProt ID #Q9Y679

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

Species Cross-Reactivity: H

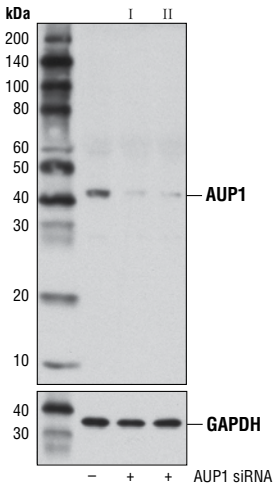
Description: SignalSilence® AUP1 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit AUP1 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: Ancient ubiquitous protein 1 (AUP1) is a component of the ER-associated protein degradation (ERAD) machinery responsible for the ubiquitin-mediated degradation of misfolded proteins (1). AUP1 protein contains four conserved domains, with a long, amino-terminal stretch of hydrophobic amino acids followed by an acyltransferase domain (2). Amino-terminal protein sequences direct localization of AUP1 to both the ER and to cytosolic lipid droplets (3). The AUP1 CUE domain binds ubiquitin (4), while the G2BR domain allows for association between AUP1 and E2 conjugating enzyme UBE2G2 (5,6). The presence of these binding domains suggests a central role for AUP1 in the ubiquitination-mediated protein degradation (2). Research studies indicate that AUP1 recruits UBE2G2 to cytosolic lipid droplets, ER-derived organelles that are sites for storage and hydrolysis of neutral lipids. Inhibition of AUP1 protein function results in decreased ubiquitin-mediated degradation of several proteins, including the cholesterol biosynthetic enzyme HMG-CoA-reductase and the cholesterol synthesis regulator INSIG1 (6).

Directions for Use: CST recommends transfection with 100 nM SignalSilence® AUP1 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.

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.

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.



Western blot analysis of extracts from 293 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® AUP1 siRNA I #14176 (+), or SignalSilence® AUP1 siRNA II (+), using AUP1 Antibody #14035 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The AUP1 Antibody confirms silencing of AUP1 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Storage: AUP1 siRNA II is supplied in RNAse-free water. *Aliquot and store at -20°C.*

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) Mueller, B. et al. (2008) *Proc Natl Acad Sci USA* 105, 12325-30.

(2) Spandl, J. et al. (2011) *J Biol Chem* 286, 5599-606.

(3) Stevanovic, A. and Thiele, C. (2013) *J Lipid Res* 54, 503-13.

(4) Lohmann, D. et al. (2013) *PLoS One* 8, e72453.

(5) Klemm, E.J. et al. (2011) *J Biol Chem* 286, 37602-14.

(6) Jo, Y. et al. (2013) *Mol Biol Cell* 24, 169-83.

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Cell Signaling TECHNOLOGY®

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.