

#6325 Store at -20°C

# SignalSilence® Atg4C siRNA I



✓ 10 µM in 300 µl (100 transfections)

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

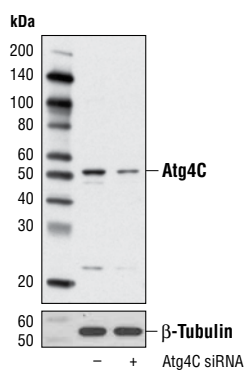
**Description:** SignalSilence® Atg4C siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Atg4C expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All 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 autophagosomal-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 carboxyl-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 and recycle the Atg8 homologue (6).

Atg4C-deficient mice display a tissue-specific decrease in LC3 lipidation only when under stressful conditions such as prolonged starvation. Mutant mice also exhibit increased susceptibility to the development of chemical carcinogen induced fibrosarcomas suggesting that Atg4C may contribute to events associated with tumor progression (7).

**Directions for Use:** CST recommends transfection with 100 nM Atg4C 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® Atg4C siRNA I (+), using Atg4C Antibody #5262 (upper) or β-Tubulin (9F3) Rabbit mAb #2128 (lower). The Atg4C Antibody confirms silencing of Atg4C expression, while the β-Tubulin (9F3) Rabbit mAb is used as a loading control.

Entrez-Gene ID #84938  
Swiss-Prot Acc. #Q96DT6

**Storage:** Atg4C siRNA I 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) 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.
- (7) Mariño, G. et al. (2007) *J Biol Chem* 282, 18573-83.

© 2010 Cell Signaling Technology, Inc.

**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.