

Atg16 siRNA (m): sc-72581

BACKGROUND

WD-repeats are motifs that are found in a variety of proteins and are characterized by a conserved core of 40-60 amino acids that commonly form a tertiary propeller structure. While proteins that contain WD-repeats participate in a wide range of cellular functions, they are generally involved in regulatory mechanisms concerning chromatin assembly, cell cycle control, signal transduction, RNA processing, apoptosis and vesicular trafficking. Atg16, also known as ATG16L1, IBD10 or WDR30, is a 607 amino acid protein that localizes to both the cytoplasm and the peripheral membrane and contains seven WD-repeats. Existing as a homooligomer, Atg16 interacts with APG5 and plays a crucial role in autophagy, the major intracellular degradation system that delivers cytoplasmic proteins to lysosomes for destruction. Genetic variations in the gene encoding Atg16 are associated with susceptibility to inflammatory bowel disease type 10 (IBD10), a chronic relapsing intestinal inflammation. Multiple isoforms of Atg16 exist due to alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 6107678. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Mizushima, N., et al. 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J. Cell Sci.* 116: 1679-1688.
3. Zheng, H., et al. 2004. Cloning and analysis of human Apg16L. *DNA Seq.* 15: 303-305.
4. Massey, D.C. and Parkes, M. 2007. Genome-wide association scanning highlights two autophagy genes, ATG16L1 and IRGM, as being significantly associated with Crohn's disease. *Autophagy* 3: 649-651.

CHROMOSOMAL LOCATION

Genetic locus: Atg1611 (mouse) mapping to 1 D.

PRODUCT

Atg16 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 Atg16 shRNA Plasmid (m): sc-72581-SH and Atg16 shRNA (m) Lentiviral Particles: sc-72581-V as alternate gene silencing products.

For independent verification of Atg16 (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-72581A, sc-72581B and sc-72581C.

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.

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

Atg16 siRNA (m) is recommended for the inhibition of Atg16 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.

GENE EXPRESSION MONITORING

Atg16 (E-10): sc-393274 is recommended as a control antibody for monitoring of Atg16 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Atg16 gene expression knockdown using RT-PCR Primer: Atg16 (m)-PR: sc-72581-PR (20 μ l, 597 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.