SignalSilence® ULK1 siRNA I

#7000 Store at -20°C

☑ 10 µM in 300 µl (3 nmol)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355) orders@cellsignal.com
Support ■ 877-678-TECH (8324) info@cellsignal.com
Web ■ www.cellsignal.com

rev. 02/17/16

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

Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® ULK1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit ULK1 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.

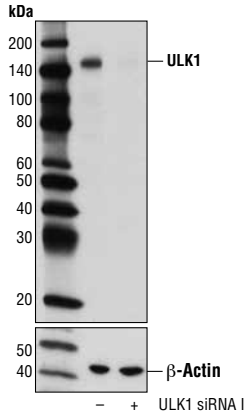
Background: Two related serine/threonine kinases, UNC-51-like kinase 1 and 2 (ULK1, ULK2), were discovered as mammalian homologs of the *C. elegans* gene UNC-51 in which mutants exhibited abnormal axonal extension and growth (1-4). Both proteins are widely expressed and contain an amino-terminal kinase domain followed by a central proline/serine rich domain and a highly conserved carboxy-terminal domain. The roles of ULK1 and ULK2 in axon growth have been linked to studies showing that the kinases are localized to neuronal growth cones and are involved in endocytosis of critical growth factors, such as NGF (5). Yeast two-hybrid studies found ULK1/2 associated with modulators of the endocytic pathway, SynGap and syntenin (6). Structural similarity of ULK1/2 has also been recognized with the yeast autophagy protein Atg1/Apg1 (7). Knockdown experiments using siRNA demonstrated that ULK1 is essential for autophagy (8), a catabolic process for the degradation of bulk cytoplasmic contents (9,10). It appears that Atg1/ULK1 can act as a convergence point for multiple signals that control autophagy (11), and can bind to several autophagy-related (Atg) proteins, regulating phosphorylation states and protein trafficking (12-16).

AMPK, activated during low nutrient conditions, directly phosphorylates ULK1 at multiple sites including Ser317, Ser555, and Ser777 (17,18). Conversely, mTOR, which is a regulator of cell growth and an inhibitor of autophagy, phosphorylates ULK1 at Ser757 and disrupts the interaction between ULK1 and AMPK (17).

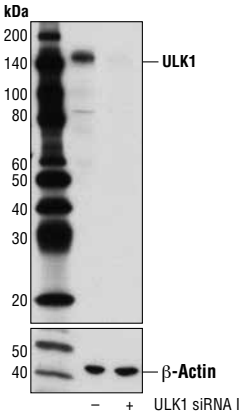
Specificity/Sensitivity: SignalSilence® ULK1 siRNA I inhibits human and monkey ULK1 expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® ULK1 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 RD cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® ULK1 siRNA I (+), using ULK1 (D9D7) Rabbit mAb #6439 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The ULK1 (D9D7) Rabbit mAb confirms silencing of ULK1 expression, while the β-Actin (D6A8) 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 #8408
Swiss-Prot Acc. #O75385

Storage: ULK1 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) Ogura, K. et al. (1994) *Genes Dev* 8, 2389-400.
(2) Kuroyanagi, H. et al. (1998) *Genomics* 51, 76-85.
(3) Yan, J. et al. (1998) *Biochem Biophys Res Commun* 246, 222-7.
(4) Yan, J. et al. (1999) *Oncogene* 18, 5850-9.
(5) Zhou, X. et al. (2007) *Proc Natl Acad Sci USA* 104, 5842-7.
(6) Tomoda, T. et al. (2004) *Genes Dev* 18, 541-58.
(7) Matsuura, A. et al. (1997) *Gene* 192, 245-50.
(8) Chan, E.Y. et al. (2007) *J Biol Chem* 282, 25464-74.
(9) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot Cell* 1, 11-21.
(10) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ* 12 Suppl 2, 1509-18.
(11) Stephan, J.S. and Herman, P.K. (2006) *Autophagy* 2, 146-8.
(12) Okazaki, N. et al. (2000) *Brain Res Mol Brain Res* 85, 1-12.
(13) Young, A.R. et al. (2006) *J Cell Sci* 119, 3888-900.
(14) Kamada, Y. et al. (2000) *J Cell Biol* 150, 1507-13.
(15) Lee, S.B. et al. (2007) *EMBO Rep* 8, 360-5.
(16) Hara, T. et al. (2008) *J Cell Biol* 181, 497-510.
(17) Kim, J. et al. (2011) *Nat Cell Biol* 13, 132-41.
(18) Egan, D.F. et al. (2011) *Science* 331, 456-61.

◀ *Western blot analysis of extracts from RD cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® ULK1 siRNA I (+), using ULK1 (D8H5) Rabbit mAb #8054 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The ULK1 (D8H5) Rabbit mAb confirms silencing of ULK1 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.*

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.