SignalSilence® Atg4A siRNA I

#6427 Store at -20°C

☑ 10 µM in 300 µl
(100 transfections)

Cell Signaling TECHNOLOGY®

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

rev. 02/10/16

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

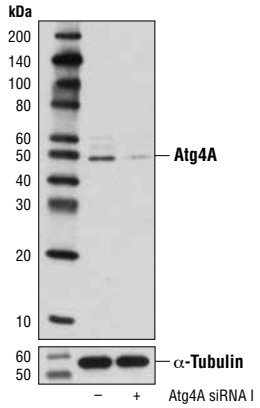
Species Cross-Reactivity: H

Description: SignalSilence® Atg4A siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Atg4A 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: Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents. Control of autophagy was largely discovered in yeast and involves proteins encoded by a set of autophagy-related genes (Atg) (1). Formation of autophagic vesicles requires a pair of essential ubiquitin-like conjugation systems, Atg12-Atg5 and Atg8-phosphatidylethanolamine (Atg8-PE), which are widely conserved in eukaryotes (2). Numerous mammalian counterparts to yeast Atg proteins have been described, including three Atg8 proteins (GATE-16, GABARAP, and LC3) and four Atg4 homologues (Atg4A/autophagin-2, Atg4B/autophagin-1, Atg4C/autophagin-3, and Atg4D/autophagin-4) (3-5). The cysteine protease Atg4 is pivotal to autophagosome membrane generation and regulation. Atg4 primes the Atg8 homologue for lipidation by cleaving its carboxy terminus and exposing its glycine residue for E1-like enzyme Atg7. The Atg8 homologue is transferred to the E2-like enzyme Atg3 before forming the Atg8-PE conjugate. During later stages of autophagy, Atg4 can reverse this lipidation event by cleaving PE, thereby recycling the Atg8 homologue (6).

Directions for Use: CST recommends transfection with 100 nM Atg4A 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.

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 HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Atg4A siRNA I (+), using Atg4A (D62C10) Rabbit mAb #7613 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The Atg4A (D62C10) Rabbit mAb confirms silencing of Atg4A expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.

Entrez-Gene ID #115201
Swiss-Prot Acc. #Q8WYN0

Storage: Atg4A siRNA I is supplied in RNAse-free water. *Aliquot and store at -20ºC.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot Cell* 1, 11-21.

(2) Ohsumi, Y. (2001) *Nat Rev Mol Cell Biol* 2, 211-6.

(3) Kabeya, Y. et al. (2000) *EMBO J* 19, 5720-8.

(4) Kabeya, Y. et al. (2004) *J Cell Sci* 117, 2805-12.

(5) Mariño, G. et al. (2003) *J Biol Chem* 278, 3671-8.

(6) Sou, Y.S. et al. (2008) *Mol Biol Cell* 19, 4762-75.

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.