

SignalSilence® Atg13 siRNA I

✓ 10 µM in 300 µl (3 nmol)

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New 10/12

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

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

Description: SignalSilence® Atg13 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Atg13 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.

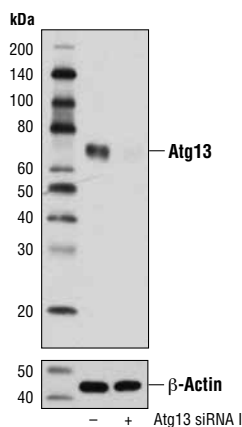
Background: Autophagy is a catabolic process for the autophagosomal-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation, but has also been associated with a number of physiological processes including development, differentiation, neurodegeneration, infection, and cancer (3). The molecular machinery of autophagy was largely discovered in yeast and referred to as autophagy-related (Atg) genes.

Atg13/Apg13 was originally identified in yeast as a constitutively expressed protein that was genetically linked to Atg1/Apg1, a protein kinase required for autophagy (4). Over-expression of Atg1 suppresses the defects in autophagy observed in Atg13 mutants (4). Autophagy requires a direct association between Atg1 and Atg13, and is inhibited by TOR-dependent phosphorylation of Atg13 under high nutrient conditions (5). Similarly, mammalian Atg13 forms a complex with the Atg1 homologues ULK1/2, along with FIP200, localizes to autophagic isolation membranes, and regulates autophagosome biogenesis (6-8). mTOR phosphorylates both Atg13 and ULK1, suppressing ULK1 kinase activity and autophagy (7-9). ULK1 can directly phosphorylate Atg13 at a yet unidentified site, presumably to promote autophagy (7,8). Additional studies suggest that Atg13 and FIP200 can function independently of ULK1 and ULK2 to induce autophagy through an unknown mechanism (10).

Specificity/Sensitivity: SignalSilence® Atg13 siRNA I inhibits human, mouse, and monkey Atg13 expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Atg13 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® Atg13 siRNA I (+), using Atg13 Antibody #6940 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The Atg13 Antibody confirms silencing of Atg13 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 #9776
Swiss-Prot Acc. #075143

Storage: Atg13 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) Funakoshi, T. et al. (1997) *Gene* 192, 207-13.
- (5) Kamada, Y. et al. (2000) *J Cell Biol* 150, 1507-13.
- (6) Ganley, I.G. et al. (2009) *J Biol Chem* 284, 12297-305.
- (7) Hosokawa, N. et al. (2009) *Mol Biol Cell* 20, 1981-91.
- (8) Jung, C.H. et al. (2009) *Mol Biol Cell* 20, 1992-2003.
- (9) Kim, J. et al. (2011) *Nat Cell Biol* 13, 132-41.
- (10) Alers, S. et al. (2011) *Autophagy* 7, 1423-33.