

Atg12 siRNA (m): sc-72579

BACKGROUND

Atg12 (autophagy-related protein 12), also known as APG12, APG12L, FBR93 or HAPG12, is a 140 amino acid protein that is ubiquitously expressed and belongs to the Atg12 family of proteins. Atg12 is a homolog of the yeast protein Apg12 that participates in autophagy. Autophagy is a membrane trafficking mechanism that delivers cytoplasmic cargo to the vacuole/lysosome for degradation and recycling. In yeast, autophagy requires a protein conjugation system consisting of Apg12 covalently bound at the carboxy terminal glycine to lysine 149 of Apg5. Similarly in humans, Atg12 is essential for autophagy and localizes to the cytoplasm where it is covalently bound to APG5, a conjugation reaction that requires APG7, Atg10 and ATP. The Atg12-APG5 conjugate functions as an important regulator of the autophagic process and is required for the change in membrane morphology and development of autophagosomes. Due to alternative splicing events, two Atg12 isoforms exist.

REFERENCES

1. Ueno, K., et al. 1998. Cloning and tissue expression of cDNAs from chromosome 5q21-22 which is frequently deleted in advanced lung cancer. *Hum. Genet.* 102: 63-68.
2. Mizushima, N., et al. 1998. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. *J. Biol. Chem.* 273: 33889-33892.
3. Mizushima, N., et al. 1998. A protein conjugation system essential for autophagy. *Nature* 395: 395-398.

CHROMOSOMAL LOCATION

Genetic locus: Atg12 (mouse) mapping to 18 C.

PRODUCT

Atg12 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 Atg12 shRNA Plasmid (m): sc-72579-SH and Atg12 shRNA (m) Lentiviral Particles: sc-72579-V as alternate gene silencing products.

For independent verification of Atg12 (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-72579A, sc-72579B and sc-72579C.

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.

RESEARCH USE

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

APPLICATIONS

Atg12 siRNA (m) is recommended for the inhibition of Atg12 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

Atg12 (C-6): sc-271688 is recommended as a control antibody for monitoring of Atg12 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 Atg12 gene expression knockdown using RT-PCR Primer: Atg12 (m)-PR: sc-72579-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. Rangaraju, S., et al. 2010. Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. *J. Neurosci.* 30: 11388-11397.
2. Fan, X., et al. 2017. Foot-and-mouth disease virus infection suppresses autophagy and NF- κ B antiviral responses via degradation of ATG5-ATG12 by 3C^{pro}. *Cell Death Dis.* 8: e2561.

PROTOCOLS

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