

Atg4D siRNA (m): sc-141325

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 predominantly 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 cysteine 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.* 59: 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 (mouse) mapping to 9 A3.

PRODUCT

Atg4D siRNA (m) is a target-specific 19-25 nt siRNA 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 Atg4D shRNA Plasmid (m): sc-141325-SH and Atg4D shRNA (m) Lentiviral Particles: sc-141325-V as alternate gene silencing products.

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

Atg4D siRNA (m) is recommended for the inhibition of Atg4D 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 Atg4D gene expression knockdown using RT-PCR Primer: Atg4D (m)-PR: sc-141325-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.