SANTA CRUZ BIOTECHNOLOGY, INC.

Atg4b siRNA (h): sc-72584

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. Atg4b (ATG4 autophagy related 4 homolog B), also known as APG4B, AUTL1 or KIAA0943, is a 393 amino acid protein that localizes to the cytoplasm and and belongs to the peptidase C54 family. Expressed in heart, pancreas, brain, liver and skeletal muscle, Atg4b 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. Atg4b 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. Hemelaar, J., et al. 2003. A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE16, MAP1LC3, GABARAP, and Apg8L. J. Biol. Chem. 278: 51841-51850.
3. Tanida, I., et al. 2004. HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl-termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and $GABA_A$ receptor-associated protein-phospholipid conjugates. J. Biol. Chem. 279: 36268-36276.
4. Kabeya, Y., et al. 2004. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. J. Cell Sci. 117: 2805-2812.

CHROMOSOMAL LOCATION

Genetic locus: ATG4B (human) mapping to 2q37.3.

PRODUCT

Atg4b 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 Atg4b shRNA Plasmid (h): sc-72584-SH and Atg4b shRNA (h) Lentiviral Particles: sc-72584-V as alternate gene silencing products.

For independent verification of Atg4b (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-72584A, sc-72584B and sc-72584C.

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

Atg4b siRNA (h) is recommended for the inhibition of Atg4b 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

Atg4b (231CT21.1.7): sc-517310 is recommended as a control antibody for monitoring of Atg4b 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Atg4b gene expression knockdown using RT-PCR Primer: Atg4b (h)-PR: sc-72584-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. Li, M., et al. 2012. A high-throughput FRET-based assay for determination of Atg4 activity. Autophagy 8: 401-412.
2. Lin, Y.X., et al. 2017. An *in situ* intracellular self-assembly strategy for quantitatively and temporally monitoring autophagy. ACS Nano 11: 1826-1839.
3. Shin, J.H., et al. 2019. Down-regulated TMED10 in Alzheimer disease induces autophagy via ATG4B activation. Autophagy 1: 1-11.
4. You, F.F., et al. 2021. ATG 4B serves a crucial role in RCE-4-induced inhibition of the Bcl-2-Beclin 1 complex in cervical cancer Ca Ski cells. Int. J. Mol. Sci. 22: 12302.
5. Jianbing, H., et al. 2022. The effect of allograft inflammatory factor-1 on inflammation, oxidative stress, and autophagy via miR-34a/ATG4B pathway in diabetic kidney disease. Oxid. Med. Cell. Longev. 2022: 1668000.

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.