

Atg9a siRNA (m): sc-72587

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, enwrapping 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., Chan, E.Y., Hu, X.W., Köchl, R., Crawshaw, S.G., High, S., Hailey, D.W., Lippincott-Schwartz, J. and Tooze, S.A. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* 119: 3888-3900.
3. Webber, J.L., Young, A.R. and Tooze, S.A. 2007. Atg9 trafficking in Mammalian cells. *Autophagy* 3: 54-56.
4. He, C. and Klionsky, D.J. 2007. Atg9 trafficking in autophagy-related pathways. *Autophagy* 3: 271-274.
5. Legakis, J.E., Yen, W.L. and Klionsky, D.J. 2007. A cycling protein complex required for selective autophagy. *Autophagy* 3: 422-432.

CHROMOSOMAL LOCATION

Genetic locus: Atg9a (mouse) mapping to 1 C3.

PRODUCT

Atg9a siRNA (m) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Atg9a shRNA Plasmid (m): sc-72587-SH and Atg9a shRNA (m) Lentiviral Particles: sc-72587-V as alternate gene silencing products.

For independent verification of Atg9a (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72587A, sc-72587B and sc-72587C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

APPLICATIONS

Atg9a siRNA (m) is recommended for the inhibition of Atg9a expression in mouse 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 μ M in 66 μ 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Atg9a gene expression knockdown using RT-PCR Primer: Atg9a (m)-PR: sc-72587-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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