

SignalSilence® UCHL1 siRNA I

✓ 10 µM in 300 µl (3 nmol)

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New 11/12

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

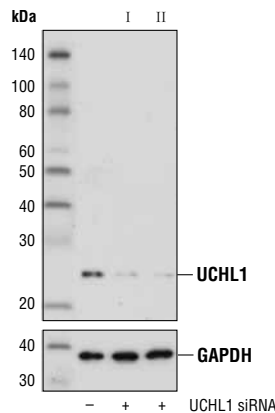
Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® UCHL1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit UCHL1 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 Ubiquitin C-terminal Hydrolase (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.

UCHL1 (PGP 9.5/PARK5) functions as a deubiquitinating enzyme and monoubiquitin stabilizer. *In vitro* studies have demonstrated that UCHL1 can hydrolyze isopeptide bonds between the C-terminal glycine of Ub and the ε-amino group of lysine on target proteins. UCHL1 is also involved in the cotranslational processing of pro-ubiquitin and ribosomal proteins translated as ubiquitin fusions (5-7). Mice deficient in UCHL1 experience spasticity, suggesting that UCHL1 activity is required for the normal structure and function of the neuromuscular junction (5-7). Research studies have implicated loss of UCHL1 expression in numerous human malignancies, such as prostate, colorectal, renal, and breast carcinomas. Investigators have shown that in breast carcinomas, loss of UCHL1 expression can be attributed to hyper-methylation of the *UCHL1* promoter (8). While loss of UCHL1 expression is implicated in human carcinogenesis, mutation of UCHL1 has been implicated in neurodegenerative diseases such as Parkinson's and Alzheimer's (6,7).

Specificity/Sensitivity: SignalSilence® UCHL1 siRNA I inhibits human and monkey UCHL1 expression.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® UCHL1 siRNA I (+), or SignalSilence® UCHL1 siRNA II #12415 (+), using UCHL1 (D8R2I) XP® Rabbit mAb #11896 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The UCHL1 (D8R2I) XP® Rabbit mAb confirms silencing of UCHL1 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® UCHL1 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.

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 #7345
Swiss-Prot Acc. #P09936

Storage: UCHL1 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) 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) Todi, S.V. and Paulson, H.L. (2011) *Trends Neurosci*, 34, 370-382.
- (6) Setsuie, R. and Wada, K. *Neurochem Int* 51, 105-11.
- (7) Day, I.N. and Thompson, R.J. (2010) *Prog Neurobiol* 90, 327-62.
- (8) Xiang, T. et al. (2012) *PLoS One* 7, e29783.