

# Atg9a siRNA (h): sc-72586

## BACKGROUND

Autophagy, a process that results in the lysosomal-dependent degradation of cytosolic compartments, is carried out by the autophagosome, which is a double-membrane vesicle whose formation is catalyzed by several autophagy-related gene (Atg) proteins. Atg9a (autophagy-related protein 9A), also known as APG9-like 1, is a 839 amino acid, multi-pass membrane protein that localizes to the pre-autophagosomal structure (PAS). Isolation membranes are suggested to originate from the PAS, enveloping cytoplasmic components to form a double membrane autophagosome, which then fuses with the vacuole. Ubiquitously expressed in human adult tissues, Atg9a cycles between the Golgi and endosomes and, with the autophagosome-specific marker LC3, plays a critical role in starvation-induced autophagosome formation. Three isoforms of Atg9a exist as a result of alternative splicing events.

## REFERENCES

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2. Young, A.R., et al. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* 119: 3888-3900.
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4. He, C., et al. 2007. Atg9 trafficking in autophagy-related pathways. *Autophagy* 3: 271-274.
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9. Korolchuk, V.I., et al. 2009. A novel link between autophagy and the ubiquitin-proteasome system. *Autophagy* 5: 862-863.

## CHROMOSOMAL LOCATION

Genetic locus: ATG9A (human) mapping to 2q35.

## PRODUCT

Atg9a siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Atg9a shRNA Plasmid (h): sc-72586-SH and Atg9a shRNA (h) Lentiviral Particles: sc-72586-V as alternate gene silencing products.

For independent verification of Atg9a (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72586A, sc-72586B and sc-72586C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Atg9a siRNA (h) is recommended for the inhibition of Atg9a expression in human cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10  $\mu$ M in 66  $\mu$ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

Atg9a (F-3): sc-518179 is recommended as a control antibody for monitoring of Atg9a gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Atg9a gene expression knockdown using RT-PCR Primer: Atg9a (h)-PR: sc-72586-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Mohamud, Y., et al. 2020. Coxsackievirus infection induces a non-canonical autophagy independent of the ULK and PI3K complexes. *Sci. Rep.* 10: 19068.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.