

Atg3 siRNA (m): sc-72583

BACKGROUND

Atg3 (autophagy-related protein 3), also known as APG3-like, hAPG3 or PC3-96, is an E2-like enzyme that localizes to the cytoplasm and is expressed in a variety of tissues with predominant levels found in kidney, placenta, liver, heart and skeletal muscle. Atg3 catalyzes the formation of the Atg8-phosphatidylethanolamine (Atg8-PE) conjugate, a reaction that is essential for autophagy (a cellular process that allows for the degradation of organelles and bulk cellular proteins). The process of forming the Atg8-PE conjugate begins with the removal of the C-terminal arginine residue of Atg8 by Atg4, a cysteine protease. The, now exposed, glycine residue is then activated by Atg7 and is then transferred to Atg3 for the final conjugation to PE. This last step can be accelerated by the presence of the Atg12-Atg5 conjugate which functions similarly to an E3 enzyme.

REFERENCES

1. Tanida, I., et al. 2002. Human Apg3p/Aut1p homologue is an authentic E2 enzyme for multiple substrates, GATE-16, GABARAP, and MAP LC3, and facilitates the conjugation of hApg12p to hApg5p. *J. Biol. Chem.* 277: 13739-13744.
2. Wu, B.X., et al. 2006. The rat Apg3p/Aut1p homolog is upregulated by ischemic preconditioning in the retina. *Mol. Vis.* 12: 1292-1302.
3. Yamada, Y., et al. 2006. Crystallization and preliminary X-ray analysis of Atg3. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 62: 1016-1017.
4. Yamada, Y., et al. 2007. The crystal structure of Atg3, an autophagy-related ubiquitin carrier protein (E2) enzyme that mediates Atg8 lipidation. *J. Biol. Chem.* 282: 8036-8043.

CHROMOSOMAL LOCATION

Genetic locus: Atg3 (mouse) mapping to 16 B5.

PRODUCT

Atg3 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 Atg3 shRNA Plasmid (m): sc-72583-SH and Atg3 shRNA (m) Lentiviral Particles: sc-72583-V as alternate gene silencing products.

For independent verification of Atg3 (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-72583A, sc-72583B and sc-72583C.

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

Atg3 siRNA (m) is recommended for the inhibition of Atg3 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

Atg3 (A-3): sc-393660 is recommended as a control antibody for monitoring of Atg3 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 Atg3 gene expression knockdown using RT-PCR Primer: Atg3 (m)-PR: sc-72583-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Phadwal, K., et al. 2022. Metformin protects against vascular calcification through the selective degradation of Runx2 by the p62 autophagy receptor. *J. Cell. Physiol.* E-published.

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.