

#7339 Store at -20°C

# SignalSilence® UCHL3 siRNA II



✓ 10 µM in 300 µl (100 Transfections)

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

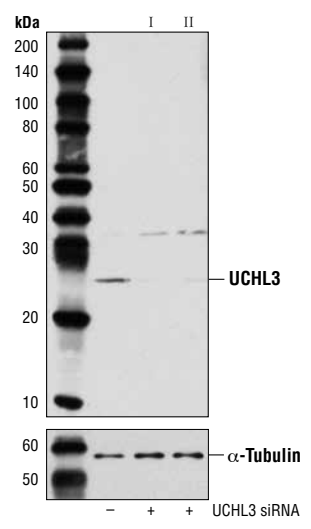
### Species Cross-Reactivity: H

**Description:** SignalSilence® UCHL3 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit UCHL3 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:** Protein ubiquitination and deubiquitination are reversible processes catalyzed by ubiquitinating enzymes (UBEs) and deubiquitinating enzymes (DUBs) (1,2). DUBs are categorized into 5 subfamilies: USP, UCH, OTU, MJD, and JAMM. UCHL1, UCHL3, UCHL5/UCH37, and BRCA-1-associated protein-1 (BAP1) belong to the UCH family of DUBs, which all possess a conserved catalytic domain (UCH domain) of about 230 amino acids. UCHL5 and BAP1 have unique extended C-terminal tails. UCHL1 is abundantly expressed in neuronal tissues and testes, while UCHL3 expression is more widely distributed (3,4). Although UCHL1 and UCHL3 are the most closely related UCH family members with about 53% identity, their biochemical properties differ in that UCHL1 binds monoubiquitin and UCHL3 shows dual specificity toward both ubiquitin (Ub) and NEDD8, a Ub-like molecule. In particular, UCHL3 functions as a Ub hydrolase involved in the processing of both Ub precursors and ubiquitinated substrates, generating free monomeric Ub. This is accomplished through the ability of UCHL3 to recognize and hydrolyze isopeptide bonds at the C-terminal glycine of either Ub or NEDD8 (5-7). Recent functional studies have identified UCHL3 as a critical regulator of adipogenesis through its ability to promote IGF-IR and insulin receptor signaling (8). Furthermore, UCHL3 has been shown to promote deubiquitination, recycling, and cell surface expression of the epithelial sodium channel (9).

**Directions for Use:** CST recommends transfection with 100 nM SignalSilence® UCHL3 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.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® UCHL3 siRNA I #7511 (+) or SignalSilence® UCHL3 siRNA II (+), using UCHL3 Antibody #3525 (upper) or  $\alpha$ -Tubulin (11H10) Rabbit mAb #2125 (lower). The UCHL3 Antibody confirms silencing of UCHL3 expression, while the  $\alpha$ -Tubulin (11H10) Rabbit mAb is used as a loading control.

Entrez-Gene ID #7347  
Swiss-Prot Acc. #P15374

**Storage:** UCHL3 siRNA II is supplied in RNase-free water. Aliquot and store at -20°C.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

### Background References:

- (1) Nijman, S.M. et al. (2005) *Cell* 123, 773-86.
- (2) Nalepa, G. et al. (2006) *Nat Rev Drug Discov* 5, 596-613.
- (3) Leroy, E. et al. (1998) *Nature* 395, 451-2.
- (4) Kurihara, L.J. et al. (2001) *Hum Mol Genet* 10, 1963-70.
- (5) Osaka, H. et al. (2003) *Hum Mol Genet* 12, 1945-58.
- (6) Wada, H. et al. (1998) *Biochem Biophys Res Commun* 251, 688-92.
- (7) Kwon, J. (2007) *Exp Anim* 56, 71-7.
- (8) Suzuki, M. et al. (2009) *Endocrinology* 150, 5230-9.
- (9) Butterworth, M.B. et al. (2007) *J Biol Chem* 282, 37885-93.

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**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.