SANTA CRUZ BIOTECHNOLOGY, INC.

Atg4a siRNA (h): sc-91197

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The Power to Question

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. Atg4a (ATG4 autophagy related 4 homolog A), also known as APG4A or AUTL2, is a 398 amino acid protein that localizes to the cytoplasm and belongs to the peptidase C54 family. Expressed in a variety of tissues, including brain, skeletal muscle and fetal liver, Atg4a functions as a cysteine protease that cleaves the C-terminal part of target proteins, such as GABARAP and MAP1LC3, and plays an essential role in autophagy. Atg4a exists as multiple alternatively spliced isoforms and is functionally inhibited by N-ethylmaleimide.

REFERENCES

1. Mariño, G., et al. 2003. Human autophagins, a family of cysteine proteinases potentially implicated in cell degradation by autophagy. J. Biol. Chem. 278: 3671-3678.
2. Scherz-Shouval, R., et al. 2003. The COOH terminus of GATE-16, an intra-Golgi transport modulator, is cleaved by the human cysteine protease HsApg4A. J. Biol. Chem. 278: 14053-14058.
3. Kabeya, Y., et al. 2004. LC3, GABARAP and GATE-16 localize to autophagosomal membrane depending on form-II formation. J. Cell Sci. 117: 2805-2812.
4. Tanida, I., et al. 2006. Atg8L/Apg8L is the fourth mammalian modifier of mammalian Atg8 conjugation mediated by human Atg4b, Atg7 and Atg3. FEBS J. 273: 2553-2562.

CHROMOSOMAL LOCATION

Genetic locus: ATG4A (human) mapping to Xq22.3.

PRODUCT

Atg4a 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Atg4a shRNA Plasmid (h): sc-91197-SH and Atg4a shRNA (h) Lentiviral Particles: sc-91197-V as alternate gene silencing products.

For independent verification of Atg4a (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-91197A, sc-91197B and sc-91197C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Atg4a siRNA (h) is recommended for the inhibition of Atg4a 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 µM in 66 µl. 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

Atg4a (1458CT808.66.25.69): sc-517309 is recommended as a control antibody for monitoring of Atg4a 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-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Atg4a gene expression knockdown using RT-PCR Primer: Atg4a (h)-PR: sc-91197-PR (20 µl, 599 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Imai, H., et al. 2021. LPIN1 downregulation enhances anticancer activity of the novel HDAC/PI3K dual inhibitor FK-A11. Cancer Sci. 112: 792-802.
2. Fan, Y.M., et al. 2022. Coxsackievirus protease 2A targets host protease Atg4a to impair autophagy. Viruses 14: 2026.

RESEARCH USE

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

PROTOCOLS

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