# SignalSilence® UBE2S siRNA II

10 μM in 300 μl (100 transfections)

rev. 02/25/16



### Species Cross-Reactivity: H

Description: SignalSilence<sup>®</sup> UBE2S siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit UBE2S 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<sup>®</sup> siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** Protein ubiquitination requires the concerted action of the E1, E2, and E3 ubiquitin-conjugating enzymes. Ubiquitin is first activated through ATP-dependent formation of a thiol ester with ubiquitin-activating enzyme E1. The activated ubiquitin is then transferred to a thiol group of ubiquitin-carrier enzyme E2. The final step is the transfer of ubiquitin from E2 to an  $\epsilon$ -amino group of the target protein lysine residue, which is mediated by ubiquitin-ligase enzyme E3 (1).

The human anaphase promoting complex (APC/C) is a large macromolecular E3 ligase complex that is largely responsible for the timely progression through mitosis via the sequential targeting of cell cycle regulators for proteasomal degradation. Recent work has revealed that APC/C substrates are marked for proteasomal degradation during cell cycle progression through the covalent assembly of Lys11-linked ubiquitin chains, which occurs through a priming phase and elongation phase (2-5). The APC/C utilizes, in part, the UBE2C/UBCH10 E2 enzyme to prime

substrates for degradation through the covalent attachment of short Lys11-linked chains (3,6). The Lys11-specific elongating E2 enzyme, UBE2S/E2-EPF, extends these short chains into long Lys11-linked ubiquitin chains on APC/C bound substrates (2,3,7).

In addition to the well-established biochemical role for UBE2S in cell cycle regulation, this enzyme has been shown to be overexpressed in many types of human cancer (8) and has also been implicated in hypoxia signaling (9,10). Indeed, UBE2S has been reported to associate with VHL and to target it for proteasomal degradation, thereby stabilizing HIF-1 $\alpha$  (9).



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® UBE2S siRNA I #7220 (+), or SignalSilence® UBE2S siRNA II (+), using UBE2S Antibody #9630 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The UBE2S Antibody confirms silencing of UBE2S expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Directions for Use: CST recommends transfection with 100 nM SignalSilence<sup>®</sup> UBE2S 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.

**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.



 Orders

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#### Entrez-Gene ID #27338 Swiss-Prot Acc. #Q16763

**Storage:** UBE2S 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 complementary products.

#### **Background References:**

- (1) Hershko, A. (1988) J. Biol. Chem. 263, 15237-15240.
- (2) Williamson, A. et al. (2009) *Proc Natl Acad Sci USA* 106, 18213-8.
- (3) Jin, L. et al. (2008) Cell 133, 653-65.
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- (5) Song, L. and Rape, M. (2010) Mol Cell 38, 369-82.
- (6) Summers, M.K. et al. (2008) Mol Cell 31, 544-56.
- (7) Wickliffe, K.E. et al. (2011) Cell 144, 769-81.
- (8) Tedesco, D. et al. (2007) Neoplasia 9, 601-13.
- (9) Jung, C.R. et al. (2006) Nat Med 12, 809-16.

 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
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.