SANTA CRUZ BIOTECHNOLOGY, INC.

Atg4D siRNA (h): sc-97728

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. Atg4D (autophagy-related gene 4D), also known as APG4D or AUTL4, is a 474 amino acid protein that localizes to the cytoplasm and belongs to the C-54 family of cysteine proteases. Expressed predominately in skeletal muscle, but also present in testis, Atg4D functions as a cysteine protease that is required for autophagy and functions to specifically cleave the C-terminal region of target proteins, thereby allowing the target proteins to bind to autophagosomes. The enzymatic activity of Atg4D is inhibited by N-ethylmaleimide, a thiol reactive compound that is capable of modifying cystine residues in proteins and peptides.

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. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 611340. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Gajewska, M., et al. 2008. Role of autophagy in mammary gland development. J. Physiol. Pharmacol. 9: 237-249.
4. Periyasamy-Thandavan, S., et al. 2009. Autophagy: molecular machinery, regulation and implications for renal patho-physiology. Am. J. Physiol. Renal Physiol. 297: F244-F256.
5. Dwivedi, M., et al. 2009. Autophagy genes mediate the effect of calcineurin on lifespan in *C. elegans*. Autophagy 5: 604-607.
6. Dwivedi, M., et al. 2009. Autophagy—is it a preferred route for lifespan extension? BMB Rep. 42: 62-71.
7. Young, A.R., et al. 2009. Autophagy mediates the mitotic senescence transition. Genes Dev. 23: 798-803.

CHROMOSOMAL LOCATION

Genetic locus: ATG4D (human) mapping to 19p13.2.

PRODUCT

Atg4D siRNA (h) is a pool of 2 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 Atg4D shRNA Plasmid (h): sc-97728-SH and Atg4D shRNA (h) Lentiviral Particles: sc-97728-V as alternate gene silencing products.

For independent verification of Atg4D (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-97728A and sc-97728B.

RESEARCH USE

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

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

Atg4D siRNA (h) is recommended for the inhibition of Atg4D 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

Atg4D (222CT15.4.1): sc-517312 is recommended as a control antibody for monitoring of Atg4D 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 Atg4D gene expression knockdown using RT-PCR Primer: Atg4D (h)-PR: sc-97728-PR (20 µl, 598 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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