## SignalSilence® Atg5 siRNA I

 10 μM in 300 μl (100 transfections)

rev. 02/10/16



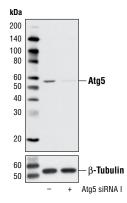
## Species Cross-Reactivity: H

**Description:** SignalSilence<sup>®</sup> Atg5 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Atg5 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<sup>®</sup> siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

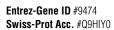
**Background:** Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, neurodegeneration, infection and cancer (3). The molecular machinery of autophagy was largely discovered in yeast and referred to as **aut**ophagy-related (Atg) genes. Formation of the autophagosome involves an ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles (4-6). This conjugation reaction is mediated by the ubiquitin-E1-like enzyme Atg7 and the E2-like enzyme Atg10 (7,8).

**Directions for Use:** CST recommends transfection with 100 nM Atg5 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® Atg5 siRNA I (+), using Atg5 Antibody #2630 (upper) or  $\beta$ -Tubulin (9F3) Rabbit mAb #2128 (lower). The Atg5 Antibody confirms silencing of Atg5 expression, while the  $\beta$ -Tubulin (9F3) Rabbit mAb is used as a loading control.



**Storage:** Atg5 siRNA I is supplied in RNAse-free water. Aliquot and store at -20°C.

Cell Signaling

Orders 877-616-CELL (2355)

Support 877-678-TECH (8324)

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## **Background References:**

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

- (2) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ* 12 Suppl 2, 1509-18.
- (3) Levine, B. and Yuan, J. (2005) J Clin Invest 115, 2679-88.
- (4) Mizushima, N. et al. (1998) J Biol Chem 273, 33889-92.

(5) Mizushima, N. et al. (1998) Nature 395, 395-8.

- (6) Suzuki, K. et al. (2001) *EMBO J* 20, 5971-81.
- (7) Tanida, I. et al. (1999) Mol Biol Cell 10, 1367-79.
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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
 Se—S. cerevisiae
 Ce—C. elegans
 Hr—horse
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.