

SignalSilence® Atg9A siRNA I



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

✓ 10 µM in 300 µl
(3 nmol)

Species Cross-Reactivity: H, (M, R, Mk)

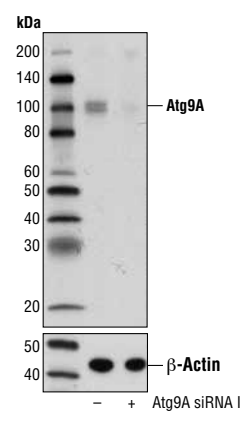
Description: SignalSilence® Atg9A siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Atg9A expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® 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 (1,2). It is generally activated by conditions of nutrient deprivation but is also 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 is directed by a number of autophagy-related (Atg) genes (4).

Atg9, one of the Atg proteins identified in yeast, is essential for autophagosome formation (5). There are two human functional orthologues based on the yeast homolog Atg9p: Atg9A, which has also been identified as Atg9L1 and mAtg9, and Atg9L2, which was first reported as nitric-oxide synthase 3 antisense (NOS3AS) (6,7). Atg9A is an integral membrane protein that is required for both the initiation and the expansion of the autophagosome (6,7). Recruitment of Atg9A to the autophagosomal membrane is dynamic and transient as Atg9A also cycles between autophagy-related structures known as omegasomes, the trans-Golgi network (TGN), and endosomes, and at no point becomes a stable component of the autophagosomal membrane (6,8). The precise regulation of Atg9A trafficking is not fully clarified, yet it is suggested to involve p38 mitogen-activated protein kinase (MAPK)-binding protein p38IP and the Beclin-1-binding protein Bif-1 (9,10).

Specificity/Sensitivity: SignalSilence® Atg9A siRNA I inhibits human, mouse, rat, and monkey Atg9A expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Atg9A 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® Atg9A siRNA I (+), using Atg9A Antibody #9730 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The Atg9A Antibody confirms silencing of Atg9A 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 #79065
Swiss-Prot Acc. #Q7Z3C6

Storage: Atg9A 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) 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) Klionsky, D.J. et al. (2003) *Dev Cell* 5, 539-45.
- (5) Noda, T. et al. (2000) *J Cell Biol* 148, 465-80.
- (6) Young, A.R. et al. (2006) *J Cell Sci* 119, 3888-900.
- (7) Yamada, T. et al. (2005) *J Biol Chem* 280, 18283-90.
- (8) Orsi, A. et al. (2012) *Mol Biol Cell* 23, 1860-73.
- (9) Webber, J.L. and Tooze, S.A. (2010) *EMBO J* 29, 27-40.
- (10) Takahashi, Y. et al. (2011) *Autophagy* 7, 61-73.